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Conjugated Linoleic Acid Suppresses Myogenic Gene Expression in a Model of Human Muscle Cell Inflammation[1,2]

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Abstract

Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, contribute to muscle wasting in inflammatory disorders, where TNFα acts to regulate myogenic genes. Conjugated linoleic acid (CLA) has shown promise as an antiproliferative and antiinflammatory agent, leading to its potential as a therapeutic agent in muscle-wasting disorders. To evaluate the effect of CLA on myogenesis during inflammation, human primary muscle cells were grown in culture and exposed to varying concentrations of TNFα and the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers. Expression of myogenic genes (Myf5, MyoD, myogenin, and myostatin) and the functional genes creatine kinase (CK) and myosin heavy chain (MHC IIx) were measured by real-time PCR. TNFα significantly downregulated MyoD and myogenin expression, whereas it increased Myf5 expression. These changes corresponded with a decrease in both CK and MHC IIx expression. Both isomers of CLA mimicked the inhibitory effect of TNFα treatment on MyoD and myogenin expression, whereas myostatin expression was diminished in the presence of both isomers of CLA either alone or in combination with TNFα. Both isomers of CLA decreased CK and MHC IIx expression. These findings demonstrate that TNFα can have specific regulatory effects on myogenic genes in primary human muscle cells. A postulated antiinflammatory role of CLA in myogenesis appears more complex, with an indication that CLA may have a negative effect on this process. J. Nutr. 138: 12–16, 2008.

Introduction

Severe muscle wasting is characteristic of many inflammatory disease states such as AIDS, sepsis, chronic heart failure, chronic obstructive airway disease, and advanced cancer, with progressive loss of weight and muscle mass having the ability to cause major functional impairment (1). Under normal conditions, muscle mass reflects the balance between rates of muscle synthesis and breakdown; however, as a consequence of a prolonged inflammatory state, rapid loss of muscle protein can occur through an imbalance between protein synthesis and protein degradation, which can have dramatic health implications in certain disease states (2).

Muscle growth is controlled by a series of basic helix-loop-helix transcription factors known collectively as the myogenic regulatory factors (3). These myogenic factors can be either positive or negative regulating factors of myoblast differentiation (4). Of the myogenic factors, MyoD, myogenin, and Myf5 exert crucial roles in the recruitment and maturation of satellite cells into myoblasts. More recently, MyoD and myogenin have been shown to be expressed in myotubes where they direct and maintain characteristics of the muscle phenotype (5). Myostatin (growth/differentiation factor 8; GDF-8) is a member of the transforming growth factor-β family of growth factors and is a negative regulator of growth and differentiation in skeletal muscle and acts specifically in developing and maturing skeletal muscle (6). Myostatin acts by inhibiting both satellite cell proliferation and differentiation through the upregulation of p21, halting the cell cycle, and inhibiting differentiation through inhibiting MyoD expression (7).

Although the molecular basis of muscle wasting in chronic inflammatory conditions such as cancer cachexia remains largely unresolved, several key mediators have been identified. The metabolic abnormalities that arise in cachexia are in part mediated by the production of proinflammatory cytokines, including tumor necrosis factor (TNF)[3]-α, interleukin (IL)-1β, IL-6, and interferon (IFN)-γ (8). These factors are produced by host immune cells in response to the tumor or by the tumor itself (9). TNFα in particular has been found to be elevated in the circulation of people with cancer and is thought to contribute to the increased muscle catabolism and loss of muscle function seen in cancer cachexia (10). Previous research suggests that impaired regeneration of skeletal muscle is a key pathogenic factor for muscle depletion in cachexia (11). It has been identified that

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[3] Abbreviations used: c9,t11 CLA, *cis*-9, *trans*-11 conjugated linoleic acid; CK, creatine kinase; CLA, conjugated linoleic acid; IFN, interferon; IL, interleukin; MHC IIx, myosin heavy chain IIx; t10,c12 CLA, *trans*-10, *cis*-12 conjugated linoleic acid; TNF, tumor necrosis factor.

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TNFα targets specific myogenic genes (MyoD and myogenin) involved in the proliferation and differentiation of skeletal muscle, thus inhibiting skeletal myogenesis in the cachectic patient (11). In vivo, TNFα has been shown to downregulate the expression of MyoD and myogenin in differentiated mouse C2C12 myotubes, thereby compromising regeneration of skeletal muscle (11,12). TNFα has also been observed to suppress both MyoD and myogenin expression through a nuclear factor κB-dependent manner in C2C12 myoblasts, hindering myogenic differentiation (12).

Currently, therapeutic interventions in cancer cachexia aimed at increasing muscle and weight are of limited benefit (13). In recent times, conjugated linoleic acid (CLA), a group of positional and geometric isomers of linoleic acid found naturally in meat and dairy products derived from ruminant animals, have shown promise in reducing inflammation and as an anticarcinogenic agent (14). Although many isomers of CLA exist, the *cis*-9, *trans*-11 (c9,t11) and *trans*-10, *cis*-12 (t10,c12) isomers are the predominant biologically active forms and exhibit numerous physiological properties such as anticarcinogenic, immunomodulatory, and antiinflammatory action (15).

There is clear evidence illustrating the dose-response, time-dependent, and isomer-specific effects of CLA on the in vitro proliferation of human tumor cells from breast, lung, colon, prostate, skin, and stomach cancer (16). Recently, it has been suggested that the antiproliferative effects of CLA arise from its antiinflammatory properties by negatively regulating the expression of proinflammatory cytokines such as TNFα, IL-1, and IL-6 (15). There is evidence to suggest that CLA supplementation is able to reduce serum TNFα levels in vivo and decrease skeletal muscle TNFα gene expression, although the underlying mechanisms are unclear (17). Due to its antiproliferative and antiinflammatory effects, CLA has the potential to be a novel dietary supplement for patients suffering from muscle wasting in chronic inflammatory related disease, particularly cancer cachexia.

The aims of this study were to characterize the effects of TNFα on myogenic gene expression in human primary skeletal muscle and to assess the impact of CLA isomers on this response. The establishment and characterization of a novel system such as primary muscle cell culture provides a platform that allows insight into muscle-wasting conditions and permits the evaluation of novel dietary factors such as CLA in potentially attenuating this response using a model that has direct human physiologic relevance.

Materials and Methods

Reagents. Lyophilized recombinant human TNFα was obtained from BioSource and suspended in PBS (Sigma Aldrich) to give a final concentration of 1 μg/μL. TNFα was subsequently diluted into a working stock of 0.01 μg in 1 μL. CLA (individual c9,t11 and t10,c12 CLA isoforms) were obtained from Adelab Scientific in lyophilized form and were dissolved in 100% v:v ethanol to give a 500-mmol/L stock solution, which was further diluted in ethanol to produce the range of test concentrations. The final concentration of ethanol in all experiments involving CLA addition was at 0.01% v:v including a vehicle control. All fatty acids were stored at –20°C.

Primary muscle cell culture. Primary muscle cells were obtained from a total of 8 healthy adult male volunteers (22.8 ± 0.5 y).

One resting muscle biopsy sample from each subject was obtained under local anesthesia (xylocaine 1%) from the vastus lateralis using the percutaneous needle biopsy technique (18) modified to include suction (19). The excised muscle tissue was immersed in ice-cold serum free α-MEM (Gibco) and digested in 0.5% trypsin/0.53 mmol/L EDTA (Gibco). The supernatant containing the myoblasts was collected and the process was repeated twice to aid digestion. Fetal bovine serum (Gibco) was added to the supernatant to a final concentration of 10%. Supernatant was filtered to remove connective tissue and centrifuged (400 × *g*; 5 min) to obtain a pellet. The resulting pellet was resuspended in 5 mL α-MEM and seeded onto an uncoated 25-cm^2 flask and incubated at 37°C for 30 min to facilitate attachment of fibroblasts. Myoblasts suspended in the medium were then seeded onto flasks coated with extracellular matrix gel (0.3% in α-MEM; Sigma) and maintained in growth medium. All experimental procedures involved in this study were approved by the Deakin University Human Research Ethics Committee.

Adult-derived myoblasts (harvested from the satellite cell population of healthy volunteers) were maintained in α-MEM supplemented with 10% fetal bovine serum, 0.5% fungizone (Gibco), and 0.5% antibiotic (penicillin-streptomycin) (Gibco) and incubated at 37°C in the presence of 5% CO_2. Myoblast differentiation was initiated by the addition of α-MEM supplemented with 2% horse serum, 0.5% fungizone, and 0.5% penicillin-streptomycin. Myotubes were treated with TNFα (10 μg/L) at either the onset of myotube differentiation or after the cells had differentiated (by incubation in differentiation medium for 4 d) and incubated in the presence of TNFα for 96 h. Myotubes were also exposed to CLA (25 μmol/L) for 96 h with or without TNFα.

RNA extraction. Total RNA was extracted from cell culture using TRI Reagent (Sigma-Aldrich). Cells were washed twice with PBS and TRI Reagent added to lyse the cells. The homogenate was collected and 200 μL/1 mL of chloroform added to the sample. After 10 min incubation and centrifugation to facilitate the separation of phases, the aqueous phase was removed and transferred to a fresh tube. RNA was precipitated with an equal volume of isopropanol and refrigerated overnight to aid in precipitation of the RNA. The following day, samples were centrifuged (7000 × *g*; 60 min) to obtain an RNA pellet. The pellet was then washed with 75% ethanol and resuspended in 5 μL nuclease-free water and stored at −80°C in small aliquots.

RNA quantification and RT. RNA integrity and quantity were assessed on an Agilent Bioanalyzer 2100 with an RNA 6000 Nano LabChip kit (Agilent Technologies). A total of 0.5 μg RNA was reverse transcribed to synthesize first-strand cDNA using the AMV reverse transcription kit (A3500; Promega). RNA was added to a master mix containing 5 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl (pH 8.8), 50 mmol/L KCl, 1% Triton X-100, 1 mmol/L of each 2-deoxynucleotide 5′-triphosphate, 20 U recombinant RNAsin ribonuclease inhibitor (40 kU/L), and 0.5 mg oligo(dT). The mixture was incubated at 42°C for 60 min before the reaction was terminated by incubation at 99°C for 5 min followed by 5 min at 4°C using the PCR express Thermal Cycler (Hybaid). The cDNA was diluted to a concentration of 1:20 in sterile milliQ water and stored at −20°C for subsequent analysis. An RT negative was obtained by not adding any RNA to an aliquot of the RT mix.

Real-time PCR analysis. To perform PCR, specific primers were designed for all genes using Primer Express software (Applied Biosystems) on sequences obtained from GenBank. The primer sequences used were: cyclophillin (NM_021130): forward primer, 5′-CATCTGCACTGCCAAGACTGA-3′, reverse primer, 5′-TTCATGCTTCTTTCACTTTG C-3′; creatine kinase (CK) (muscle; NM_001824): forward primer, 5′- GCATC TGGCACAATGAC-3′, reverse primer, 5′-GATGACCCGGAGGTGAT C-3′; myosin heavy chain (MHC IIx) (AF111785): forward primer, 5′-AAGGTCGGCAATGAGTATGTCA-3′, reverse primer, 5′- CAACCATC-CACAGGACACTCTTC-3′; Myf5 (NM_005593): forward primer, 5′-TTCTACGACGGCTCCTGCATA-3′, reverse primer, 5′-CCACTCGCGG-CACAAACT-3′; MyoD (NM_002478): forward primer, 5′-CCGCCTG AGCAAAGTAAATGA-3′, reverse primer, 5′-GCAACCGCTGGTTTG-GATT-3′; myogenin (NM_002479): forward primer, 5′- GGTGCCCAGC GAATGC-3′, reverse primer, 5′-TGATGCTGTCCACGATGGA-3′; myostatin (NM_005259): forward primer, 5′-CCAGGAGAAGATGGGCT-GAA-3′, and reverse primer, 5′-CAAGACCAAAATCCCTTCTGGAT-3′.

Where possible, primers were designed spanning intron-exon boundaries to prevent amplification of the target region from any contaminat-

ing DNA. Quantification of messenger RNA expression was performed (in triplicate) by real-time PCR using the ABI PRISM 5700 sequence detection system (Applied Biosystems) (20). For the PCR step, reaction volumes of 20 μL contained SYBR Green 1 Buffer (Applied Biosystems), forward and reverse primers, and cDNA template (diluted 1:40). All samples were conducted in triplicate. Real-time PCR was conducted for 1 cycle (50°C 2 min, 95°C 10 min) followed by 40 cycles (95°C 15 s, 60°C 60 s) and fluorescence was measured after each of the repetitive cycles. A melting point dissociation curve generated by the instrument was used to confirm that only a single product was amplified. Fluorescent emission data were captured and mRNA levels were quantitated using the critical threshold value. To compensate for variations in input RNA amounts and efficiency of RT, cyclophilin (GenBank accession no. NM_021130) mRNA was quantitated and results were normalized to these values.

Statistical analysis. Experiments examining the effects of TNFα when added to undifferentiated and differentiated myoblasts cells were analyzed for time (undifferentiated vs. differentiated cells) and treatment effects by 2-way ANOVA with post hoc paired and unpaired *t* tests where appropriate. Where data from each experimental treatment were normalized to its individual control, 1 sample *t* test was performed. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS version 14). All data are presented as means ± SEM of separate cell lines each grown individually in triplicate. Significance was considered at $P < 0.05$.

Results

Effect of TNFα on myogenic gene expression. Exposure of myotubes to TNFα (10 μg/L) for 4 d at either the onset of myotube differentiation or after the cells had differentiated resulted in a variety of changes in myogenic genes (**Table 1**). For cells incubated with TNFα at the onset of differentiation, there was a significant 72% increase in Myf5 expression with a concomitant significant decrease of 63% in myogenin expression. These aforementioned gene expression changes were also evident in myotubes that had first undergone differentiation for 4 d prior to exposure to TNFα, with a similar pattern of change with Myf5 expression significantly increasing by 69% and myogenin significantly decreasing by 63%. TNFα exposure also resulted in a significant inhibition in the expression of MyoD in differentiated myotubes by 68%. The absolute expression of MyoD, Myf5, or myogenin did not differ between similarly treated cells at the onset of myotube differentiation or after the cells had differentiated, but myostatin expression was significantly lower in both control and TNFα treated cells that had first undergone differentiation.

As a transcriptional proxy measure of functional muscle proteins expressed during differentiation, both CK and MHC IIx mRNA expression were measured under the same experimental conditions as previously described. Both CK and MHC IIx mRNA expression were significantly reduced in undifferentiated and differentiated myotubes in response to treatment with TNFα (Table 1). Total protein levels in undifferentiated and differentiated cells did not differ between treatments (data not shown).

Effect of CLA on TNFα-induced myogenic gene expression changes. To investigate the potential for individual CLA isomers to exert an inhibitory effect on the inflammatory actions of TNFα on myogenic differentiation, human primary muscle cells were incubated for 96 h in the presence or absence of combinations of TNFα (10 μg/L), c9,t11 CLA (25 μmol/L), and t10,c12 CLA (25 μmol/L) in myotubes that had undergone differentiation (**Table 2**). The addition of TNFα alone resulted in decreased expression of MyoD and myogenin, increased expression of Myf5, and no effect on Myostatin, similar to the effects shown in Table 1. Both CLA isomers, either alone or in combination with TNFα, significantly decreased the expression of MyoD and myogenin to a level similar to that in the presence of TNFα alone, whereas Myf5 expression was unaffected. Myostatin expression was unaffected by the presence of TNFα alone; however, it was diminished by the presence of both isomers of CLA, either alone or combination with TNFα.

Expression of both CK and MHC IIx was measured in response to TNFα treatment alone and in combination with both isomers of CLA (Table 2). Expression of CK was significantly decreased in response to the c9,t11 and t10,c12 isomers of CLA, whereas MHC IIx expression was significantly decreased by the addition of the t10,c12 isomer and TNFα combined. Total protein levels in treated differentiated cells did not differ from their individual controls (data not shown).

TABLE 1 Changes in myogenic and functional protein gene expression in human primary myotubes after exposure to 10 μg/L TNFα for 4 d[1,2]

	Undifferentiated cells		Differentiated cells	
Gene	Control	TNFα	Control	TNFα
		AU[3]		
MyoD	0.054 ± 0.010	0.030 ± 0.016	0.061 ± 0.016	0.019 ± 0.006*
Myogenin	0.512 ± 0.069	0.142 ± 0.124*	0.576 ± 0.154	0.212 ± 0.049*
Myf5	0.117 ± 0.025	0.223 ± 0.023*	0.064 ± 0.023	0.208 ± 0.049*
Myostatin	0.139 ± 0.036	0.134 ± 0.018	0.056 ± 0.018	0.070 ± 0.012**
CK	0.150 ± 0.013	0.063 ± 0.024*	0.122 ± 0.036	0.021 ± 0.007*
MHC IIx	0.269 ± 0.041	0.079 ± 0.033*	0.171 ± 0.066	0.023 ± 0.004*

[1] Values are means ± SEM, $n = 5$ cell lines with triplicate observations for each cell line. Time × treatment interactions were not significant, $P \geq 0.05$. *Different from respective control, $P < 0.05$; **Different from respective undifferentiated cells, $P < 0.05$.

[2] TNFα was added to cells at either the onset of myotube differentiation (cells were initially in the undifferentiated state) or after the cells had differentiated for 4 d.

[3] Arbitrary units.

Discussion

Muscle pathologies involving inflammation are the major cause of muscle weakness and loss of function seen in many chronic wasting diseases that are associated with elevated levels of circulating inflammatory mediators, including TNFα (1). In this study, differentiated primary human myotubes were used as a model to delineate the effects of TNFα on myogenic gene expression. TNFα significantly downregulated both MyoD and myogenin expression in myotubes, whereas Myf5 expression was significantly increased. These changes corresponded with decreased CK and MHC IIx mRNA expression, indicating that the effects of TNFα on myogenic gene expression could translate into changes of functional muscle protein genes expressed during differentiation.

Previous animal studies have illustrated that TNFα-induced muscle depletion is paralleled by a decrease in both MyoD and myogenin mRNA and protein expression (2,12,21). Surprisingly, Myf5 expression, a positive regulator of myogenesis, increased in response to TNFα in this study. One explanation for this may be related to the known interrelationship between Myf5 and MyoD, which are both expressed during myoblast proliferation and differentiation and play important roles in

TABLE 2 Changes in myogenic and functional protein gene expression in differentiated human primary myotubes in response to TNFα (10 μg/L), c9,t11 CLA, and t10,c12 CLA (25 μmol/L) for 4 d[1,2]

	TNFα	c9,t11 CLA	c9,t11 CLA + TNFα	t10,c12 CLA	t10,c12 CLA + TNFα
			proportion of control		
MyoD	0.502 ± 0.149*	0.602 ± 0.106*	0.273 ± 0.149*	0.594 ± 0.127	0.268 ± 0.091*
Myogenin	0.391 ± 0.185	0.427 ± 0.107*	0.303 ± 0.208	0.406 ± 0.097*	0.257 ± 0.147*
Myf5	1.789 ± 0.240	1.025 ± 0.205	1.265 ± 0.511	0.875 ± 0.141	0.567 ± 0.101
Myostatin	1.019 ± 0.398	0.525 ± 0.053*	0.448 ± 0.169	0.563 ± 0.115	0.351 ± 0.145*
CK	0.788 ± 0.146	0.479 ± 0.091*	0.556 ± 0.264	0.361 ± 0.117*	0.457 ± 0.236
MHC IIx	0.637 ± 0.231	0.551 ± 0.188	0.520 ± 0.177	0.445 ± 0.155	0.289 ± 0.128*

[1] Values are means ± SEM, $n = 3$ cell lines with triplicate observations for each cell line. *Different from individual control, $P < 0.05$.
[2] All data were normalized to separate control treatments, designated as 1.0.

maturation and commitment of satellite cells (22). In the absence or inhibition of MyoD, there is increased Myf5 mRNA expression; this may act as a functional substitute to compensate for the absence of MyoD (23). This compensatory action of Myf5 in response to TNFα-induced downregulation of MyoD may enable the myotube cells to progress through proliferation and early differentiation, albeit with reduced CK and MHC IIx mRNA expression. Interestingly, it has previously been reported that both MyoD and myogenin transcriptionally activate muscle-derived CK (24,25); hence, a reduction in CK expression in response to TNFα in this study confirms this link in a human muscle cell model. The decreased structural protein marker expression has also been observed at a posttranscriptional level, where TNFα has been shown to inhibit the expression of MHC in both differentiating murine C2C12 and primary human myoblasts (26,27).

It was surprising that expression of myostatin, a negative regulator of growth and differentiation in skeletal muscle, was unaltered by TNFα treatment. Interestingly, however, absolute myostatin expression was reduced in control and TNFα-treated cells after the cells had differentiated, indicating that myostatin expression is highest in early stage proliferation and differentiation rather than in late differentiation. Overexpression of myostatin in mice has been found to induce dramatic cachexia-like muscle wasting; however, serum levels of IL-6 and TNFα were not changed, so TNFα may not be a direct mediator of myostatin expression (28).

It has been suggested that the antiproliferative effects of CLA arise from its antiinflammatory properties by negatively regulating the expression of proinflammatory cytokines such as TNFα, IL-1, and IL-6 (15). This study indicates that in response to treatment with either isomer of CLA alone or in combination with TNFα, effects on myogenic gene expression are mixed, with CLA unable to attenuate the TNFα-induced reduction in MyoD and myogenin gene expression. In fact, expression of these genes in the presence of CLA mimicked the inhibitory effect of TNFα treatment alone. Furthermore, decreased CK and MHC IIx mRNA expression in response to individual CLA isomers alone or in combination with TNFα were also found, similar to the effects of TNFα alone. This finding is supported by the previously described inhibitory effect of the t10,c12 isomer of CLA on muscle cell differentiation, as measured by decreased CK activity, in rodent L6 skeletal muscle cells (29). In contrast, stimulatory effects of both the c9,t11 isomer (doses up to 50 μmol/L) and linoleic acid have been found; however, at higher concentrations, c9,t11 CLA inhibited differentiation (29,30). Interestingly, expression of myostatin, which was not affected by TNFα, was significantly diminished in the presence of either isomer of CLA. This suggests that a diminution in myostatin expression by CLA may increase muscle growth and could partly explain the favorable effects that CLA has on body composition (31).

While divergent effects of CLA isomers previously have been reported in both in vitro and in vivo murine and human models, in this study, the actions of both isomers of CLA were similar. The possibility of further post-transcriptional events occurring that could result in divergence of CLA action on functional protein measures of muscle myogenesis cannot be discounted. The effects of CLA on gene expression in our cell system are unlikely to be a result of apoptotic or cell proliferation effects. Total cellular protein, a gross measure of cell viability and cell number, was similar for all treatment conditions, indicating no gross effects on cell viability. Furthermore, myogenic and functional genes expressions were reported relative to a housekeeping gene (cyclophilin) to account for differences in cell number due to seeding and growth rate differences. Therefore, the gene expression changes observed were not due to changes in the proliferation rates of cells.

Although it has been postulated that CLA can modulate the expression of TNFα through the transcription factor nuclear factor κB (32), it is possible that CLA may act through different mechanisms/regulatory pathways that are further dependent on the isomeric form of CLA. Postulated modes of action of CLA include effects on regulation of genes involved in arachidonic acid metabolism, resulting in attenuation of inducible eicosanoids involved in inflammatory events, modulation of genes involved in apoptosis and cell cycle control, or direct modulation of expression of inflammatory genes (33). Considering that CLA could decrease CK and MHC IIx expression in primary muscle cells, the well-documented antiproliferative effects of CLA on tumor cells may actually reflect a similar situation in muscle cells, meaning that CLA has little therapeutic benefit in increasing muscle growth in vivo and may, in fact, be detrimental in inflammatory muscle-wasting conditions. One strength of this study is that the responses observed were in the face of exposure to physiologic relevant concentrations of TNFα and CLA. Similar levels of TNFα have been found in the serum of patients with cancer cachexia (34) and the dose of CLA used was consistent with concentrations in human serum (35).

In summary, this study demonstrates that TNFα has specific regulatory effects on myogenic genes in a human in vitro muscle cell system. Changes in myogenic gene expression in response to TNFα are likely an important part of muscle wasting seen in chronic inflammatory conditions such as cancer cachexia; however, more functional measures need to be studied to further delineate the effects of TNFα on inflammatory-related muscle

wasting. Although a postulated antiinflammatory role of CLA in myogenesis appears more complex, there is an indication that CLA may have a negative effect on myogenesis, at least in vitro. The establishment and characterization of a primary human muscle cell model of myogenesis during inflammation has application in evaluating novel dietary factors such as CLA in conditions such as inflammatory muscle wasting.

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