Conjugated Linoleic Acid’s Lack of Attenuation of Hyperpnea-Induced Bronchoconstriction in Asthmatic Individuals in the Short Term

Jonathon L. Stickford, Timothy D. Mickleborough, Alyce D. Fly, and Joel M. Stager

Purpose: Conjugated linoleic acid (CLA) has been reported to modify the inflammatory response associated with allergic airway disease, primarily in animal models. To extend these observations to humans, the effect of short-term CLA supplementation on the severity of exercise-induced bronchoconstriction (EIB) was investigated in asthmatics.

Methods: Six subjects with physician-diagnosed asthma and EIB began the study on their usual diet, to which was added 4.8 g CLA/d for 8 wk. Pulmonary-function tests were administered before and after eucapnic voluntary hyperventilation (EVH) challenge at the commencement (Week 0) and conclusion of the treatment period (Week 8). Pre- and 90 min post-EVH challenge, urine was assayed for the presence of cysteinyl leukotrienes (LT) C4–E4 and 9α,11β-prostaglandin (PG) F2.

Results: Pre- to post-EVH forced expiratory volume in 1 s (FEV1) did not significantly differ (p > .05) from Week 8 to Week 0. The pre- to post-EVH decline in FEV1 at Week 8 (–29.6% ± 6.6%) was not significantly different (p > .05) from that at Week 0 (–32.0% ± 5.5%). Area under the curve of FEV1 plotted against time from zero to 60 min (AUC0–60) was unaltered at Week 8 (–931% ± 350% change per minute) compared with Week 0 (–1,090% ± 270% change per minute). CLA supplementation did not alter forced midexpiratory flow, forced vital capacity (FVC), or FEV1/FVC. In addition, post-EVH urinary LTC4–E4 and 9α,11β-PGF2α were unchanged after CLA supplementation.

Conclusion: Daily supplementation of 4.8 g CLA for 8 wk does not attenuate airway inflammation or hyperpnea-induced bronchoconstriction in asthmatic individuals.

Keywords: diet, exercise-induced asthma, inflammation

Exercise-induced bronchoconstriction (EIB) is a transient narrowing of the bronchial airways during (Beck, Offord, & Scanlon, 1994) or after (Anderson & Holzer, 2000) vigorous exercise, which results in an airflow limitation. Although the exact prevalence is unknown, EIB is estimated to affect up to 90% of all asthmatic individuals and 12–15% of the U.S. population (Anderson, 1985; Rupp, 1996). The pathophysiology of EIB likely involves mechanistic pathways, but it is generally accepted that exercise-induced hyperpnea plays an important role as an initiating stimulus through mucosal cooling and dehydration (Anderson & Kippelen, 2005). The role and release of inflammatory mediators in the pathogenesis of EIB have been well documented (Beasley et al., 1987; Hallstrand et al., 2005; O’Byrne, 1997). Tumor-necrosis factor-α (TNF-α), interleukin-1 (IL-1), and histamine are released from mast cells after airway trauma and initiate the inflammatory response (Hallstrand et al., 2005). In addition, arachidonic metabolites such as the cysteinyl (Cyst) 4-series leukotrienes (LT; LTC4, LTD4, LTE4) and the prostaglandin (PG) D2 urinary metabolite 9α,11β-PGF2α have been implicated in the pathogenesis of EIB (O’Byrne, 1997).

There have been significant advances in asthma therapy during the last decade, but treatment is far from ideal. β2-adrenoceptor agonists, mast-cell stabilizers, corticosteroids, and leukotriene modifiers are some of the typical pharmacological medications used to treat EIB. Clinical responses to current therapy such as LT modifiers and corticosteroids are heterogeneous, and even with optimal treatment, there is a substantial burden of unaddressed disease. Although daily asthma medications such as leukotriene modifiers provide only modest protection against symptoms (Storms, 2003), prolonged use of several medications can result in reduced effectiveness or tachyphylaxis (Hancox et al., 2002). Of major importance to athletes with EIB is that a number of medications are currently banned from use during athletic competitions by governing bodies, making effective treatment difficult. Therefore, alternative means of treatment are necessary. There is now convincing evidence that dietary modification has the potential to reduce the severity of this condition (Mickleborough & Gotshall, 2004; Tecklenburg, Mickleborough, Fly, Bai, & Stager, 2007).
Conjugated linoleic acids (CLAs) are positional and geometric isomers of linoleic acid (18:2n-6) found mainly in ruminant products. Animal research has shown that CLA has the ability to modulate the immune response. It has been shown to down-regulate TNF-α synthesis in rat macrophage cultures ex vivo (Turek, Li, Schoenlein, Allen, & Watkins, 1998) and decrease endotoxin-induced production of TNF-α in vivo (Yang & Cook, 2003). In addition, CLA-fed guinea pigs exhibited significantly less histamine and prostanoid release from trachea (Whigham et al., 2001) and Cyst-LT release from lung tissue when superfused with antigen (Whigham et al., 2002).

CLA has been observed to down-regulate inflammatory cytokine and eicosanoid synthesis, including mediators implicated in the pathogenesis of EIB, thus making it a viable dietary supplement for the treatment of EIB in humans. To date, however, there is little experimental evidence for this dietary treatment strategy. Therefore, the aim of the current study was to examine the effect of 8 weeks of CLA supplementation on pulmonary function and urinary markers of airway inflammation in subjects with asthma and EIB. We hypothesized that CLA supplementation would attenuate the bronchoconstrictive and inflammatory response typically observed in asthmatic subjects with EIB.

Methods

Subjects

Six subjects (3 male, 3 female; 21.2 ± 2.6 years; 176 ± 11 cm) with physician-diagnosed asthma and EIB from a university population and the local community participated in this study. The study’s protocols and procedures were approved by the university’s institutional review board before initiation. All subjects completed health, medication, and food-frequency questionnaires and gave written informed consent before enrollment in the study.

Subjects were recreationally active and concurrently undergoing treatment for mild to moderate persistent asthma, with a forced expiratory volume in 1 s (FEV1) greater than 70% of predicted (American Thoracic Society, 1995). They reported a history of chest tightness, shortness of breath, and intermittent wheezing after exercise, relieved by bronchodilator therapy. Two of the 6 subjects were taking maintenance medication throughout the course of the study (n = 1, loratadine, an antihistamine; n = 1, fluticasone propionate, an inhaled corticosteroid). All subjects continued to use their short-acting β2-agonist bronchodilator as needed during the course of the study. To identify any treatment effects, we had subjects refrain from using their short-acting β2-agonist for at least 12 hr, antihistamines for 48 hr, and inhaled corticosteroids for 4 days before eucapnic voluntary hyperventilation (EVH) testing (Anderson, Argyros, Magnussen, & Holzer, 2001; Baumann, Rundell, Evans, & Levine, 2005). In addition, they refrained from ingesting coffee and alcohol for 8 hr and performing physical exercise for 24 hr before testing. To identify any treatment effects, we had subjects refrain from using their short-acting β2-agonist for at least 12 hr, antihistamines for 48 hr, and inhaled corticosteroids for 4 days before eucapnic voluntary hyperventilation (EVH) testing (Anderson, Argyros, Magnussen, & Holzer, 2001; Baumann, Rundell, Evans, & Levine, 2005). In addition, they refrained from ingesting coffee and alcohol for 8 hr and performing physical exercise for 24 hr before testing.

Study Design and Protocol

Before they enrolled in the study, a screening test was administered to all subjects to detect the presence of EIB, which was indicated by a more than 10% decrease in post-EVH FEV1 compared with pre-EVH testing values (Anderson, 1985; Eggleston, 1979). An EVH challenge test was used as a surrogate for exercise to detect EIB (Anderson et al., 2001). In addition, subjects were administered log sheets on which to record their use of all medication throughout the course of the study. The number of times a subject used his or her bronchodilator inhaler was totaled on a weekly basis for the duration of the study. On arrival at the laboratory for each visit, subjects completed a food-frequency questionnaire (FFQ; Fred Hutchinson Cancer Research Center, Seattle, WA) to monitor dietary habits.

The study was conducted as a dietary intervention trial over 8 consecutive weeks, with each subject serving as his or her own control. Subjects entered the study on their usual diet and received an additional CLA supplement (Tonalin, Cognis Corp., La Grange, IL) for the duration of the study. CLA-supplement composition is displayed in Table 1. Subjects consumed two capsules, each containing 0.8 g CLA in free-fatty-acid form derived from safflower oil, three times daily, at breakfast, lunch, and dinner (total of 4.8 g/day). This dose and regimen have previously been shown to promote a physiological response by inducing lipid peroxidation (Basu, Smedman, & Vessby, 2000) and inhibiting stimulated plasma blood mononuclear cell IL-2 secretion in humans (Mullen et al., 2007). A placebo treatment arm was not incorporated in the study design because it has been demonstrated previously that olive oil does not modulate airway function in asthmatic individuals (Mickleborough, Lindley, Ionescu, & Fly, 2006; Mickleborough, Murray, Ionescu, & Lindley, 2003). Adherence to the treatment regimen was monitored by asking the subjects to document the dose of capsules consumed daily and to return any unused capsules. For the purpose of the study a compliance rate of >90% was considered acceptable. In addition, subjects were contacted on a biweekly basis by e-mail to encourage treatment compliance.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Content (mg)</th>
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<tbody>
<tr>
<td>cis-9, trans-11 CLA</td>
<td>380</td>
</tr>
<tr>
<td>trans-10, cis-12 CLA</td>
<td>380</td>
</tr>
<tr>
<td>Unidentified CLA</td>
<td>40</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>120</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>40</td>
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<tr>
<td>Stearic acid</td>
<td>30</td>
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<tr>
<td>Linoleic acid</td>
<td>10</td>
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<tr>
<td>Total</td>
<td>1,000</td>
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Subjects underwent complete pre- and post-EVH pulmonary-function tests (1, 5, 10, 15, 30, 45, and 60 min) at the commencement (Week 0) and conclusion (Week 8) of the supplementation period. Urine samples were collected pre-EVH and 90 min post-EVH at each testing date for the analysis of proinflammatory mediators.

**FFQ**

Nutrient-intake data were collected using an FFQ developed by the Nutrition Assessment Shared Resource of the Fred Hutchinson Cancer Research Center (FHCRC). This questionnaire has been shown to be valid and reliable in the collection of dietary data (Willett et al., 1985). Subjects completed the GSEL version of the questionnaire at Week 0 and Week 8 to assess any possible changes in dietary factors. FFQ data were sent to the FHCRC for analysis, and the results were accessed by the researcher through a secure Web site.

**EVH Test**

The EVH test was used as a surrogate for an exercise challenge test. The test required each subject to inspire dry air (relative humidity <10%) at room temperature (22 °C) containing 21% oxygen, 5% carbon dioxide, and the balance nitrogen. The subjects breathed at a target minute ventilation rate of 30 × FEV₁ for 6 min (Anderson et al., 2001). They inspired air from a 150-L Douglas bag attached to the inspiratory port of a two-way breathing valve (Model 2600, Hans Rudolph, Inc., Shawnee, KS) connected to a rubber mouthpiece. A nose clip was worn for the full duration of the test. Minute ventilation ($V吸入$) was measured throughout the 6-min test using a computer-interfaced metabolic cart (Vmax 22, SensorMedics Corp., Yorba Linda, CA) to verify the target $V吸入$.

**Pulmonary Function**

The subjects performed pre- and post-EVH pulmonary-function tests (forced vital capacity [FVC], FEV₁, and midexpiratory flow [FEF₂₅₋₇₅%]) in accordance with the spirometry standards of the American Thoracic Society (1995). The percentage decline in FEV₁ at each time point from the baseline value was calculated using the following equation: Percent decline = (highest pre-EVH FEV₁ – lowest post-EVH FEV₁ at each time point)/highest pre-EVH FEV₁. The maximum percentage decline in FEV₁ was determined as the largest value obtained during each trial. In addition, the area under the curve of the percentage decline in FEV₁ versus time was calculated using trapezoidal integration and used to assess the total bronchoconstrictive response to the EVH challenge (Makker, Lau, Thomson, Binks, & Holgate, 1993).

**Urinary LTC₄–E₄ and 9α,11β-PGF₂α Analyses**

Urine was collected pre-EVH and at 90-min post-EVH during each laboratory visit. All subjects drank 800 ml of water before the EVH challenge to enhance diuresis after the tests. Three 1-ml samples were transferred to microcentrifuge tubes and stored at −80 °C until analysis of inflammatory mediators. The urine concentrations of LTC₄–LTE₄ and 9α,11β-PGF₂α were determined using enzyme immunoassay techniques (Cayman Chemical, Ann Arbor, MI). The microplates were washed using an ELx405 automated plate washer (Bio-Tek Instruments, Inc., Winooski, VT) and read at 410 nm using a Powerwave XS spectrophotometer (Bio-Tek Instruments). The Cyst-LT assay is based on the competition between Cyst-LTs and a Cyst-LT-acetylcholinesterase conjugate (Cyst-LT tracer) for a limited amount of Cyst-LT antiserum. The antiserum Cyst-LT complex binds to a mouse monoclonal antirabbit antibody that has previously attached to the well. The antibody cross-reacted with LTC₄ (100%), LTD₄ (100%), LTE₄ (67%), LTD₃ (61%), LTC₃ (54%), LTE₃ (41%), and N-acetyl-LTE₄ (10.5%) and below 0.01% for other primary eicosanoid metabolites. The intra- and interassay coefficient of variation (CV) for the Cyst-LT enzyme immunoassay kit was <15%. The 9α,11β-PGF₂α assay is based on the competition between 11β-PGF₂α and an 11β-PGF₂α-acetylcholinesterase conjugate (11β-PGF₂α tracer) for a limited number of 11β-PGF₂α-specific rabbit-antiserum-binding sites. The antibody cross-reacted with 2.3 dinor-11β-PGF₂α (10%) and 11β-13, 14-dihydro-15-keto-PGF₂α (0.5%) and below 0.01% for all other primary eicosanoid metabolites. Intra- and interassay CVs for the 9α,11β-PGF₂α assay were <15%. The concentration of both eicosanoids was adjusted for creatinine concentration (Cayman Chemicals, Ann Arbor, MI). The intra- and interassay CVs for creatinine were <6%.

**Statistical Analysis**

Data were analyzed using SPSS version 16 statistical software (SPSS Inc., Chicago, IL). They were assessed for normality using the Kolmogorov–Smirnov test. Homogeneity of variance was determined using Levene’s test. Pulmonary function, inflammatory-mediator concentration, and dietary nutrient intake data at Week 0 and Week 8 were compared using paired $t$ tests. Pairwise comparisons with a Bonferroni adjustment to maintain an overall Type I error rate of 5% ($p < .05$) were used to isolate differences. Bronchodilator use was assessed using repeated-measures ANOVA. Mauchly’s test was used to determine whether the assumption of sphericity was violated and confirmed that the assumption was met. Data are expressed as $M ± SEM$.

**Results**

**Dietary Intake and Compliance**

From Week 0 to Week 8 no measurable changes in dietary frequencies were observed. Carbohydrate, protein, fat, caffeine, sodium, and ascorbic acid intake
from the FFQ revealed no significant difference \((p > .05)\) from Week 0 to Week 8. Likewise, no significant differences \((p > .05)\) were detected for intake of arachidonic, eicosapentaenoic, and docosahexaenoic acid between Week 0 and Week 8. Subject adherence to the treatment regimens was confirmed; pill counts at the end of each supplement period showed that capsules were consumed regularly.

**Pulmonary Function**

Pre-EVH values for FVC, FEV\(_1\), FEV\(_1\)/FVC, and FEF\(_{25-75}\)% were within normal predicted values (Table 2; Morris, 1976). All subjects exhibited >80% predicted FVC and FEV\(_1\). Pre-EVH pulmonary-function measures were not significantly different \((p > .05)\) from Week 0 to Week 8. EVH \(V_E\) did not significantly differ \((p > .05)\) from Week 0 (117.1 ± 12.5 L/min) to Week 8 (114.6 ± 13.4 L/min).

There were no significant differences \((p > .05)\) observed between Weeks 0 and 8 for post-EVH FEV\(_1\) in each time period. Percentage decline in FEV\(_1\) across time is shown in Figure 1. The maximum percentage decline in FEV\(_1\) also did not significantly differ \((p > .05)\) between Week 0 and Week 8 (−32% ± 6% decline vs. −30% ± 7% decline, respectively; Figure 2). The total bronchoconstrictive response, as determined by the AUC\(_{0-60}\), was −931% ± 270% decline per minute at Week 8 and did not significantly differ \((p > .05)\) from the −1,090% ± 350% decline per minute at Week 0. Furthermore, similar patterns were observed for the post-EVH percentage decline in FVC, FEV\(_1\)/FVC, and FEF\(_{25-75}\)%.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Week 0</th>
<th>Week 8</th>
</tr>
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<tbody>
<tr>
<td>FVC (L)</td>
<td>5.28 ± 0.63</td>
<td>5.09 ± 0.58</td>
</tr>
<tr>
<td>% predicted</td>
<td>103.5 ± 7.4</td>
<td>99.6 ± 5.8</td>
</tr>
<tr>
<td>FEV(_1) (L)</td>
<td>4.10 ± 0.52</td>
<td>4.00 ± 0.42</td>
</tr>
<tr>
<td>% predicted</td>
<td>99.9 ± 7.8</td>
<td>97.6 ± 4.8</td>
</tr>
<tr>
<td>FEV(_1)/FVC (%)</td>
<td>77.6 ± 2.7</td>
<td>79.1 ± 3.1</td>
</tr>
<tr>
<td>% predicted</td>
<td>97.0 ± 3.3</td>
<td>98.9 ± 3.7</td>
</tr>
<tr>
<td>FEF(_{25-75})% (L/s)</td>
<td>3.66 ± 0.57</td>
<td>3.55 ± 0.37</td>
</tr>
<tr>
<td>% predicted</td>
<td>81.3 ± 10.1</td>
<td>79.5 ± 6.1</td>
</tr>
</tbody>
</table>

**Note.** FVC = forced vital capacity; FEV\(_1\) = forced expiratory volume in 1 s; FEF\(_{25-75}\)% = forced midexpiratory flow. There were no significant differences \((p > .05)\) for any variables between time points.

Figure 1 — The percentage decline of forced expiratory volume in 1 s (FEV\(_1\)) across time from before to after eucapnic voluntary hyperventilation (EVH) at Weeks 0 and 8. No significant differences \((p > .05)\) were found between Week 0 and Week 8 at any time point. Error bars represent SEM.
Mean urinary levels of LTC₄–E₄ and 9α,11β-PGF₂α were shown in Figure 3. Pre-EVH measures of LTC₄–E₄ and 9α,11β-PGF₂α were not significantly different (p > .05) between Week 0 and Week 8. Post-EVH urinary measures of LTC₄–E₄ and 9α,11β-PGF₂α were 876 ± 326 and 843 ± 186 pg/mg creatinine, respectively, at Week 0. After CLA supplementation, post-EVH urinary measures of LTC₄–E₄ and 9α,11β-PGF₂α were 1,071 ± 320 and 1,226 ± 265 pg/mg creatinine, respectively. Post-EVH measures of LTC₄–E₄ and 9α,11β-PGF₂α were not significantly altered (p > .05) by the CLA supplementation.

**Discussion**

The current study has demonstrated that 8 weeks of CLA supplementation (4.8 g/day) does not attenuate airway inflammation or the bronchoconstrictive response to an EVH challenge in asthmatic individuals. This is supported by the lack of significant difference observed for all measures of pulmonary function in response to the EVH challenge after CLA supplementation compared with baseline. Further support is provided by the lack of difference in the total bronchoconstrictive response, as measured by the AUC₀–₆₀, after CLA supplementation compared with baseline. There were no changes in urinary inflammatory-mediator concentrations measured in response to the EVH challenge from Week 0 to Week 8. In addition, weekly bronchodilator use failed to improve over the study duration. These findings are not likely to be caused by changes in the provoking stimulus (Vₑ during EVH challenge testing) or nutrient intake, because no differences were observed in these measures between the beginning and end of the treatment period.

The CLA dosage used in the current study was 4.8 g/day administered over 8 weeks. It is possible that the dose or the duration of the study was insufficient to detect differences in airway hyperresponsiveness as a result of the CLA supplementation. In animal studies, Whigham et al. (2001; 2002) used a diet consisting of 0.25 g CLA/100 g food, or 0.25 wt%, to evoke a significant reduction in prostanoid levels. The current study used a mean value of 1.1 g CLA/100 g food, or 1.1 wt%, but failed to observe a significant reduction in eicosanoid levels. These findings suggest that the inability of CLA to exert a significant effect on pulmonary function and inflammatory-mediator release may be a result of the duration of the study period (8 weeks) and not the CLA dosage in the current study. Ip, Jiang, Thompson, and Scimeca (1997) observed that the maximum CLA concentration occurred in mammary-tissue phospholipids after 8 weeks in mice when fed 1.0 wt% CLA. It is possible that this duration (8 weeks) used in a murine model is insufficient for incorporation of CLA into human bronchial epithelial tissue, but the optimum dosage and duration for incorporation of CLA into human tissue are at present unknown.

The isomer occurring at the highest level in the diet is cis-9,trans-11 CLA (Chin, Liu, Storkson, Ha, & Pariza, 1992). Most of the research conducted on humans with CLA supplementation has used the cis-9,trans-11 and trans-10,cis-12 isomers in a 1:1 ratio (Salas-Salvadó, Márquez-Sandoval, & Bulló, 2006). The current study used approximately equal amounts of the
two isomers because this combination has been used in previous research and they are the two most abundant isomers found in commercial sources. However, it has not yet been determined which isomers have the greatest physiological benefit and what interactions, if any, that take place between the various isomers when ingested in combination. Some investigators argue that the main biologically active isomer is cis-9,trans-11 CLA (Jaudszus, Krokowski, & Mockel, 2008), and Nugteren (1970) was able to show that trans-10,cis-12 CLA may be responsible for some of the proposed immunomodulatory effects. It might even be possible for each isomer to yield different or even antagonistic effects, because evidence suggests that different isomers have different health effects (Pariza, Park, & Cook, 2001).

In a study investigating the effect of CLA on Type 1 hypersensitivity, a decrease in histamine and PGE$_2$ release from CLA-fed guinea pig trachea was documented when it was superfused with antigen (Whigham et al., 2001). In addition, polygraph tests revealed no increase in contractile response of superfused trachea from CLA-fed guinea pigs stimulated with antigen. In another study by Whigham et al. (2002), all the prostanooids measured from tracheas (thromboxane B$_2$,6-keto PGF$_{2\alpha}$, PGF$_{2\alpha}$, PGE$_2$, and PGD$_2$) and four of five from lungs (thromboxane B$_2$,6-keto PGF$_{2\alpha}$, PGF$_{2\alpha}$, and PGD$_2$) of CLA-fed guinea pigs were significantly lower than in control in response to antigen challenge. In addition, there was a reduction in lung-tissue Cyst-LTs after antigen stimulation in CLA-fed guinea pigs compared with control.

The animal studies demonstrated that CLA reduces the prostanooid levels in trachea and lung tissues (Whigham et al., 2001; Whigham et al., 2002), as well as eosinophil and cytokine activity in bronchoalveolar lavage fluid (Jaudszus et al., 2008). The inability of CLA to exert similar effects on eicosanoid levels in the current study could be a result of a different sample medium or differences between species. For example, despite observing an increase in the CLA concentration of human plasma blood mononuclear cell lipids after a 63-day supplementation period (3.9 g/day), Kelley et al. (2001) observed no change in cytokine and eicosanoid secretion when stimulated in vitro.

The mechanism by which CLA exerts its proposed effect on the inflammatory response is unclear. However, CLA has been hypothesized to exert its effect through the down-regulation of the cyclo-oxygenase and 5-lipoxygenase (5-LOX) pathways or phospholipase A$_2$ enzyme activity (Whigham et al., 2002). Results from Nugteren (1970) provide additional support for this hypothesis. As such, the eicosanoid pathway may be modified by enzymatic inhibition during CLA supplementation of the trans-10,cis-12 isomer. In contrast, a study conducted by Stachowska et al. (2007) measured LTB$_4$ concentration from human macrophages incubated with CLA to determine 5-LOX activity. No significant difference was detected between the control and the CLA groups. Therefore, CLA had no effect on 5-LOX expression in macrophages, which may explain the unaltered inflammatory mediator levels measured in the current study.
CLA may directly affect gene expression of inflammatory cytokines by activating transcription factors such as peroxisome proliferator-activated receptors (PPARs). Yu, Correll, and Vanden Heuvel (2002) observed a decrease in IL-1β and TNF-α and activation of PPAR-γ when RAW mouse macrophage cells were treated with various CLA isomers. In an attempt to identify the mechanism by which cis-9,trans-11 CLA exerted its anti-inflammatory effect, Jaudszus, Foerster, Kroege, Wolf, and Jähr (2005) added GW9662, a PPAR-γ antagonist, to cell cultures while measuring IL-8 production levels. The data demonstrated that PPAR-γ inhibition abolishes the CLA-induced reduction in IL-8 in human bronchial epithelial cell in vitro, indicating that the cis-9,trans-11 isomer may exert some of its effect through PPAR-γ.

The results obtained in the current study are in agreement with measured values observed in asthmatic individuals under similar testing conditions. The pre-EVH pulmonary-function values measured in the current study were similar to those previously reported (Mickleborough et al., 2003; Rundell, Spiering, Baumann, & Evans, 2005). Likewise, target ventilation levels during the EVH test were similar to those reported by Kippelen et al. (2010) for asthmatic individuals (100 ± 4 L/min). In response to the EVH challenge, subjects in the current study exhibited a decrease in postchallenge pulmonary function comparable to those previously reported in asthmatics receiving placebo treatment (−23% ± 4%; Kippelen et al., 2010). The pre-EVH urinary 9α,11β-PGF2α level observed in the current study is in accordance with previous research (910 ± 80 pg/mg creatinine; Bochenek, Nagraba, Nizankowska, & Szczeklik, 2003). Post-EVH urinary inflammatory mediator secretion was unaltered by the treatment, which is expected given the pulmonary-function observations of the current study. Therefore, the finding that CLA had no effect on hyperpnea-induced bronchoconstriction and airway inflammation is unlikely to be caused by an insufficient challenge to the airways to induce bronchoconstriction in the current study.

**Conclusion**

In conclusion, this study has shown that a relatively short, 8-week supplementation of 4.8 g CLA/day (50% cis-9,trans-11; 50% trans-10,cis-12) does not reduce the bronchoconstrictive response to EVH challenge and airway inflammation in mild to moderate asthmatics with EIB.

**Acknowledgments**

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**References**


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