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Original Article

# Inflammatory and growth factor response to continuous and intermittent exercise in youth with cystic fibrosis☆

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**Abstract**

*Background:* Children with cystic fibrosis (CF) tend to suffer from chronic systemic inflammation and may have impaired growth associated with muscle catabolism. Therefore, investigating which type of exercise can elicit an anabolic response with minimal inflammation is of clinical value.
*Methods:* Twelve children with CF (mean±SD; age: 14.7±2.3 years, predicted $FEV_1$: 90.0±21.6%) and biological age-matched controls (age: 13.9±2.1 years) completed moderate-intensity, continuous exercise (MICE) and high-intensity, intermittent exercise (HIIE) on separate days. During each exercise, blood was drawn at various time points and analyzed for immune cells, inflammatory cytokines, and growth mediators.
*Results:* At rest, children with CF had higher concentrations of neutrophils and IL-6 compared with controls. In children with CF, HIIE did not affect immune cell subsets or cytokines: TNF-α, IL-6, and tumor necrosis factor-like weak inducer of apoptosis (TWEAK). All immune cell subsets and IL-6 increased significantly with MICE in both groups. Growth hormone (GH) increased with both types of exercise, with a greater change from rest during MICE.
*Conclusions:* HIIE was a sufficient stimulus to increase GH in children with CF, without affecting systemic inflammation.


*Keywords:* Immune cells; Cytokines; Moderate intensity; High intensity; TWEAK

## 1. Introduction

Children with cystic fibrosis (CF) are known to experience chronic systemic inflammation [1], which has been associated with increased protein breakdown and lower fat-free mass [2]. Chronic pulmonary infection [3] and pancreatic insufficiency leading to malnutrition [4] can also result in reduced fat free mass. Exercise is a non-pharmacological approach to attenuating protein breakdown [5] and increasing fat-free mass in humans [6]. In healthy children, however, acute bouts of exercise are known to activate the same inflammatory mediators involved in the pathology of CF [1]. The effect of exercise on inflammation raises the possibility that some forms of exercise should be avoided by children with CF. Alternatively, anabolic processes are also triggered by exercise, which stimulates the release of growth factors [1]. The anabolic effects of exercise are likely to be beneficial to children with CF since poor muscular development and retention is a common characteristic of the disease [7].

While many research studies have shown that exercise imparts numerous benefits to children with CF [8–11], the inflammatory and growth factor response to exercise has not been

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investigated extensively. The only known study reported a greater increase in inflammatory cytokines (e.g., interleukin 6 [IL-6] and tumor necrosis factor alpha [TNF-α]), but a similar growth hormone (GH) response in children with CF compared to healthy children [1].

GH is a growth mediator, and binding to its receptor leads to the production of insulin-like growth factor 1 (IGF-1) in muscle [12]. IGF-1 is responsible for activating growth pathways [13], as well as preventing muscle degradation [14]. GH has been traditionally given for catch up height; however, the evidence now points to broader functional effects of GH, such as improved strength, and muscle mass [15]. In the specific context of children with CF, exogenously-administered GH also has a direct effect on whole body protein balance [16]. In this way, GH has an anabolic effect on the muscle, potentially through reduced inflammation [16]. It is therefore intriguing to think that regular exercise-induced production of GH could have similar endogenous benefits.

The relationship between IL-6 and muscle catabolism is equivocal. Some studies have reported that IL-6 is associated with reduced growth rates and skeletal muscle development [17–19], while others have found no change in the rate of muscle protein breakdown [20,21]. Despite conflicting findings, IL-6 is capable of promoting protein degradation [22] and baseline concentrations are higher at rest in children with CF than healthy children [1], making it a cytokine of interest.

The inflammatory cytokine, TNF-α, promotes skeletal muscle protein degradation and inhibits skeletal muscle growth and regeneration. This is achieved by its ability to reduce circulating GH and IGF-1 concentrations [23], promote GH resistance in skeletal muscle [12,24], inhibit muscle production of IGF-1 [12,25], and contribute to lower IGF-1 protein content in muscles [23]. In addition, increased levels of TNF-α increase rates of muscle protein degradation [26]. Given the catabolic effects of TNF-α, elevating levels should be avoided to promote muscle growth.

Tumor necrosis factor related weak inducer of apoptosis (TWEAK) is a novel cytokine recently identified in humans [27]. It has been classified as a member of the TNF cytokine family for having similar TNF properties, such as providing long-range signaling [27]; however, unlike TNF-α, TWEAK has been found to have longer lasting effects [28]. TWEAK is involved in skeletal muscle degradation. Mice treated with TWEAK and mice genetically modified to over express TWEAK have lower body and skeletal muscle mass [29]. Furthermore, TWEAK is involved in the inflammatory response in human bronchial epithelial cell lines [30]. Due to its catabolic effects on muscle and inflammatory effects in the lungs, the exercise response to TWEAK may be clinically relevant to the CF population.

Given that many exercise recommendations for children with CF commonly take the form of endurance training to improve aerobic fitness [8,10], it is reasonable to better understand how this form of exercise affects inflammatory and growth mediators. In adult studies, several inflammatory mediators are thought to be more duration-dependent than intensity-dependent, such as neutrophils [31] and IL-6 [32]. Brief but intense bouts of exercise reflect the natural physical activity patterns of young children [33–35], with some evidence that children with CF receive greater enjoyment from this type of exercise [9]. We were therefore interested in examining the inflammatory and growth factor response to this type of exercise (brief but intense), which more closely reflects the natural behaviors and activity patterns of young children and may, therefore, be more conducive to anabolic processes [36]. Indeed, the health benefits of high intensity interval training among adults have received considerable attention in recent years [37–39]; high intensity interval training is essentially replicating the natural physical activity patterns of children. Thus, further study of this type of exercise may facilitate the prescription of time-efficient exercise applicable to a broad age range of patients. Moreover, continuous exercise therapies are usually drawn from prescriptive approaches for adults that may not be suitable, or enjoyable, for children and, therefore, difficult to incorporate into their daily life.

The objectives of this study were 1) to determine the effect of moderate-intensity, continuous exercise (MICE) and high-intensity, intermittent exercise (HIIE) on the inflammatory and growth factor response in children with CF, and 2) to compare these responses with healthy matched-controls. We hypothesized that 1) HIIE would produce a lower inflammatory response and a greater growth factor response in children with CF, because this exercise environment would be most conducive to growth, and 2) children with CF would have a greater inflammatory response and similar growth factor response compared to matched-controls.

## 2. Methods

### 2.1. Participants

Twelve children with CF (2 females) and twelve healthy matched-controls participated in this study. Children who could not perform reproducible pulmonary function tests were excluded from the study. Participants' characteristics are shown in Table 1. Children with CF and controls were matched by gender and biological age, as determined by the estimated years from the age of peak height velocity (PHV) [40]. Seven patients with CF were on non-steroidal anti-inflammatory drugs (NSAIDs) and/or inhaled, or nasal spray corticosteroids. One participant was diagnosed with cirrhosis of the liver. Baseline values for immune cells, cytokines, and growth factors are shown in Table 2.

### 2.2. Experimental design and exercise testing

The study procedures were approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board. Participants completed three visits. Visit 1: informed assent and consent was obtained from participants and guardians, respectively, followed by self-assessment of sexual maturity [41], height measurements, bioelectrical impedance analysis for body composition, a standard spirometry test, and an aerobic fitness test on a cycle ergometer using the *McMaster All-Out Progressive Continuous Cycling Test* to determine peak oxygen uptake ($VO_{2peak}$) and peak mechanical power (PMP).

Table 1
Participants' characteristics.

| | CF (n=12) | Matched-controls (n=12) |
|---|---|---|
| Chronological age (years) | 14.7±2.3 (11.3 to 17.5) | 13.9±2.1 (10.5 to 17.5) |
| Estimated years from age PHV | 0.6±1.6 (−2.5 to 2.7) | 0.7±1.5 (−2.5 to 2.7) |
| Height (m) | 1.6±0.1 (1.42 to 1.77) | 1.7±0.1 (1.45 to 1.83) |
| Height (z-score) | −0.5±0.5 (−1.86 to 0.92)** | 0.5±1.0 (−1.64 to 1.41) |
| Weight (kg) | 49.5±11.3 (37.5 to 67.2) | 55.0±9.1 (35.7 to 65.6) |
| Weight (z-score) | −0.03±0.7 (−1.75 to 1.44)** | 1.3±1.2 (−1.59 to 1.28) |
| FFM (kg) | 39.4±10.0 (26.6 to 52.8) | 44.6±9.1 (29.0 to 57.2) ‡ |
| FM (kg) | 9.5±3.1 (5.7 to 17.8) | 10.7±5.4 (4.4 to 21.2) ‡ |
| FEV1 (% predicted) | 90.3±22.0 (38 to 123) | 93.4±7.4 (84 to 109) |
| $VO_{2peak}$ (mL/kg/min) | 47.3±6.4 (35.9 to 55.3) * | 53.1±6.7 (44.5 to 65.5) |
| $VO_{2peak}$% predicted (mL/kg/min) | 101.4±17.8 (73 to 141) * | 114.9±11.1 (96 to 135) |
| PMP (W) | 162±46 (93 to 240) ** | 220±54 (121±284) |
| PMP (W/kg) | 3.2±0.4 (2.5 to 3.7) ** | 4.0±0.7 (2.8 to 5.2) |

Values are mean±SD with range in parentheses. PHV: peak height velocity. FFM: fat free mass. FM: fat mass. $FEV_1$: forced expiratory volume in 1 s. $VO_{2peak}$: peak oxygen consumption. PMP: peak mechanical power. W: watts. Height and weight z-scores were calculated using reference values of weight-for-age and stature-for-age from the Centers for Disease Control and Prevention [52]. Reference data for $FEV_1$ was obtained from Wang et al. [53]. Percent predicted of $VO_{2peak}$ was calculated using reference data obtained from our laboratory. Tanner stage assessment based on self-assessment of pubic hair development for males and breast development for females [41]. Significant difference between groups at *$p<0.05$, **$p<0.01$; ‡n=11 for matched-controls.

Visits 2 and 3 were scheduled at the same time of day for each individual child and always between afternoon and early evening hours. Visits 2 and 3 were scheduled a week apart and consisted of either MICE (2×30-min bouts of cycling at an intensity equivalent to 50% PMP, with 6 min rest between bouts) or HIIE (6 series of 4×15 s bouts performed at 100% PMP, with 1 min of rest between bouts and 6 min of rest between each series). Gas was collected during MICE at four different times, intermittently, throughout the session for 6 min at a time. By design, MICE and HIIE were not matched for energy expenditure.

### 2.3. Blood collection

At Visits 2 and 3, an indwelling catheter was placed in an arm vein of the child after 10 min of supine rest. Blood samples were collected at rest (REST), at the mid-point of exercise (EX-MID), immediately after exercise (EX-END), following 30 min of recovery (REC-30), and following 60 min of recovery (REC-60); a portion of these samples was processed to extract plasma. Additional blood samples were collected at REST, EX-END, and REC-60 from which serum was extracted.

### 2.4. Blood analysis

Total leukocytes, neutrophils, lymphocytes, and monocytes were analyzed using an automated Coulter counter at the McMaster University Medical Centre Core Laboratory. Plasma samples were analyzed for IL-6, TNF-α, GH, and IGF-1, while serum samples were analyzed for TWEAK using commercially-available enzyme-linked immunosorbent assays (ELISA).

### 2.5. Statistics

Independent *t*-tests (PASW version 18.0, SPSS, Inc., Chicago, IL) were used to compare variables including $FEV_1$, $VO_{2peak}$, and PMP. In addition, the power output between MICE and HIIE was evaluated using dependent *t*-tests for children with CF and controls separately. Two-way ANOVAs (Statistica version 5.0, Statsoft, Inc., Tulsa, OK) were performed on baseline concentrations (2 factors: group [CF, control] by exercise type [MICE, HIIE]) to determine differences between groups, between exercise types, or between groups and exercise types. One-way repeated measures ANOVAs were performed on absolute concentrations of all blood markers in response to each exercise in children with CF and matched-controls, separately. When an exercise effect was present, two-way repeated measures ANOVAs were performed on the magnitude of change (Δ) from REST values between [1] MICE and HIIE in children with CF, and [2] children with CF and matched-controls separately for MICE and HIIE. In addition, comparisons between children with CF on corticosteroid and/or NSAIDs (n=7),

Table 2
Average baseline (REST) concentrations of immune cells, cytokines, and growth factors.

| | CF (n=12) | Matched-controls (n=12) |
|---|---|---|
| Leukocytes | 8.3±3.1 (4.5 to 14.2)* | 5.7±1.5 (4.5 to 9.2) |
| Neutrophils | 5.5±3.0 (2.8 to 11.5)** | 2.8±10 (1.9 to 5.4) |
| Lymphocytes | 2.0±0.6 (1.0 to 3.2) | 2.2±0.5 (1.7 to 3.1) |
| Monocytes | 0.6±0.2 (0.3 to 1.2) | 0.5±0.2 (0.3 to 0.9) |
| IL-6 | 5.3±6.0 (0.8 to 20.5)* | 1.2±0.9 (0.5 to 3.7) |
| TNF- α | 1.0±1.1 (0.04 to 3.9) | 1.1±0.3 (0.6 to 1.6) |
| TWEAK | 972.8±281.8 (626.6 to 1140.6) | 953.4±228.5 (595.9 to 1361.50) |
| GH | 3.1±3.5 (0.06 to 10.6) | 3.0±2.9 (0.1 to 8.4) |
| IGF-1 | 206.7±57.4 (70.9 to 269.3) | 210.1±65.5 (73.9 to 296.3) |

Values are displayed as mean±SD with range in parentheses. Immune cell counts are expressed in $10^9$/L. Inflammatory cytokines are expressed in pg/mL. Growth factors are expressed in ng/mL. IL-6: interleukin 6. TNF-α: tumor necrosis factor alpha. TWEAK: tumor necrosis related weak inducer of apoptosis. GH: growth hormone, IGF-1: insulin-like growth factor 1. Significant differences *$p<0.05$, **$p<0.01$ between CF and controls.

children with CF not on corticosteroid and/or NSAIDs (n=5), and the matched-controls (n=12) were performed on the magnitude of change from REST values using two-way repeated measures ANOVAs on all blood markers. Tukey's HSD *post hoc* analysis, which accounts for multiple mean comparisons, was performed for all significant ANOVA tests to determine specific mean differences. Alpha level was set at $p<0.05$.

## 3. Results

### 3.1. Baseline (resting) concentrations

Our participants had similar levels of all blood markers (GH, IGF-1, TNF-α, IL-6 and TWEAK) at the start of both experimental sessions (i.e., REST). Thus, samples were pooled separately for children with CF and controls to compare CF and control values at REST. At REST, children with CF had higher average concentrations of leukocytes, neutrophils, and IL-6 compared to controls (Table 2).

### 3.2. Exercise intensity (power output)

The power output for children with CF during MICE and HIIE was 1.6±0.2 W/kg and 3.3±0.4 W/kg, respectively. For matched controls, MICE was 2.0±0.4 W/kg and HIIE was 4.1±0.7 W/kg. Significant differences were seen between MICE and HIIE in children with CF ($p<0.001$) and in matched-controls ($p<0.001$). Oxygen consumption during MICE was ~65% $VO_{2peak}$ for both groups.

### 3.3. Cystic fibrosis — MICE versus HIIE

*Immune cell subsets.* Children with CF had an increase from REST in all immune cell subsets with MICE, while only the total leukocyte pool and not individual subsets was increased in response to HIIE (Table 3). In MICE, the magnitude of change from REST was greater than HIIE in leukocytes at REC-30 and REC-60 (Fig. 1A); in neutrophils at REC-30 and REC-60 (Fig. 1B); and in lymphocytes at EX-END (Fig. 1C). There were no differences in the magnitude of change from REST in monocytes between exercises (Fig. 1D).

*Cytokines.* In MICE, IL-6 concentrations at EX-END, REC-30, and REC-60 were greater than at REST, while HIIE had no effect on IL-6 (Table 4). The magnitude of change from REST in IL-6 was significantly greater for MICE than HIIE (main effect for exercise, $p<0.05$) (Fig. 2); however, there was no exercise type × time interaction. Exercise had no effect on TNF-α or TWEAK.

*Growth factors.* MICE and HIIE stimulated an increase in circulating GH from REST at EX-MID, and MICE continued to increase GH from REST at EX-END, while HIIE did not (Fig. 3A). In MICE, the magnitude of change from REST was

Table 3
Immune response to MICE and HIIE in children with CF and matched-controls.

| | REST | EX-MID | EX-END | REC-30 | REC-60 |
|---|---|---|---|---|---|
| *MICE* | | | | | |
| Leukocytes | | | | | |
| CF | 8.36±3.04 | 9.03±3.18 | 10.26±2.68** | 10.09±3.39** | 10.74±3.50*** |
| Controls | 5.47±1.40 | 6.98±1.99** | 8.09±2.75*** | 7.27±2.52*** | 8.22±2.88*** |
| Neutrophils | | | | | |
| CF | 5.41±2.96 | 5.87±3.01 | 6.48±2.62 | 7.06±3.01** | 8.10±3.24*** |
| Controls | 2.56±0.89 | 3.27±1.22 | 3.87±1.59** | 4.33±2.13*** | 5.34±2.57*** |
| Lymphocytes | | | | | |
| CF | 2.14±0.78 | 2.38±0.75 | 2.83±0.88*** | 2.19±0.69 | 1.99±0.65 |
| Controls | 2.21±0.58 | 2.88±0.95* | 3.22±1.34*** | 2.26±0.72 | 2.27±0.70 |
| Monocytes | | | | | |
| CF | 0.60±0.19 | 0.61±0.21 | 0.78±0.22* | 0.67±0.23 | 0.61±0.28 |
| Controls | 0.46±0.15 | 0.57±0.16* | 0.68±0.18*** | 0.49±0.13 | 0.52±0.16 |
| *HIIE* | | | | | |
| Leukocytes | | | | | |
| CF | 8.28±3.74 | 8.79±3.61 | 8.96±3.65* | 8.23±3.38 | 8.32±3.20 |
| Controls | 5.90±1.76 | 6.56±1.67** | 6.72±1.83*** | 6.01±1.85 | 6.33±1.74 |
| Neutrophils | | | | | |
| CF | 5.50±3.67 | 5.78±3.53 | 5.93±3.55 | 5.40±3.34 | 5.41±3.07 |
| Controls | 3.06±1.49 | 3.39±1.34 | 3.33±1.54 | 3.17±1.33 | 3.33±1.30 |
| Lymphocytes | | | | | |
| CF | 1.92±0.53 | 2.05±0.52 | 2.07±0.53 | 1.99±0.50 | 1.98±0.49 |
| Controls | 2.09±0.46 | 2.34±0.49 | 2.41±0.57* | 2.11±0.52 | 2.07±0.67 |
| Monocytes | | | | | |
| CF | 0.60±0.26 | 0.67±0.24 | 0.65±0.27 | 0.63±0.19 | 0.68±0.22 |
| Controls | 0.50±0.20 | 0.54±0.20 | 0.70±0.55 | 0.46±0.20 | 0.54±0.1 |

Values are displayed as mean±SD. Immune cell counts are expressed in $10^9$/L. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. REST: pre-exercise, EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Significant differences *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to REST values.

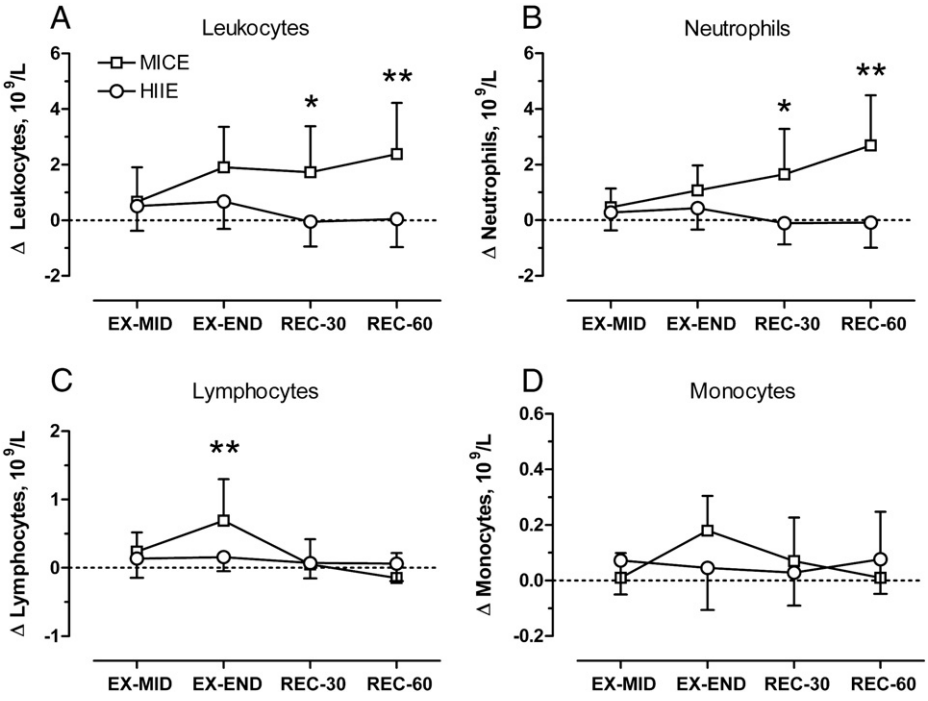


Fig. 1. Change in immune cells in response to MICE and HIIE in children with CF. (A) Leukocytes, (B) Neutrophils, (C) Lymphocytes, and (D) Monocytes. Values are displayed as mean ± SD. Δ: change in concentration from REST. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Δ between MICE and HIIE is significantly different at $*p<0.01$, $**p<0.001$ for the specific time point indicated.

greater than HIIE at EX-MID and EX-END (Fig. 3B). Neither type of exercise had an effect on IGF-1.

### 3.4. CF versus matched-controls — MICE

*Immune cell subsets.* In children with CF, leukocytes at EX-END, REC-30, and REC-60; neutrophils at REC-30 and REC-60; lymphocytes at EX-END; and monocytes at EX-END were greater than at REST. In the matched-controls, leukocytes at EX-MID, EX-END, REC-30, and REC-60; neutrophils at EX-END, REC-30, and REC-60; lymphocytes at EX-MID and EX-END; and monocytes at EX-MID and EX-END were greater than at REST (Table 3). The magnitude of change from REST for each immune cell subset (at each time point) was not different between groups.

*Cytokines*. In children with CF, IL-6 at EX-END, REC-30, and REC-60 was greater than at REST (Table 4). In the matched-controls, IL-6 at EX-END, REC-30 and REC-60 was greater than at REST. The magnitude of change from REST for IL-6 was not different between groups. MICE had no effect on TNF-α or TWEAK in either group.

*Growth factors*. In children with CF as in matched-contorls, GH at EX-MID and EX-END was greater than at REST (Table 4). The magnitude of change from REST for GH was not different between groups. MICE had no effect on IGF-1 in either group.

### 3.5. CF versus matched-controls — HIIE

*Immune cell subsets.* In children with CF, leukocytes at EX-END was greater than at REST (Table 3). In matched-controls, leukocytes at EX-MID and EX-END; and lymphocytes at EX-END were greater than at REST. The magnitude of change from REST for leukocytes and lymphocytes was not different between groups. HIIE did not affect neutrophils, lymphocytes, or monocytes in children with CF, and did not affect neutrophils or monocytes in the matched-controls.

*Cytokines.* In children with CF, none of the measured inflammatory cytokines were affected by HIIE (Table 4). In the matched-control group, IL-6 at REC-60 was greater than at REST, and TWEAK at REC-60 was lower than at REST. Despite the absence of an effect of HIIE on inflammatory cytokines in children with CF, the magnitudes of change from REST for IL-6 and TWEAK were not statistically different between groups (Fig. 4A and 4.B). HIIE had no effect on TNF-α in either group.

*Growth factors.* In children with CF, GH at EX-MID was greater than at REST. In the matched-controls, GH at EX-MID and EX-END was greater than at REST (Table 4). Despite the absent of an increase in GH at EX-END in children with CF, the magnitude of change from REST for GH was not statistically different between groups (Fig. 4C). HIIE did not have an effect on IGF-1 in either group.

### 3.6. Effects of anti-inflammatory drugs

The magnitudes of change from REST for immune cells, cytokines, or growth factors are presented in Tables 5 and 6 for the three groups, and for MICE and HIIE. The use of NSAIDs and/or corticosteroids did not affect the immune cell, cytokine, or growth factor response to exercise.

Table 4
Inflammatory cytokine and growth factor response to MICE and HIIE in children with CF and matched-control.

| | REST | EX-MID | EX-END | REC-30 | REC-60 |
|---|---|---|---|---|---|
| *MICE* | | | | | |
| IL-6 | | | | | |
| CF | 4.12±5.65 | 4.45±5.27 | 5.90±6.44* | 6.76±6.83*** | 6.14±6.08** |
| Controls | 0.90±0.51 | 1.06±0.51 | 2.26±0.58*** | 2.92±0.52*** | 2.47±0.47*** |
| TNF-α | | | | | |
| CF | 1.06±1.15 | 1.17±1.11 | 1.15±1.08 | 1.28±1.29 | 1.19±1.10 |
| Controls | 1.04±0.32 | 1.09±0.40 | 1.20±0.51 | 1.17±0.49 | 1.13±0.37 |
| TWEAK | | | | | |
| CF | 939.57±318.80 | – | 901.93±191.24 | – | 844.51±209.57 |
| Controls | 916.55±261.03 | – | 963.65±203.03 | – | 900.49±215.55 |
| GH | | | | | |
| CF | 2.30±4.54 | 20.43±19.73*** | 15.49±13.33** | 5.56±4.05 | 2.89±2.46 |
| Controls | 3.16±4.75 | 16.13±8.26*** | 16.21±9.08*** | 7.71±7.28 | 4.00±5.14 |
| IGF-1 | | | | | |
| CF | 201.96±56.42 | 190.72±52.17 | 199.15±59.61 | 193.93±53.37 | 190.12±53.79 |
| Controls | 205.08±65.99 | 196.53±63.57 | 199.72±66.06 | 206.09±70.07 | 201.55±70.57 |
| *HIIE* | | | | | |
| IL-6 | | | | | |
| CF | 6.56±7.75 | 5.29±5.71 | 5.37±5.39 | 5.57±5.49 | 5.47±5.52 |
| Controls | 1.51±1.57 | 1.45±1.57 | 1.68±1.81 | 1.78±1.63 | 1.89±1.61** |
| TNF-α | | | | | |
| CF | 1.03±1.02 | 1.25±0.94 | 1.32±1.13 | 1.19±1.16 | 1.17±1.10 |
| Controls | 1.10±0.38 | 1.17±0.48 | 1.11±0.46 | 1.21±0.50 | 1.18±0.46 |
| TWEAK | | | | | |
| CF | 1005.95±337.06 | – | 862.82±339.76 | – | 821.39±276.18 |
| Controls | 990.24±241.90 | – | 888.12±246.50 | – | 807.79±271.32* |
| GH | | | | | |
| CF | 3.99±4.50 | 9.61±5.64*** | 7.20±3.41 | 3.14±2.37 | 1.59±1.80 |
| Controls | 2.85±3.47 | 11.06±9.42** | 11.34±6.70** | 5.72±4.02 | 2.16±1.23 |
| IGF-1 | | | | | |
| CF | 211.53±64.14 | 209.19±63.65 | 214.18±65.19 | 211.94±66.22 | 210.83±64.79 |
| Controls | 215.09±70.00 | 215.06±72.52 | 215.97±68.79 | 223.71±69.45 | 217.52±66.94 |

Values are displayed as mean±SD. Inflammatory cytokines are expressed in pg/mL. Growth factors are expressed in ng/mL. IL-6: interleukin 6. TNF-α: tumor necrosis factor alpha. TWEAK: tumor necrosis related weak inducer of apoptosis. GH: growth hormone, IGF-1: insulin-like growth factor 1. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. REST: pre-exercise, EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Significant differences *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to REST values.

## 4. Discussion

The main findings from this study demonstrate that in children with CF HIIE elicited a lower inflammatory and growth factor response compared to MICE. In HIIE, the only effect was an increase in the total leukocyte pool, but when examined separately, the individual components of the leukocyte population did not increase significantly with exercise. Conversely, MICE elicited an inflammatory response with an increase from rest in all immune cells, and a greater change in leukocytes and neutrophils when compared with HIIE, supporting our hypothesis. These results may be due to the difference in duration between the two types of exercise as neutrophilia during exercise is thought to be duration-dependent rather than intensity-dependent [31]. However, while our CF patients and matched-controls completed 6 min of intermittent exercise with no change in neutrophils, a study conducted by Rosa et al. [42] reported increases in neutrophils immediately after 6 min of continuous exercise at 70% $VO_{2max}$ in healthy children and in children with exercise-induced asthma. Thus, even if total duration of exercise is the same, continuous exercise is more likely to increase neutrophils than intermittent exercise.

The inflammatory mediators IL-6, TNF-α, and TWEAK have been shown to inhibit growth and promote muscle protein

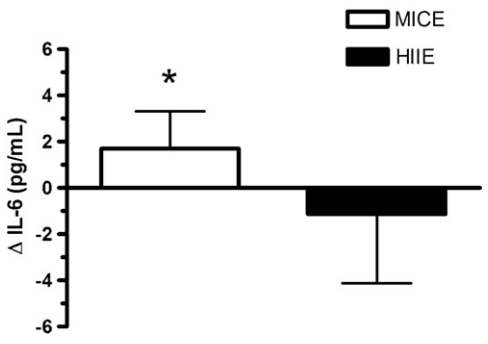


Fig. 2. Average magnitude of change in IL-6 in response to MICE and HIIE in children with CF. Values are displayed as mean±SD. Δ: change in concentration from REST values. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. Δ between MICE and HIIE is significantly different at *$p<0.05$.

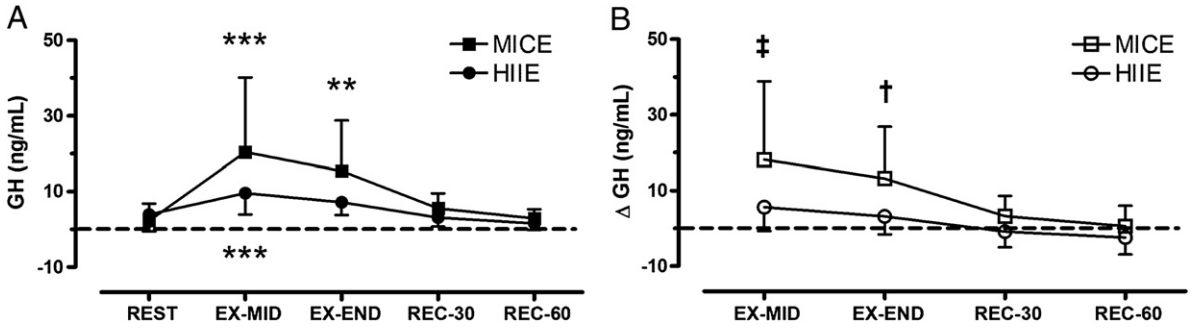


Fig. 3. Growth hormone (GH) response to MICE and HIIE in children with CF. (A) Absolute concentration in GH response, (B) Change in GH from REST. Values are displayed as mean±SD. Δ: change in concentration from REST values. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. REST: pre-exercise, EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Concentration is significantly different at $^{**}p<0.01$, $^{***}p<0.001$ from REST. Δ between MICE and HIIE are significantly different at $\dagger p<0.01$, $\ddagger p<0.001$ for the specific time point indicated.

catabolism [22–26,29]. The only inflammatory cytokine to be altered by exercise in children with CF was IL-6 in response to MICE. The absence of an IL-6 response to HIIE may prove to be beneficial to CF patients suffering from systemic inflammation, including our participants as they already had higher resting levels of IL-6 compared to matched-controls. However, exercise-induced production of muscle-derived IL-6 is thought to be anti-inflammatory [43]. Thus, the paradoxical roles of IL-6 make it difficult to fully understand its effect under exercise conditions, especially in a clinical population such as CF. More research is needed to determine whether exercise-induced increases in IL-6 play a more pro-inflammatory or anti-inflammatory role in the CF population. Regardless, HIIE did not exacerbate existing levels of circulating cytokines in patients.

The growth factors GH and IGF-1 of patients with CF are of interest because circulating levels of IGF-1, for example, tend to be lower than in healthy individuals [44] and GH treatment increases lean muscle mass and reduces muscle catabolism in children with CF [16]. To our knowledge, this is the first study that has compared the GH response to moderate-intensity, continuous exercise and high-intensity, intermittent exercise in children with CF. Contrary to our hypothesis, the change in GH from REST was greater for MICE. The highest GH level we measured was at EX-MID for both exercises; and although the magnitude of GH secretion at this time point was greater for MICE, further calculations found that the amount secreted per min of exercise was greater for HIIE. An increase of ~1.9 ng/ml/min of exercise was observed for HIIE and ~0.6 ng/ml/min for MICE, which would indicate that the intermittent and more intense exercise was more efficient at inducing GH release. These results are supported by findings in the adult literature that suggest that when aerobic and anaerobic exercises are matched for duration and work accomplished, anaerobic exercise results in greater levels of GH secretion [45].

To our knowledge, no exercise studies have observed an increase in IGF-1 in children. This is consistent with findings from the current study as no exercise effects were seen with IGF-1. In adults, IGF-1 can increase 10 min into a bout of exercise followed by a decrease back to baseline during exercise; IGF-1 mobilization during exercise has been suggested to be

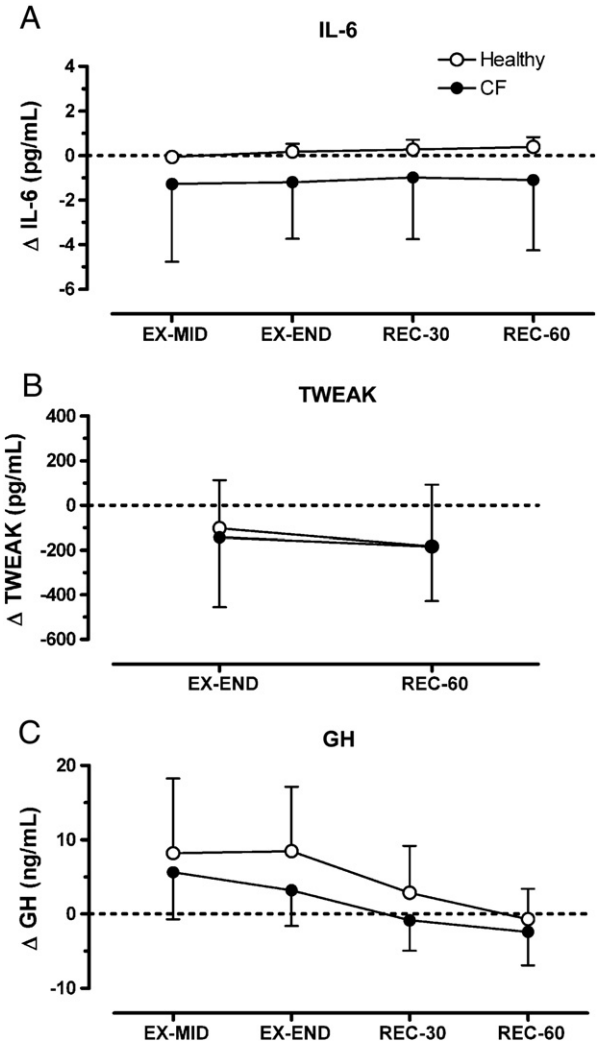


Fig. 4. Change in IL-6 (A), TWEAK (B), and GH (C) in response to HIIE in children with CF and matched-controls. Values are displayed as mean±SD. Δ: change in concentration from REST values. EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. The magnitudes of change from REST were similar in all markers during HIIE.

Table 5
Effect of corticosteriods and/or non-steriodial anti-inflammatory drugs on the magnitude of change from REST in immune cells in response to MICE and HIIE.

| | EX-MID | EX-END | REC-30 | REC-60 | *p* value for main effect for group | *p* value for group by time interaction |
|---|---|---|---|---|---|---|
| *MICE* | | | | | | |
| Δ Leukocytes | | | | | | |
| CF on drugs | 0.96±1.23 | 2.19±1.53 | 1.52±1.15 | 2.23±1.49 | 0.51 | 0.71 |
| CF not on drugs | 0.26±1.26 | 1.50±1.38 | 2.02±2.31 | 2.60±2.44 | | |
| Controls | 1.52±0.91 | 2.62±1.77 | 1.80±1.58 | 2.75±1.90 | | |
| Δ Neutrophils | | | | | | |
| CF on drugs | 0.52±0.80 | 0.82±0.96 | 1.21±1.11 | 2.45±1.40 | 0.79 | 0.91 |
| CF not on drugs | 0.37±0.53 | 1.43±0.75 | 2.26±2.17 | 3.04±2.39 | | |
| Controls | 0.72±0.43 | 1.31±0.90 | 1.78±1.50 | 2.79±1.96 | | |
| Δ Lymphocytes | | | | | | |
| CF on drugs | 0.39±0.21 | 0.97±0.55 | 0.15±0.23 | −0.11±0.18 | 0.30 | 0.64 |
| CF not on drugs | 0.03±0.23 | 0.30±0.50 | −0.09±0.51 | −0.19±0.57 | | |
| Controls | 0.67±0.54 | 1.01±0.91 | 0.05±0.38 | 0.07±0.61 | | |
| Δ Monocytes | | | | | | |
| CF on drugs | 0.02±0.08 | 0.23±0.13 | 0.07±0.18 | −0.05±0.27 | 0.62 | 0.49 |
| CF not on drugs | 0.00±0.11 | 0.10±0.06 | 0.08±0.13 | 0.10±0.16 | | |
| Controls | 0.11±0.11 | 0.23±0.15 | 0.03±0.11 | 0.07±0.10 | | |
| *HIIE* | | | | | | |
| Δ Leukocytes | | | | | | |
| CF on drugs | 0.41±0.87 | 0.54±0.94 | −0.33±0.71 | −0.23±1.14 | 0.39 | 0.66 |
| CF not on drugs | 0.66±1.01 | 0.88±1.15 | 0.34±1.06 | 0.45±0.76 | | |
| Controls | 0.66±0.54 | 0.82±0.73 | 0.11±0.40 | 0.43±0.53 | | |
| Δ Neutrophils | | | | | | |
| CF on drugs | 0.04±0.47 | 0.21±0.70 | −0.48±0.52 | −0.45±0.97 | 0.06 | 0.29 |
| CF not on drugs | 0.62±0.76 | 0.74±0.85 | 0.43±0.77 | 0.42±0.54 | | |
| Controls | 0.33±0.39 | 0.27±0.62 | 0.11±0.44 | 0.28±0.58 | | |
| Δ Lymphocytes | | | | | | |
| CF on drugs | 0.24±0.27 | 0.24±0.20 | 0.13±0.21 | 0.14±0.24 | 0.42 | 0.36 |
| CF not on drugs | −0.01±0.25 | 0.05±0.18 | −0.02±0.23 | −0.04±0.27 | | |
| Controls | 0.25±0.22 | 0.32±0.31 | 0.02±0.30 | −0.02±0.56 | | |
| Δ Monocytes | | | | | | |
| CF on drugs | 0.12±0.14 | 0.05±0.18 | 0.04±0.14 | 0.10±0.15 | 0.82 | 0.56 |
| CF not on drugs | 0.01±0.08 | 0.03±0.12 | 0.01±0.08 | 0.05±0.09 | | |
| Controls | 0.04±0.08 | 0.20±0.55 | −0.04±0.07 | 0.04±0.08 | | |

Values are displayed as mean±SD. Immune cell counts are expressed in $10^9$/L. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Δ: change in concentration from resting values. Children with CF on corticosteroid and/or NSAIDs: n=7, children with CF not on corticosteroid and/or NSAIDs: n=5, matched-controls: n=12.

GH-independent [46]. The earliest blood sample taken in the current study was 22 min after the start of intermittent exercise (considering exercise+rest time). It may have been more appropriate to have samples taken earlier into the exercise session in order to see changes in IGF-1. IGF-1 may be a more adequate indicator of growth promotion than GH since IGF-1 directly influences growth by initiating cellular changes [13]. Future studies will need to account for the possibly transient increase in IGF-1 and schedule blood samples around this increase in order to more clearly assess the IGF-1 response to exercise, as this has not yet been established in the pediatric exercise literature.

In comparing responses between children with CF and healthy matched-controls, our hypothesis that children with CF would have a greater inflammatory response and similar growth factor response compared to matched-controls, based on a previous study [1], was not supported. Instead, exercise responses at certain time points were seen in the matched-controls, but absent in children with CF. In addition, GH returned to resting concentrations earlier in the CF group. The absence of a response to exercise at certain time points may in part be due to the NSAIDs and corticosteroid medication taken by some children with CF, as these drugs are known to alter inflammation [1]. However, unlike the work of Tirakitsoontorn et al. [1], we found no difference in the cytokine response to exercise between children with CF on NSAIDs and/or corticosteroid medication and matched-controls or patients with CF not on these medications. Despite the absence of some exercise responses in children with CF that were seen in controls, the magnitude of change from REST in all blood markers was similar between groups.

To our knowledge, this is the first study to report the effects of exercise on circulating TWEAK concentrations. Resting levels of TWEAK have been measured in humans with and without chronic inflammation [47]. Levels are elevated in adults with rheumatoid arthritis [47] compared to controls, suggesting that TWEAK may be involved in the pathology of these diseases. In fact, TWEAK is involved in skeletal muscle degradation [29]. Results from the current study indicate that high-intensity intermittent exercise can reduce circulating TWEAK levels. Furthermore, the decrease in TWEAK with HIIE was observed in our matched-

Table 6
Effect of corticosteriods and/or non-steroidal anti-inflammatory drugs on the change in inflammatory cytokine and growth factors from baseline for MICE and HIIE.

| | EX-MID | EX-END | REC-30 | REC-60 | *p* value for main effect for group | *p* value for group by time interaction |
|---|---|---|---|---|---|---|
| *MICE* | | | | | | |
| Δ GH | | | | | | |
| CF on drugs | 17.31±16.00 | 11.34±7.36 | 3.64±4.45 | 1.18±3.22 | 0.94 | 0.73 |
| CF not on drugs | 19.28±28.17 | 15.76±20.48 | 2.72±7.01 | −0.24±8.05 | | |
| Controls | 12.96±9.48 | 13.05±11.13 | 4.54±9.63 | 0.83±7.61 | | |
| Δ IGF-1 | | | | | | |
| CF on drugs | −13.48±17.08 | −6.36±21.92 | −7.86±26.88 | −16.97±14.62 | 0.75 | 0.07 |
| CF not on drugs | −8.09±23.29 | 2.17±28.73 | −8.25±8.19 | −4.65±21.74 | | |
| Controls | −8.56±13.74 | −5.36±13.58 | 1.01±16.31 | −3.53±12.27 | | |
| Δ IL-6 | | | | | | |
| CF on drugs | 0.19±0.88 | 1.90±1.86 | 2.92±2.17 | 2.52±3.09 | 0.45 | 0.82 |
| CF not on drugs | 0.54±0.48 | 1.61±1.08 | 2.26±1.28 | 1.31 ±0.76 | | |
| Controls | 0.16±0.17 | 1.36±0.47 | 2.02±0.70 | 1.56±0.66 | | |
| Δ TNF-α | | | | | | |
| CF on drugs | 0.12±0.27 | 0.13±0.23 | 0.17±0.33 | 0.18±0.28 | 0.61 | 0.15 |
| CF not on drugs | 0.09±0.15 | 0.03±0.26 | 0.28±0.20 | 0.06±0.26 | | |
| Controls | 0.05±0.21 | 0.17±0.27 | 0.14±0.27 | 0.10±0.15 | | |
| Δ TWEAK | | | | | | |
| CF on drugs | – | −85.72±221.65 | – | −81.56±216.00 | 0.42 | 0.40 |
| CF not on drugs | – | 29.66±278.68 | – | −113.96±301.44 | | |
| Controls | – | 47.10±161.21 | – | −16.06±190.19 | | |
| *HIIE* | | | | | | |
| Δ GH | | | | | | |
| CF on drugs | 5.78±6.61 | 3.96±5.46 | −0.39±4.55 | −1.74±4.52 | 0.36 | 0.78 |
| CF not on drugs | 5.41±6.60 | 2.18±4.06 | −1.49±3.85 | 3.32±4.81 | | |
| Controls | 8.21±10.07 | 8.49±8.65 | 2.87±6.32 | −0.69±4.09 | | |
| Δ IGF-1 | | | | | | |
| CF on drugs | 3.79±18.48 | 4.80±19.05 | 14.04±31.39 | 0.70±23.24 | 0.52 | 0.22 |
| CF not on drugs | −10.93±25.38 | −0.36±8.52 | −18.70±37.06 | −2.66±17.72 | | |
| Controls | −0.04±24.61 | 0.88±30.90 | 8.62±25.68 | 2.42±21.10 | | |
| Δ IL-6 | | | | | | |
| CF on drugs | −1.96±4.57 | −1.19±3.18 | −1.68±3.52 | −2.09±3.78 | 0.06 | 0.98 |
| CF not on drugs | −0.30±0.52 | −0.19±0.61 | −0.02±0.65 | 0.30±1.32 | | |
| Controls | −0.06±0.20 | 0.18±0.35 | 0.28±0.44 | 0.39±0.44 | | |
| Δ TNF-α | | | | | | |
| CF on drugs | 0.29±0.25 | 0.39±0.69 | 0.13±0.27 | 0.20±0.25 | 0.19 | 0.40 |
| CF not on drugs | 0.14±0.20 | 0.16±0.33 | 0.21±0.27 | 0.07±0.26 | | |
| Controls | 0.07±0.20 | 0.01±0.23 | 0.11±0.17 | 0.08±0.28 | | |
| Δ TWEAK | | | | | | |
| CF on drugs | – | −104.63±337.47 | – | −73.17±246.13 | 0.43 | 0.37 |
| CF not on drugs | – | −197.03±303.14 | – | 340.51±144.80 | | |
| Controls | – | −102.12±214.37 | – | −182.45±275.39 | | |

Values are displayed as mean±SD. Inflammatory cytokines are expressed in pg/mL. Growth factors are expressed in ng/mL. GH: growth hormone, IGF-1: insulin-like growth factor 1, IL-6: interleukin 6. TNF-α: tumor necrosis factor alpha, TWEAK: tumor necrosis related weak inducer of apoptosis. MICE: moderate-intensity, continuous exercise. HIIE: high-intensity, intermittent exercise. EX-MID: midpoint of exercise, EX-END: end of exercise, REC-30: 30 min into recovery, REC-60: 60 min into recovery. Δ: change in concentration from resting values. Children with CF on corticosteroid and/or NSAIDs: n=7, children with CF not on corticosteroid and/or NSAIDs: n=5, matched-controls: n=12.

control group, but not in children with CF, suggesting that a decrease in TWEAK is a normal response to exercise.

Findings from this study contrast with those of the only other known study on the inflammatory and growth factor response to exercise in children with CF, insofar as we did not observe an exaggerated inflammatory response in children with CF compared to matched-controls, or an exaggerated response in children with CF taking NAISD/corticosteroids. The differences between studies may be due to several reasons. First, our children with CF had better $FEV_1$ values compared to the participants of Tirakitsoontorn et al. [1]. While that study reported no significant correlation between the IL-6 response to exercise and $FEV_1$ in children with CF, our results suggest otherwise ($r=-0.646$, $p<0.05$). Second, Tirakitsoontorn et al. [1] did not report $FEV_1$ values for their controls, thus, it is unknown whether pulmonary function in the CF patients and controls were different. Third, our matched-controls and children with CF were matched by biological maturity, while Tirakitsoontorn et al. [1] did not report if, or how, their groups were matched. Controlling for biological age is important because the inflammatory response to exercise is influenced by maturity status [48].

## 5. Limitations and future directions

On average, our patients were similar in pulmonary function to matched-controls and had normal percent predicted values of aerobic fitness. Thus, the clinical application of the results from this study may only be appropriate for children with CF with a good current health status. Another limitation of our study is that we did not measure GH more frequently to capture its pulsatility. It is possible that the effects of exercise on GH could be observed well-after 1 h of recovery, although such measurements were beyond the focus of this study.

Although this study provides novel information on the inflammatory and growth factor response to exercise in children with CF, results are limited to acute bouts of exercise. Further steps should be taken to determine the inflammatory and growth factor response to different forms of exercise training. Exercise can lower inflammation at rest in adults with a chronic inflammatory disease [49], a finding that would prove to be especially beneficial if true in pediatric patients with chronic inflammatory diseases such as CF. There have been no studies on the effects of exercise training on inflammatory and growth factors in children with an inflammatory disease [50]; thus, exploration of this area will provide insight into optimal types of exercise programs that can reduce inflammation while promoting growth. It may also be possible to determine whether exercise-induced changes in inflammation actually have a benefit to tissue adaptation. In addition, since many training studies with CF patients have focused on slowing the decline, maintaining or improving $FEV_1$ and $VO_{2peak}$ [8–10,51], efforts should be made to investigate which type of exercise is best at maintaining or improving $FEV_1$ and $VO_{2peak}$ while reducing inflammation and improving growth.

## 6. Conclusion

In assessing the inflammatory and growth factor responses to continuous versus intermittent exercise, it was found that intermittent exercise did not elicit an inflammatory response, but is capable of elevating circulating GH above resting levels. These findings demonstrate that exercise designed to mimic the natural physical activity pattern of children does not result in major immune or inflammatory perturbation but does induce an increased GH, which has previously been associated with muscle growth and retention in children with CF. Although larger increases in GH were observed with continuous exercise, the increases in immune cell counts and IL-6 were also greater. Therefore, exercise prescriptions aimed at avoiding or minimizing inflammation while promoting growth should further investigate incorporating short bouts of intermittent, high-intensity exercise.

## Acknowledgments

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