Title
Fitness, acute exercise, and anabolic and catabolic mediators in cystic fibrosis

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Exercise can stimulate catabolic inflammatory cytokines even in healthy children. For patients with cystic fibrosis (CF), this may be problematic because CF is characterized by increased inflammation and suppressed growth. We examined fitness and the response to brief exercise of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), insulinlike growth factor-I (IGF-I), and IGF binding protein-1 (IGFBP-1) in 14 subjects with CF (10.5 ± 0.8 yr of age), 9 of whom were treated with ibuprofen, and 14 healthy control subjects (11.6 ± 0.5 yr of age, NS). Subjects performed brief intermittent, constant work rate protocol (scaled to each individual’s exercise capacity) with blood and urine sampling. Peak \( V_{O_2} \) was correlated with IGF-I (\( r = 0.68, p < 0.01 \)) in control subjects but not in subjects with CF. In subjects with CF, baseline IL-6 was 79% greater (\( p < 0.05 \)) and IGF-I was 47% lower than in control subjects (\( p < 0.05 \)). Post hoc analysis revealed a progressive increase in the IL-6 response to exercise, with the lowest increase observed in control subjects (11.8 ± 4.6 pg/L/kJ), higher increases in patients with CF treated with ibuprofen (23.4 ± 7.7 pg/L/kJ), and highest in subjects with CF not receiving ibuprofen (29.2 ± 7.5 pg/L/kJ). Qualitatively similar results were observed for TNF-α. Exercise also significantly increased IGFBP-1 in both control subjects and subjects with CF. Brief exercise can increase even chronically elevated inflammatory mediators in CF, and this response may be attenuated by ibuprofen.

**Keywords:** exercise; cytokines; insulinlike growth factor; cystic fibrosis; inflammation

The clinician attempting to prescribe a program of exercise training for children and adolescents with cystic fibrosis (CF) faces a dilemma. Exercise may promote health in CF in part by stimulating growth factors and tissue anabolism (enhanced bone mineralization, increased muscle hypertrophy, mitochondrial density and capillarization, and increased insulin sensitivity) (1, 2). However, even in healthy children, it is now known that the very same process of exercise, if sufficiently intense, can stimulate inflammatory cytokines and lead to a catabolic state (3–6). Finding the optimal level of physical activity in children and adolescents with CF is difficult because the underlying disease is associated with increased basal energy expenditure (7, 8), hypoxemia, malnutrition, and inflammation, all of which promote tissue catabolism even at rest. The major objective of this research was to better understand the relationship of fitness to specific catabolic and anabolic mediators in subjects with CF and to test the effect of a brief bout of intense exercise on these mediators. We focused on the anabolic growth hormone→insulinlike growth factor-I system (GH→IGF-I), a system of growth hormones and mediators that modulates growth in many tissues and is known to be influenced by exercise and training. For catabolic mediators, we chose to examine the proinflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). These latter cytokines (1) are specifically known to inhibit many aspects of the GH→IGF-I axis (9); (2) are involved in inflammatory responses associated with CF (10–12); and (3) can also be stimulated by physical exercise even in healthy subjects (3, 13).

Because the balance of circulating catabolic and anabolic mediators is altered in CF (i.e., IGF-I is low; TNF-α and IL-6 are elevated), we hypothesized that the relationship between fitness and these mediators would be altered as well. Moreover, we predicted that the inflammatory response to acute exercise in children with CF would be depressed because the subjects with CF have chronically elevated TNF-α and IL-6, which could chronically stimulate anti-inflammatory mechanisms such as increased IL-1 receptor antagonist (IL-1ra). This, in turn, would attenuate the additional inflammatory stimulation that might be caused by exercise. Finally, because many children with CF are routinely treated with nonsteroidal anti-inflammatory agents such as ibuprofen (14, 15), we performed (following the suggestion of one of the study’s referees) a post hoc analysis of the data to determine the influence of this medication on the inflammatory response to exercise in these patients.

**METHODS**

The study was approved by the Institutional Review Board. Informed consent and assent were obtained from each subject or from his or her parent or legally authorized representative. Standard, calibrated scales and stadiometers were used to determine height, weight, body mass index (wt/ht\(^2\) = BMI), and BMI for age percentile (16). In the subjects with CF, standard spirometry was performed to determine the FEV\(_1\) as percent predicted. Pulmonary status was assessed by history.

**Exercise Protocols**

Each subject underwent two separate exercise testing sessions performed on different days. First, we used a ramp-type progressive exercise test on an electronically braked cycle ergometer used extensively in children and adolescents (17). The second session consisted of a series of 10 2-min bouts of constant-work rate cycle ergometry with 1-min resting intervals between each exercise bout. The work rate was individualized for each subject by finding the work rate corresponding to 50% of the difference between the anaerobic or lactate threshold (determined noninvasively from the ramp test) and peak \( V_{O_2} \) (18, 19). Children often do not sustain constant exercise for more than several minutes at a time. The total duration of the second exercise protocol was 30 min (20 min of cycle ergometer exercise interspersed with 10 min of rest).

We calculated total external work performed by each subject, i.e., power times duration (kJ) and normalized the total work performed to body mass. Finally, we measured the end-exercise heart rate of each subject during the second exercise session.
Blood and Urine Sampling Protocols

An indwelling venous catheter in the antecubital area was used to collect blood samples at preexercise, during the last (tenth) 2-min exercise bout, 5 min after exercise, and 60 min after exercise. We chose to examine the 60 min time point because it is known that systemic cytokine responses to exercise can occur well after the cessation of exercise (20). Urine samples were obtained at preexercise, 5 min after exercise, and 60 min after exercise.

Blood and Urine Measurements

Plasma lactate was measured enzymatically. Albumin was measured using standard colorimetric techniques. Growth hormone (GH) serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA). GH binding protein (GHBP) was measured using the ligand-mediated immunofunctional assay (21). IGF-I was extracted from binding proteins (IGFBPks) using the acid-ethanol extraction method (22) and measured by a two-site immunoradiometric assay (IRMA). IGFBP-1 was measured by coated-tube IRMA. We used ELISA for all of the cytokine measurements. In order to normalize for deviations in urine concentration, urine cytokine values were normalized to creatinine (3, 23).

Statistical Analysis

Two-sample t-tests were used to determine baseline differences in anthropometric variables, fitness variables, circulating albumin, circulating components of the GH→IGF-I axis, and cytokines between control and CF subjects prior to the exercise protocol. Correlations were used to describe the relationship between circulating anabolic and catabolic factors and indexes of fitness and between FEV1 (% predicted) and both baseline and mediator responses to exercise. Repeated measures analysis of variance (ANOVA) was used to analyze serum and urine values in response to the exercise bout. Between-subject tests were used to compare overall response differences between the control and CF groups across the time points. Single degree of freedom orthogonal polynomials over time were used to characterize possible changes caused by exercise, i.e., linear and quadratic changes across time. These polynomials were examined for both designs with all time points and designs with differences from baseline (for each subject).

A difference between the control and CF groups in the response pattern caused by exercise was tested using the interaction between each polynomial and the between subjects factor.

We also examined post hoc the effect of ibuprofen (nonsteroidal anti-inflammatory drug; NSAID) use on anabolic and catabolic mediators in the CF and control subjects. First, we analyzed preexercise differences among the ordered means of three groups (i.e., healthy control subjects, subjects with CF using ibuprofen, and subjects with CF not using ibuprofen). We used one-way analysis of variance (ANOVA) with a test of a linear contrast across the ordered group means. An analysis of changes in anabolic and inflammatory mediators from preexercise to peak values during or after exercise was also performed using a one-way ANOVA. Tests of linear contrasts and pairwise mean comparisons (using the Bonferroni correction) were executed on the means of the change scores. Statistical significance was taken at the p < 0.05 level. Data are presented as mean ± SEM.

RESULTS

Baseline Data

Subjects. Fourteen outpatient subjects with CF (eight female) 7 to 17 yr of age and 14 healthy children 8 to 15 yr of age (seven female) volunteered for the study. The subjects with CF tended to have relatively mild manifestations of CF, and all were known to be compliant with respect to pancreatic enzyme supplementation and antibiotic usage. Nine of the subjects with CF were receiving ibuprofen at the time of the study. The remainder were unable to tolerate ibuprofen and were receiving no systemic NSAIDs. Six of the subjects with CF and five of the control subjects were prepubertal. None of the subjects with CF was receiving hormonal replacement therapy.

Age, Weight, Height, and BMI

There was no significant difference in age and height between control and CF groups. Body weight, BMI, and BMC for age percentile were significantly higher in control subjects than in subjects with CF (Table 1). The post hoc analysis showed that BMI percentile was influenced by ibuprofen (Figure 1): BMI for age percentile was highest in control subjects, lower in ibuprofen treated subjects with CF, and lowest in ibuprofen untreated subjects with CF (p < 0.008).

Peak VO₂, Peak Work Rate, FEV₁, and Serum Albumin

Peak VO₂, peak VO₂ corrected for body weight, and peak work rate were significantly higher in the control group than in the CF group. No differences in serum albumin were observed between CF and control subjects. No statistically significant effects of ibuprofen were observed in these variables. Mean and range of the FEV₁ values are also shown in Table 1. FEV₁ was 74 ± 3% predicted in the subjects with CF not receiving ibuprofen and 89 ± 6% in the subjects with CF who did use ibuprofen (p = 0.11).

Influence of Ibuprofen on Baseline Cytokines and Growth Factors

The post hoc analysis of ibuprofen use revealed significant patterns (p < 0.05) (Figure 1). IGF-I and GHBP levels were greatest in control subjects, lower in subjects with CF who used ibuprofen, and lowest in subjects with CF unable to use ibuprofen. A mirror image was observed for serum IL-6; values were lowest in control subjects, higher in subjects with CF using ibuprofen, and highest in subjects with CF unable to use ibuprofen.

Correlations among Cytokines, Growth Factors, and Fitness Variables and FEV₁

There were no significant correlations between FEV₁ and IL-6, TNF-α, or IL-1β in the subjects with CF. Differences in the correlation between growth factors, cytokines, and fitness variables were observed between control subjects and subjects with CF at baseline. Peak VO₂ was positively correlated with IGF-I in control subjects (r = 0.68, p < 0.01), but not in subjects with CF (Figure 2). IGF-I was correlated with body weight in both control subjects (r = 0.86, p < 0.0005) and subjects with CF (r = 0.59, p < 0.05). IGF-I was correlated with body height in control subjects (r = 0.83, p < 0.0005), but no

<table>
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<th>TABLE 1. ANTHROPOMETRIC, PULMONARY FUNCTION, PEAK VO₂ PEAK WORK RATE, AND SERUM ALBUMIN DATA IN SUBJECTS WITH CYSTIC FIBROSIS AND IN CONTROL SUBJECTS*</th>
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Definition of abbreviations: BMI = body mass index; V̇O₂ = oxygen consumption.
*The data are expressed as mean ± SD.
†p < 0.01.
‡p < 0.001.
³p < 0.0001.
correlation between IGF-I and height was observed in subjects with CF. Although TNF-α was not correlated with IGF-I in control subjects, a negative relationship was found between these two variables in the subjects with CF (r = −0.57, p < 0.05).

**Effect of Brief Exercise**

Total work and heart rate (Figure 3). Total work performed on the second session was significantly greater in the control subjects. Control subjects did significantly more work even when corrected for body weight. Despite the differences in absolute and relative work, both control and CF groups reached the same heart rate by end-exercise. No effect of ibuprofen utilization was observed.

*Plasma lactate and serum growth hormone (Figure 4).* Plasma lactate and serum GH increased significantly during exercise in both control and CF groups (p < 0.001). There were no significant between-group differences in these variables at any time. No effect of ibuprofen was observed.

*Serum cytokines (Figure 5).* Exercise led to a significant increase in IL-6 in both control and CF group during the 90-min observation period (p < 0.002). The post hoc analysis revealed a significant effect (p < 0.05) of ibuprofen use when the data were expressed either as an absolute increase in IL-6 or normalized to the work performed. The increases in IL-6 levels were smallest in control subjects; higher in subjects with CF who used ibuprofen, and highest in subjects with CF unable to use ibuprofen.

In contrast to IL-6, the peak values for TNF-α were found immediately after exercise. Similar to IL-6, the post hoc analysis revealed a significant effect of ibuprofen use when the data were expressed either as an absolute increase or normalized to the work performed. The increases in TNF-α levels were smallest in control subjects, higher in subjects with CF who used ibuprofen, and highest in subjects with CF unable to use ibuprofen.

Finally, for circulating IL-1β and IL-1ra, there were no baseline differences between control and CF groups (IL-1β, 1.47 ± 0.76 versus 0.66 ± 0.2 pg/ml; IL-1ra, 290.6 ± 35.9 versus 352.4 ± 58.1 pg/ml in control and CF groups, respectively), and no effect of exercise was found in either group.

**Urine Cytokines**

Cytokines measured in the urine were, as noted, normalized to urine creatinine levels. There was great individual variability in these responses, but despite this, certain significant patterns were observed. Baseline urine IL-6 in subjects with CF (7.51 ± 2.42 pg/mg) was significantly higher than in control subjects (1.60 ± 0.37 pg/mg; p < 0.05). Exercise led to an increase in IL-6 in both groups (overall percent increase was 218 ± 181.8% and 30 ± 69.6%, p < 0.05, in control and CF groups, respectively), and like the serum IL-6 response to exercise, the peak values were observed at 60 min after exercise. The magnitude of the response did not statistically differ between the two groups, and post hoc analysis revealed no effect of ibuprofen use.

There were no differences in baseline urine TNF-α (CF, 1.12 ± 0.20 pg/mg; control, 1.08 ± 0.22 pg/mg). Exercise led to an increase in urine TNF-α in both groups (overall percent increase was 339 ± 148.9% and 88 ± 59.6%, p < 0.05, in control and CF groups, respectively). The magnitude of the TNF-α response did not statistically differ between the two groups. Finally, baseline urine IL-1ra in subjects with CF (654.7 ± 126.9 pg/mg) was significantly lower than in control subjects (1,383.8 ± 287.9 pg/mg, p < 0.05). These values remained unchanged from baseline and significantly lower than in control subjects throughout exercise and recovery (p < 0.05).

**Serum Growth Factors**

Exercise led to a small but significant decrease in IGF-I in both control and CF groups, which was not observed until 60...
min after exercise (p < 0.001). The magnitude of the response did not significantly differ between the two groups (overall percent decrease was 6.5 ± 2.4% in control subjects and 8.3 ± 3.6% in subjects with CF). There was no difference in baseline IGFBP-1 (CF, 28.6 ± 7.6 ng/ml; control, 31.8 ± 7.8 ng/ml). Exercise led to a significant increase in IGFBP-1 in both groups (overall percent increase was 55.3 ± 35.6% and 140.7 ± 42.4%, p < 0.05 in control and CF groups, respectively), which were found 60 min after exercise. The magnitude of the response did not statistically differ between the two groups, and no effect of ibuprofen use was observed. Finally, GHBP which was, as noted, lower in subjects with CF was not influenced by exercise in either group.

**DISCUSSION**

These data demonstrate an abnormal relationship among fitness, IGF-I, and TNF-α in patients with CF. Although our data corroborated recent findings by other investigators of reduced circulating IGF-I and elevated inflammatory cytokines in subjects with CF (24–26), our study failed to support the hypothesis that the inflammatory response to brief exercise was blunted in subjects with CF. In fact, a post hoc analysis suggested that the increase in IL-6 and TNF-α after exercise was ordered, with the highest values found in subjects with CF not receiving ibuprofen, lower in subjects with CF treated with ibuprofen, and lowest in control subjects. Exercise-associated increases in GH and IGFBP-1 were also observed in both control and CF groups, and the magnitude of the response was similar in subjects with CF and control subjects even though the work performed was substantially lower in the subjects with CF.

The normally high correlation between IGF-I and peak VO₂ has been observed in a number of populations of healthy children and adults (4, 27), and we found this to be the case in the control subjects in the present study as well. In contrast, peak VO₂ and IGF-I were not correlated in the subjects with CF (Figure 1). Although we found strong correlations between IGF-I and other indices of body size such as height and weight in control subjects, only a modest correlation between weight (but not height) and IGF-I was observed in the subjects with CF.

The mechanism responsible for this alteration in the relationship between IGF-I, fitness, and body size is not clear. Studies in children with other chronic diseases characterized by elevated circulating levels of inflammatory mediators such as juvenile rheumatoid arthritis also show reduced fitness and IGF-I (28, 29); and inflammatory cytokines like IL-6 and TNF-α can directly inhibit bioactivity of the GH→IGF-I axis (9, 30, 31). Circulating IL-6 was substantially elevated in the subjects with CF. Moreover, baseline levels of circulating TNF-α had a significant inverse relationship with IGF-I in subjects with CF but not control subjects. TNF-α and IL-6 are known to directly cause muscle atrophy in experimental models (32, 33). These observations suggest the possibility that the chronic catabolic influence of these inflammatory mediators may inhibit normal muscle development in patients with CF, and, along with nutritional and other mechanisms, lead to generally reduced fitness and an abnormal relationship between fitness, growth, and IGF-I. Whether or not the low levels of circulating IGF-I contribute to recent observations from this laboratory of a muscle-related abnormality in oxygen metabolism during exercise in patients with CF (34) is not known.

The mechanism responsible for the elevated baseline inflammatory cytokines in patients with CF is not known but is likely related to chronic lung disease and a “spillover” of these agents from infected sites in the lung to the central circulation. Like other pathologic situations in which inflammatory mediators are elevated and IGF-I is reduced, e.g., trauma, burns, sepsis (35), the subjects with CF demonstrated evidence of GH resistance, i.e., normal GH response to physiologic stimuli (Figure 3) with low GHBP (36). GHBP is known to be the extracellular component of the GH receptor molecule, and its levels in the bloodstream are felt by a number of investigators to reflect overall GH receptor numbers (37, 38).
In recent years, high dose ibuprofen has been used in patients with mild CF to attenuate the deterioration in lung function associated with chronic inflammatory disease. Although assessing the effect of ibuprofen was not originally a major hypothesis of this study, we noted that five of the 14 subjects with CF could not tolerate this regimen and were not treated with ibuprofen at the time of the study. Post hoc analysis was remarkable in that a clear effect of ibuprofen on growth mediators, inflammatory cytokines, and body composition was observed at baseline. The finding of increased IL-6 in subjects with CF, particularly in those not receiving ibuprofen, supports the notion that inflammatory cytokine inhibition of the GH→IGF-I axis may be playing a role in the mechanisms of the GH resistance in the subjects with CF and contributing to reduced IGF-I.

These findings may reflect more than just biochemical alterations as indicated by the fact that BMI percentile followed the same pattern as IGF-I and GHBP. (BMI in absolute value is age-dependent; thus, we focused on the BMI percentile because it allowed us to assess body composition normalized to an age standard.) In chronic disease, the lower relative BMI likely indicates an overall reduction in lean body mass, probably the result of the continuous antianabolic activity of the proinflammatory cytokines. Although malabsorption is often a component of CF symptomatology, we found no difference in circulating albumin between CF and control groups, supporting the notion that specific catabolic effects of proinflammatory cytokines are playing a role in the low BMIs observed in the subjects with CF. Further, the data support the notion that these effects can be ameliorated by ibuprofen.

As seen in Figures 3 and 4, we achieved the goal of ensuring that the work performed was appropriate for each individual’s exercise capacity: lactate levels (a widely accepted indicator of the metabolic stress of exercise) were the same in both groups. However, the work required by the subjects with CF to achieve this lactate level was significantly lower than in control subjects both in absolute terms and when the work performed was normalized to body weight (Figure 2).

In contrast to our hypothesis, the inflammatory response to exercise was not blunted in subjects with CF. Exercise-associated increases in IL-6 and TNF-α were observed in urine and blood in both groups. Even relatively mild, intermittent exercise can lead to measurable changes associated with increased inflammation in both healthy children and subjects with CF, but the post hoc analysis of the effect of ibuprofen suggested that subjects with CF achieved the higher levels of inflammation than control subjects with relatively less work. The time course of inflammatory mediators in response to exercise are known to vary and may actually peak well after the end of the exercise bout (20). Our observation of seemingly delayed post-exercise increases of IL-6 and IGFBP-1 in the CF and control groups corroborates the experience of previous investigations in adults and children. The mechanism for the prolonged time course is not yet understood.

Treatment with ibuprofen attenuated the inflammatory response to exercise in CF. Recent studies in healthy subjects by Pizza and coworkers (39) demonstrated that ibuprofen can reduce creatine kinase levels in the blood after eccentric exercise, i.e., a type of exercise known to induce muscle damage (40). However, to our knowledge, the acute effect of ibuprofen on inflammatory responses to exercise in children or adolescents has never been studied. The current observations suggest the hypothesis that a benefit of ibuprofen in patients with CF might be to attenuate inflammatory responses to exercise, but this clearly needs to be tested rigorously.

We also found that the magnitude of the change in both TNF-α and IL-6 was greater in urine than in serum. This is consistent with what we recently observed in healthy children in whom the changes in urine levels after exercise were about twice that found in the serum. This is not entirely surprising. Cytokines are cleared from the systemic circulation into the urine “pool”; thus, the cytokine concentration in the urine reflects the integral of cytokine transfer during the interval between voids. Our data do suggest that sampling from urine may, under the right experimental circumstances, serve as a reasonable alternative to blood sampling when attempting to determine the qualitative inflammatory cytokine response to exercise in children.

Previous data from this and other laboratories suggest a bi-phasic circulating IGF-I response to exercise characterized by a small initial increase and, ultimately, a decrease in IGF-I as exercise proceeds (3, 41). Indeed, in the present study we found small but significant decreases in circulating IGF-I levels in both CF and control groups 60 min after the exercise was completed, and there was no difference in the magnitude of this response between groups. The mechanism of this small, acute reduction in IGF-I has yet to be elucidated but might be related to increased inflammatory cytokines and IGFBP-1. IGFBP-1, known to inhibit IGF-I anabolic function, increased robustly after exercise in both control subjects and subjects with CF, an observation recently made in this and other laboratories in healthy subjects (3).

As noted, inflammatory cytokines do inhibit IGF-I production and stimulate IGFBP-1 (31, 42). IGFBP-1 levels are known to be inversely correlated with levels of insulin (43). However, previous studies in adults have demonstrated that prolonged heavy exercise leads to reductions in IGF-I and to large, acute increases in IGFBP-1 (44–46), which do not appear to be related to either insulin or glucose levels.

The therapeutic implications of our findings that brief exercise acutely increases circulating proinflammatory cytokines in subjects with CF are not yet clear. Although elevations in inflammatory cytokines commonly indicate pathologic states, studies in healthy adults and children indicate that exercise is a natural stimulator of these substances. This suggests that factors such as IL-6 and TNF-α may play a necessary role in the healthy adaptation to exercise, e.g., it is known that angiogenesis, an important component of the fitness response, is stimulated by inflammatory cytokines (47). Conversely, the finding that relatively less exercise in subjects with CF can additionally increase already elevated circulating levels of these mediators suggests that the beneficial range of physical activity may be narrower than in healthy control subjects. What is needed now is to determine whether or not there exists an optimal level of physical activity and/or training in CF in which anabolic mediators are increased and catabolic agents diminished. The post hoc analysis of the effects of ibuprofen on attenuating inflammatory responses to exercise is intriguing, but the long-term therapeutic impact of this finding has yet to be determined.

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