Medicina Sportiva
Med Sport 12 (3): 92-98, 2008
DOI: 10.2478/v10036-008-0018-6

GROWTH-RELATED CHANGES IN THE ACUTE IMMUNE RESPONSE TO EXERCISE IN HEALTHY BOYS

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Abstract

Previous cross-sectional comparisons have found that the magnitude of exercise-induced changes in some immune factors is related to age and maturation in healthy children.

The aim of this paper is to describe growth-related changes in the acute immune response to exercise in the same children in a longitudinal manner.

Methods: On two occasions (T1 and T2) separated by ~2 years, seven boys (initially 9.6 ± 0.5 years) performed cycling exercise for 60 min at an intensity equivalent to 70% of their peak aerobic power. Before, immediately after and 60 min after (REC) exercise, blood samples were collected and analyzed for cellular and soluble components of the immune system known to be responsive to exercise.

Results: The magnitude of the neutrophil response to exercise tended ($p = 0.08$) to be greater at T2 vs. T1 by 64%. The boys experienced exercise-induced suppression of lymphocyte ($p = 0.03$) and T cell ($p = 0.004$) counts by 11% and 13%, respectively, during REC at T2, but not T1. Boys who experienced the greatest gain in height from T1 to T2 demonstrated the greatest increase in the degree of T cell suppression during REC ($r = 0.80$, $p = 0.03$). At T2, IL-6 remained 88% above resting values during REC, but was unchanged from rest at T1.

Conclusion: Consistent with previous cross-sectional studies, we conclude that the acute immune response to exercise is related to growth in healthy boys.

Key words: *exercise, immune, children, maturation*

Introduction

In the last two decades, considerable advances have been made in our understanding of the acute and chronic effects of exercise on the immune system in adults. Unfortunately, similar progress in pediatric exercise immunology has not kept pace (1). Given the significant development of the immune system during childhood (2-5), it is reasonable to expect that acute and chronic immune effects of exercise may have unique implications to the growing child. When one further considers the association between the immune system and health complications such as obesity, insulin resistance, and atherosclerosis, it is important to explore the impact of exercise on immune system activation during growth, as the results are likely to have important implications for children with a chronic disease.

Revelations that skeletal muscle plays an important immunological role (e.g., cytokine production) (6) raise the possibility that contractile activity during growth may help regulate immune development. Although a number of studies have reported child-specific immune responses to exercise (1), few have accounted for the effects of chronological and/or biological age. In a series of cross-sectional studies, we found that the impact of acute exercise on the magnitude of the natural killer (NK) cell and IL-6 response, for example, was lower in boys *versus* men and that the recovery of neutrophils, IL-6 and T cells following exercise was faster in the boys (7). To further explore these age-related differences, we tested boys of different pubertal stages and chronological ages. We confirmed that the magnitude of the NK cell response to exercise increased with progressing physical maturity (8) and that neutrophil recovery was faster in less mature and younger boys (9). Thus, our findings suggested that young children may be resistant to major exercise-induced perturbations to the immune system, in particular components of the innate immune system.

Although the biological significance of these findings is unclear, it has been hypothesized that activation of the immune system during exercise in children may be sufficient to assist in immune development and physiological adaptation (e.g., muscle growth) without surpassing a critical threshold, which could lead to immune suppression or impairment of the anabolic effects of exercise (1). Such an idea would be particularly intriguing for children with a chronic disease characterized by a significant inflammatory burden (e.g., juvenile arthritis). A limitation in previous studies (7-9), however, has been their cross-sectional design, because true effects of growth are best determined by studying the same individuals over time. In this paper, we describe the acute immune response to exercise in boys tested on two occasions separated by ~2 years,

allowing us to assess, for the first time, growth-related changes. Based on our previous cross-sectional studies (7-9), we hypothesized that as the boys matured they would experience greater perturbation to the immune system in response to standardized exercise.

Materials and Methods

Study design and subjects. This study compared the immune-related responses to exercise in the same boys on two occasions (referred to as T1 and T2), separated by ~2 years. Detailed descriptions of the experimental procedures have been published elsewhere (7;9). Each subject completed a preliminary visit to determine their peak aerobic power ($\dot{V}O_{2peak}$) and anthropometric characteristics. On a subsequent experimental visit, they performed 60 min of cycling at 70% of their $\dot{V}O_{2peak}$.

Seven boys participated and all procedures were approved by the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board. All subjects were healthy with no recent history of allergies and were not taking any medication. Pubertal status of each boy was assessed based on pubic hair development according to Tanner (10). We routinely use self-assessment of secondary sex characteristics in our subjects as this has been shown to be valid and reliable (11) and avoids feelings of embarrassment in a research setting. Table 1 summarizes the boys' physical, pubertal, and fitness characteristics on both testing occasions. After the purpose, procedures and risks of the study were explained, the boys agreed verbally to participate and their parent then signed a written informed consent on each occasion.

Table 1. *Group characteristics*

	T1	T2
Age, yr	9.6 ± 0.5	12 ± 0.0*
Height, m	1.41 ± 0.08	1.55 ± 0.09*
Body weight, kg	36.2 ± 4.8	46.2 ± 9.2*
Body fat, %	16.7 ± 7.0	17.5 ± 7.5
FFM, kg	29.5 ± 2.4	37.8 ± 6.6*
Pubertal status	1–2	1–5
$\dot{V}O_{2peak}$, L/min	1.62 ± 0.25	2.11 ± 0.47*
$\dot{V}O_{2peak}$, mL/kg·min	45.0 ± 4.9	46.1 ± 8.1
HR_{max}, beats/min	196 ± 5	200 ± 9

Values are means ± SD or range in the case of pubertal status. T1, 1st testing occasion; T2, 2nd testing occasion; FFM, fat-free mass; $\dot{V}O_{2peak}$, peak aerobic power; HR_{max}, maximal heart rate. *Significantly different from T1, $p < 0.05$.

Preliminary visit. An initial visit was conducted at least one week prior to the experimental trial in order to measure body height and weight using standard procedures. The same model of bio-electric impedance analyzer (101A, RJL Systems, Clinton Twp., MI) was used on both testing occasions to measure whole body resistance and reactance, with an accuracy of ±0.5% and ±1%, respectively. Fat-free mass (FFM) was then estimated using the equation of Houtkooper et al. (12), with a prediction error of 2.1 kg. At T1, $\dot{V}O_{2peak}$ was determined on a mechanically braked cycle ergometer (Fleisch-Metabo) with measurements of $\dot{V}O_2$ and CO_2 production made continuously using a metabolic cart (Vmax29, SensorMedics). At T2, $\dot{V}O_{2peak}$ was determined on a Monark (Ergomedic 818E, Monark, Sweden) mechanically braked cycle ergometer with continuous collection of expired air analyzed for O_2 (Beckman O_2 analyzer OM-11, Beckman Inc., CA, U.S.A.) and CO_2 (HP47210A capnometer, Hewlett Packard, CA, U.S.A.). Details of the testing procedure are given elsewhere (7,9), and on both occasions the highest 30-sec $\dot{V}O_2$ was taken as the $\dot{V}O_{2peak}$. At T2, we performed biological calibrations of the above equipment by conducting $\dot{V}O_{2peak}$ tests on adults and children of various body sizes, but who did not participate in either study. The variability in $\dot{V}O_{2peak}$ values from the different equipment was less than 5%. A Polar heart rate (HR) monitor was used to record HR throughout each $\dot{V}O_{2peak}$ test.

Experimental visit. During the 24-h prior to their experimental visit, strenuous activity was avoided by all subjects. Upon arrival to the laboratory (~07:30), subjects rested (~20 min) before a pre-exercise blood sample was drawn. To avoid dehydration, fluid intake in the form of flavoured water was provided in a volume equivalent to 40 mL/kg body weight intermittently throughout the session. Thirty min after the first blood sample, subjects started cycling at a power output equivalent to 70% of their predetermined $\dot{V}O_{2peak}$ for two 30-min bouts separated by 5 min of rest. Immediately after exercise, another blood sample was drawn while subjects remained seated on the cycle ergometer. HR was monitored throughout the session. Subjects remained seated in the laboratory for 60 min after exercise, but were allowed to empty their bladder if necessary. At the end of the 60-min recovery period, a final blood sample was drawn.

Complete blood counts and immunophenotyping. Whole blood treated with EDTA was analyzed for total leukocytes, neutrophils, lymphocytes, monocytes, hemoglobin and hematocrit using an automated Coulter counter. EDTA-treated whole blood was used to determine lymphocyte subsets. At T1, 10 μL of monoclonal antibody, directly conjugated with FITC (Anti-CD3) or PE (anti-CD16/CD56), were mixed with 100 μL of whole blood to assess total T cells ($CD3^+$) and NK cells ($CD3^-CD16^+CD56^+$). At T2, 10 μL of monoclonal antibody, directly conjugated with Per-CP (Anti-CD3), FITC (anti-CD16), and PE (anti-CD56), were mixed with 100 μL of whole blood to assess total T

cells ($CD3^+$) and NK cells ($CD3^-CD16^+CD56^+$). All reagents were purchased from Becton Dickinson. Data acquisition was performed with a FACScan flow cytometer and CELLQuest software (Becton Dickinson). WinMDI 2.8 software (Joseph Trotter, The Scripps Research Institute, CA) was used to gate the lymphocyte population using forward-scatter vs. side-scatter characteristics and 10 000 events in this gate were counted per sample. Whole blood cell concentrations were corrected for exercise-induced changes in blood volume according to Dill and Costill (13).

Cytokine analyses. Whole blood treated with EDTA was centrifuged and the plasma was stored at –50°C until analyzed. ELISA kits (R&D Systems, Minneapolis, MN) were used to determine plasma concentrations of IL-6 (Cat. No. HS600B) and TNF-α (Cat. No. HSTA00C) in duplicate. The sensitivities of these kits, as reported by the manufacturer, are 0.039 pg/mL for IL-6 and 0.12 pg/mL for TNF-α. In our hands, the intra- and inter-assay CVs, respectively, are ≤ 5% and 9% for IL-6 and ≤ 4% and 12% for TNF-α. All post-exercise cytokine concentrations were adjusted for changes in plasma volume.

Statistical analyses. Data are presented as means ± SEM, unless otherwise stated. Differences in physical and fitness characteristics and physiological responses to exercise between testing occasions were analyzed by dependent t-tests. All immune data were assessed for a normal distribution, which was confirmed using the Kolmogorov-Smirnov test. Two-way repeated measures ANOVAs (testing occasion × exercise time) were used to analyze immune-related variables. Where appropriate, a Tukey's HSD post-hoc test was used to determine significance among means. STATISTICA for Windows 5.0 (StatSoft, OK) software was used for all ANOVAs; Microsoft Office Excel 2003 (Redmond, WA) software was used for t-tests. To assess the association between acute immune responses to exercise and growth-related variables (e.g., change in height between T1 and T2), Pearson correlations were calculated. In all cases, correlations were performed with GraphPad Prism 4.03 (GraphPad Software, San Diego, CA). For all statistical procedures, a p value ≤ 0.05 was considered statistically significant.

Results

On each occasion, the boys exercised at the same intensity (70.2 ± 2.0 vs. 70.6 ± 1.8 %, p = 0.90) relative to their $\dot{V}O_{2peak}$, as assessed in each year. Consistent with a similar metabolic stress of the exercise tasks on each occasion, there was no difference in the lactate response to exercise (data not shown).

Table 2 reports the cell counts for total leukocytes, neutrophils, lymphocytes and monocytes at T1 and T2. Exercise caused a significant increase in total leukocyte (p < 0.0001), neutrophil (p < 0.0001), lymphocyte (p < 0.001), and monocyte counts (p = 0.02). The neutrophil response tended (p = 0.08) to be greater at T2 vs. T1, whereas the lymphocyte response at T2 vs. T1 achieved statistical significance (p = 0.03); the degree of lymphopenia during recovery from exercise was greater at T2 vs. T1. The neutrophil to lymphocyte ratio, which reflects the overall perturbation to the innate and adaptive arms of the immune system, did not change at T1, but increased with exercise at T2 (p = 0.04).

Table 3 reports the cell proportions and counts for T cells and NK cells at T1 and T2. The proportion of T cells tended to be lower at post-exercise compared with rest (p = 0.056) at T1 and T2. On both occasions, T cell counts increased with exercise (p < 0.001), but T cell counts became suppressed during recovery from exercise at T2 but not T1 (p = 0.004). Exercise increased the proportion of NK cells, with the post-exercise value higher than at rest (p < 0.001), regardless of testing occasion (p = 0.19). Likewise, NK cell counts increased with exercise (p < 0.001) to the same extent on both testing occasions.

Table 4 reports the concentrations of IL-6 and TNF-α at T1 and T2. IL-6 did not change with exercise at T1, but was elevated above resting values during recovery from exercise at T2 (p < 0.05). There was no effect of exercise on TNF-α levels on either occasion (p ≥ 0.20 for all comparisons).

Table 2. *Total leukocyte, neutrophil, lymphocyte, and monocyte counts before, immediately after and 1 h after exercise at T1 and T2*

	T1			T2		
	Pre	Post	Recovery	Pre	Post	Recovery
Leukocytes[1]	5.34 ± 0.49	6.89 ± 0.64	6.11 ± 0.54	5.01 ± 0.29	6.72 ± 0.48	5.87 ± 0.44
Neutrophils[1]	2.31 ± 0.49	3.14 ± 0.50	3.10 ± 0.45	2.03 ± 0.20	3.40 ± 0.34	3.32 ± 0.39
Lymphocytes[1]	1.96 ± 0.15	2.47 ± 0.15†	2.09 ± 0.11	2.10 ± 0.17	2.42 ± 0.19†	1.85 ± 0.10†
Monocytes[1]	0.36 ± 0.05	0.46 ± 0.06	0.36 ± 0.05	0.37 ± 0.03	0.44 ± 0.04	0.34 ± 0.04
Neut:Lymph[1]	1.20 ± 0.23	1.28 ± 0.19	1.50 ± 0.21	0.99 ± 0.12	1.43 ± 0.16†	1.82 ± 0.24†

Values are means ± SEM in 10^9 cells/L. T1, 1st testing occasion; T2, 2nd testing occasion; Pre, before exercise; Post, immediately after exercise; Recovery, 60 min after exercise; Neut:Lymph, neutrophil to lymphocyte ratio. [1]Main effect of session time, p < 0.05. *Significantly different from T1, p < 0.05. †Significantly different from Pre, p < 0.05.

Table 3. *Cell proportions and counts of T cells and NK cells before, immediately after and 1 h after exercise at T1 and T2*

	T1			T2		
	Pre	Post	Recovery	Pre	Post	Recovery
	Cells proportions, %					
T cells	71.6 ± 1.6	70.2 ± 1.8	70.0 ± 1.8	78.2 ± 1.7	72.2 ± 3.4	75.8 ± 3.5
NK cells[1]	8.3 ± 0.7	12.1 ± 1.3	8.9 ± 1.0	7.3 ± 0.9	11.9 ± 2.1	5.0 ± 0.8
	Cell counts, 10^9/L					
T cells[1]	1.41 ± 0.12	1.72 ± 0.11†	1.47 ± 0.09	1.61 ± 0.13*	1.74 ± 0.15	1.39 ± 0.08†
NK cells[1]	0.16 ± 0.02	0.31 ± 0.05	0.19 ± 0.02	0.15 ± 0.02	0.29 ± 0.06	0.09 ± 0.02

Values are means ± SEM. T1, 1st testing occasion; T2, 2nd testing occasion; Pre, before exercise; Post, immediately after exercise; Recovery, 60 min after exercise. [1]Main effect of session time, $p < 0.05$. *Significantly different from T1, $p < 0.05$. †Significantly different from Pre, $p < 0.05$.

Table 4. *IL-6 and TNF-α before, immediately after and 1 h after exercise at T1 and T2*

	T1			T2		
	Pre	Post	Recovery	Pre	Post	Recovery
IL-6[1]	1.5 ± 0.1	2.0 ± 0.1	1.8 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	2.3 ± 0.4†
TNF-α	1.0 ± 0.2	1.5 ± 0.4	1.2 ± 0.2	1.1 ± 0.2	1.5 ± 0.3	1.1 ± 0.2

Values are means ± SEM in pg/mL. T1, 1st testing occasion; T2, 2nd testing occasion; Pre, before exercise; Post, immediately after exercise; Recovery, 60 min after exercise. [1]Main effect of session time, $p < 0.05$. †Significantly different from Pre, $p < 0.05$.

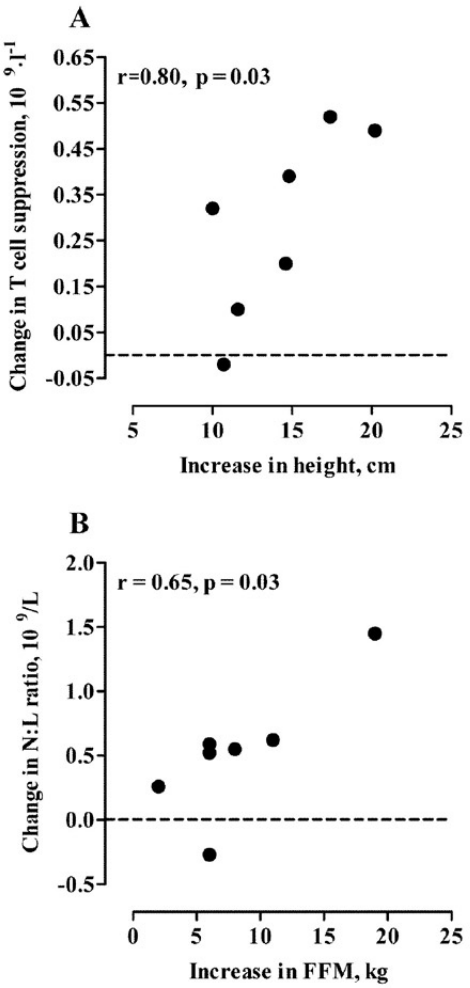

Fig. 1. *Relationship between indices of growth and changes in immune responses to exercise in seven healthy boys.* ***A)*** *change in the degree of T cell suppression during recovery from exercise and increase in height.* ***B)*** *change in the neutrophil:lymphocyte ratio during recovery from exercise and increase in fat-free mass (FFM)*

To further establish the relationship between exercise-induced changes in the immune system and growth, we performed Pearson correlations on those changes that reached statistical significance during the recovery from exercise and changes in body height, FFM, and $\dot{V}O_{2peak}$ expressed in absolute terms (i.e., L/min). In general, boys who experienced a greater degree of T cell suppression following exercise between T1 and T2 also demonstrated a greater increase in body height between testing occasions ($r = 0.80$, $p = 0.03$). This increase in height was also correlated with the increase in the neutrophil to lymphocyte ratio at T2 ($r = 0.85$, $p = 0.02$), but not with the elevated IL-6 response ($r = -0.19$, $p = 0.70$) or degree of lymphocyte suppression ($r = 0.38$, $p = 0.41$). Boys with the greatest increase in FFM also demonstrated the greatest increase in the neutrophil to lymphocyte ratio at T2 ($r = 0.68$, $p = 0.02$). Boys with a greater increase in absolute $\dot{V}O_{2peak}$ between testing occasions did not experience a greater IL-6 ($r = -0.44$, $p = 0.38$) or lymphocyte suppression ($r = 0.35$, $p = 0.44$), but tended to experience greater T cell suppression ($r = 0.71$, $p = 0.07$) and a greater elevation in the neutrophil to lymphocyte ratio ($r = 0.71$, $p = 0.07$) at T2.

Discussion

New observations that skeletal muscle is an active participant in immunological processes highlight the potential role of contractile activity in optimal immune development and function. Given the significant development of the immune system during childhood and the chronic immune activation in some pediatric diseases, the immune effects of exercise may have uni-

que implications to healthy children and those with a chronic disease. Although previous pediatric studies have assessed acute exercise-induced changes in the immune system, none have investigated the influence of growth per se on these responses in a longitudinal manner. This paper provides the first evidence for an effect of growth on the acute immune response to exercise in healthy boys.

By studying the same boys over time, we found that indices of growth were correlated with some aspects of immune activation in response to acute exercise. As expected, the boys in this study demonstrated considerable variation in growth between T1 and T2, as reflected in the change in body height (cm), total fat-free mass (kg), and $\dot{V}O_{2peak}$ (L/min). This variability in growth was associated with the magnitude of change in T cell suppression and the neutrophil to lymphocyte ratio during the recovery period following exercise. $\dot{V}O_{2peak}$ expressed in absolute terms (L/min) was used as a surrogate of growth because increases in absolute $\dot{V}O_{2peak}$ reflect growth of the cardiorespiratory system (heart and lungs) and muscle mass (14), although this variable is influenced by physical activity level, which we did not measure in this study. It should be considered that the boys in this study could have increased their physical activity level through increased sport participation, for example, over the duration of this study, without a necessary change in $\dot{V}O_{2peak}$ values. However, if an improved fitness did occur within this group of boys, we might expect a blunted immune response to exercise based on limited evidence that aerobically fit adults experience an attenuated immune-related response to standardized exercise (15). Unfortunately, there are no similar studies in children, and we actually observed exaggerated responses in these boys during the follow-up testing, suggesting that any potential impact of increased physical activity participation was not enough to outweigh the effects of growth, although more study is needed on this point. Notwithstanding these considerations, our observations are consistent with our previous cross-sectional findings that recovery of neutrophils, T cells, and IL-6 is faster in younger or less mature *versus* older or more mature individuals (7,9). The current findings thus add to the literature by providing similar observations in the same boys followed over time.

Other aspects of the immune response (e.g., NK cells) did not display changes with growth, and this shows the need for a longer follow-up period. In our previous paper investigating puberty and the NK cell response to exercise (8), maturity-related differences in the NK cell response to exercise only became apparent during late puberty (Tanner 3-5). The lack of difference in the NK cell response between testing occasions might therefore be explained by the fact that 5 out of 7 boys in the current study remained in the initial stages of sexual development during this time. This possibility is consistent with the fact that the lactate response to the exercise protocol was similar in these boys on both testing occasions and with other physiological responses to exercise (e.g., sweating), which seem to mature only during late puberty (16). It is also possible that mechanisms driving recruitment of NK cells into the peripheral circulation are different than those responsible for mobilization of neutrophils or recovery of T cells, and therefore potentially influenced differently by processes of growth.

The mechanisms for the growth-related changes observed in the acute immune response to exercise are unclear. Considering that IL-6 is produced in, and released from, contracting skeletal muscle (17) and that IL-6 can mobilize neutrophils into the peripheral circulation (18), the lower IL-6 and neutrophil responses observed at T1 may have been a consequence of lower production and/or release of IL-6 from the active muscle tissue during exercise, which may also influence the distribution of T cells (18). This possibility is supported by the significant positive correlation between an estimate of FFM and the neutrophil to lymphocyte ratio. It remains possible, however, that other growth-related changes (e.g., muscle recruitment, muscle co-activation), which we did not measure could have resulted in a greater muscle stress during the exercise, thus contributing to a greater cytokine release, for example. The lower IL-6 and neutrophil responses to exercise at T1 are indicative of an overall reduced inflammatory response to exercise which may be of clinical relevance.

A blunted inflammatory response to acute exercise in young children would suggest that the young child with a disease could acquire the multitude of health benefits from engaging in regular exercise, without further exacerbating their inflammatory condition. While this seems a reasonable hypothesis, children with cystic fibrosis (19) and children with Type 1 diabetes mellitus (20) actually experience an exaggerated cytokine response to an acute bout of exercise, compared with the response in healthy control children. However, the extent to which growth influences these responses in the child with a chronic disease is not known. Further insight into this question is likely to aid health professionals in developing age-appropriate exercise programs for children with chronic disease. It is, however, acknowledged that the type of exercise imposed in this study is not like the natural physical activity patterns of children or the clinically-based exercise prescriptions that are often delivered for children with various chronic diseases. Our purpose was not to mimic children's play, but to

apply a significant, standardized physiological stress to these boys in order to measure and describe how the immune system reacted to this stress. Indeed, the exercise model we used represents the balance between what children can be motivated to perform on the one hand and sufficient physiological stress to activate the immune system on the other. We are now planning studies designed to understand how the typical patterns of children's play activates the immune system in healthy children and children with a chronic disease.

While our findings raise a number of intriguing questions, this study has limitations. Testing on the two occasions was conducted in different laboratories and therefore different exercise equipment was used. This limitation was addressed by identifying each boy's $\dot{V}O_{2peak}$ on each occasion and setting the exercise stimulus for each occasion at the same intensity relative (70%) to the boy's $\dot{V}O_{2peak}$ in each year. A similar maximal performance was confirmed by identical maximal HR values between T1 and T2. All immune measurements were made using the same Coulter counter, the same FACScan flow cytometer, and using monoclonal antibodies purchased from the same company. Another limitation is the low number of subjects, which limits our ability to generalize the findings. Typically, a small sample size contributes to the possibility of committing a Type II statistical error (i.e., missing an effect when one is present). However, given the fact that we did find statistically significant effects among different aspects of the immune response (e.g., T cells and IL-6) we believe a Type II error is unlikely. Alternatively, we may have committed a Type I statistical error (i.e., accepting an effect when one is not present), which is controlled for by the choice of alpha (p value). Since we set alpha at 0.05 and p values for the observed effects ranged from 0.003 to 0.03 – indicating that the probability of these findings being due to chance was low – we feel a Type I error is also unlikely. Nevertheless, it will be important to confirm these findings in larger trials, with additional testing occasions to better elucidate growth rates. Notwithstanding the aforementioned limitations of this study, our findings are novel, consistent with previous cross-sectional studies, and add to the growing literature on pediatric exercise immunology (1).

In summary, this paper provides evidence that some aspects of the acute immune response to exercise are associated with normal growth in healthy boys. The extent to which these changes might influence, or be influenced by, normal immune development remain unknown. Considering the involvement of immune activation in many chronic diseases, including type 2 diabetes mellitus, among today's youth, the need to further elucidate how exercise interacts with the immune system is critical. Learning how growth impacts exercise-induced immune activation in children with chronic disease will also be important to optimize exercise prescriptions.

Acknowledgments

Data in this study were collected with the assistance of Marta Kubacki, Melanie De Jonge, Raymond Trott, Jae-Hyun Lee, Mazen Hamadeh, and Amy Mark.

Financial support was provided by the Gatorade Sports Science Institute (GSSI) and BD Biosciences.

B.W. Timmons was a recipient of an NSERC Industrial Post-graduate Scholarship sponsored by the GSSI.

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Received: June 06, 2008
Accepted: August 24, 2008
Published: September 03, 2008

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