Cellular Immunity and Inflammatory Mediator Responses to Intense Exercise in Overweight Children and Adolescents

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Background: Obesity modifies inflammatory mediators, but little is known about how obesity modifies the inflammatory responses of exercising children. This study assessed the acute effect of exercise on inflammatory mediators in overweight children.

Methods: Twenty-eight overweight (OW) youth (body mass index > 85%) and 30 normal-weight (NW) controls of the same proportions of age and gender performed 10 2-minute bouts of cycle ergometry exercise above the anaerobic threshold, with 1-minute rest intervals between bouts. Pre- and postexercise blood samples were collected for white blood cell subpopulation and inflammatory cytokines.

Results: Baseline leukocyte populations were higher in OW youth ($p < .05$). Exercise increased most leukocyte subtypes for both groups ($p < .05$). Granulocytes remained elevated 2 hours postexercise ($p < .05$) for both groups, whereas monocytes remained elevated 2 hours postexercise for the OW children. Natural killer (NK) cells dropped below baseline 2 hours postexercise. Exercise significantly decreased CD4 and CD8 cells, which remained depressed 2 hours postexercise in the OW children. Baseline levels of interleukin (IL)-6 were ≈64% higher in OW children ($p < .001$). Exercise increased IL-6 in both groups ($p < .001$), which further increased 2 hours postexercise ($p < .05$). Tumor necrosis factor α, IL-1β, and IL-1 receptor antagonist did not change with exercise.

Conclusions: Elevated baseline leukocyte subtypes and IL-6 levels in OW children suggest that childhood obesity is associated with a chronic low-grade inflammatory state. The acute inflammatory response to intense exercise appears to be similar between NW and OW children for most markers, but the depression of NK, CD4, and CD8 cells 2 hours postexercise suggests that an acute risk of mitogen-induced inflammation may exist in OW children after high-intensity exercise.

Key words: pediatric obesity, proinflammatory cytokines, leukocytes, physical activity, immune responses, cytokines

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The prevalence of childhood obesity has increased considerably worldwide in recent years, despite major efforts to promote weight reduction. Regardless of the mechanisms responsible for the rise in childhood obesity,[1] it is associated with increased risk of hyperlipidemia, hypertension, insulin resistance, diabetes, and arteriosclerosis later in life.[2,3] One of the hypothesized links between obesity and its comorbidities is an inflammatory mechanism.[4] Adipose tissue is one of the sources for a number of hormones and cytokines that are related to the inflammatory process, namely adiponectin, plasminogen activator factor 1, interleukin (IL)-6, and tissue necrosis factor α (TNF-α).[5,6] Several reports in the adult population demonstrated that circulating levels of inflammatory cytokines such as IL-6 and TNF-α, as well as some of the leukocyte subpopulations, are chronically elevated in

obesity and play an important role in the development of obesity-related complications.[4,5,7–9]

It is well established that physical activity has an important role in the prevention and treatment of obesity and coronary artery disease. Kasapis and Thompson suggested that these chronic effects are partially mediated by attenuation of the inflammatory response.[10] However, the acute effects of exercise on the inflammatory response are not always consistent with the chronic effects. Acute exercise in both adults and children results in increases in almost all subtypes of leukocytes and inflammatory cytokines.[11–13] The source of the leukocytosis appears to be the lungs, liver, and lymph nodes,[14] possibly stimulated by the catecholamines, cortisol, or growth hormone (GH).[14,15] The mechanism for the increased cytokines during exercise is of debate but could be related to catecholamines,[15,16] IL-6,[17] and the exercising muscle.[18] However, this exercise response may not be deleterious because it appears to be transient.[19,20] Also, IL-6 has anti-inflammatory and prometabolic effects,[21,22] whereas TNF-α has been consistently shown to have proinflammatory effects.[23] Thus, a marker of the inflammatory status during exercise could be the IL-6 to TNF-α ratio.[24] An exercise-induced increase in the ratio may be regarded as anti-inflammatory, whereas a decrease may be considered proinflammatory.

Obesity modifies the immunologic profiles of children.[13,25] However, little is known about the influence of acute exercise on the immune response in overweight children. Timmons showed that normal-weight boys have greater anti-inflammatory responses during exercise than men.[24] Given the antagonism between inflammation and growth, such a response is logical. However, Timmons did not report on the influence of obesity status on the exercise response. Obesity is associated with decreased exercise-associated sympathetic activity and GH response.[26,27] Since GH and the catecholamine responses during exercise have been linked to leukocyte proliferation,[14–16] the blunted hormonal response of the obese suggests that they may have a blunted immune response to exercise compared with normal-weight individuals. One may expect a reduced immunologic response to exercise in overweight children; however, to the best of our knowledge, the effect of exercise on the immunologic profile in overweight children has not been evaluated.

Therefore, the aim of the present study was to evaluate the inflammatory response to an acute, intense exercise bout in overweight and normal-weight children. Measurement of inflammatory mediators included circulating levels of IL-6, TNF-α, IL-1β, and the anti-inflammatory mediator IL-1 receptor antagonist (IL-1ra) and white blood cell (WBC) subpopulation. We hypothesized that (1) baseline levels of the inflammatory mediators and leukocyte subpopulation would be elevated in overweight children and (2) the response of the leukocytes to acute exercise would be exaggerated, whereas the responses of the inflammatory mediators would be abated in overweight children and adolescents compared with their normal-weight counterparts.

Methods

Subjects

Fifty-eight subjects (age range 8–17 years; 28 females, 30 males) were recruited by the University of California, Irvine (UCI) Pediatric Exercise Research Center to participate in this study (Table 1), and the testing sessions occurred in the General Clinical Research Center (GCRC) at the UCI Medical Center. Twenty-eight subjects were overweight (body

Table 1 Mean (± SEM) Anthropometric Characteristics of Study Participants

	Normal Weight (n = *28*)	*Overweight* (n = *30*)
Age (yr)	12.4 ± 0.5	12.2 ± 0.5
Male/female	15/13	13/17
Height (cm)	153.8 ± 3.4	156.0 ± 2.6
Body weight (kg)	44.1 ± 2.6	71.5 ± 4.4*
BMI (kg/m^2)	18.2 ± 0.5	28.7 ± 1.2*
BMI percentile	46.0 ± 4.5	96.9 ± 0.6*
Body fat (%)	18.9 ± 1.1	37.6 ± 1.6*
Lean body mass (kg)	35.7 ± 2.3	42.1 ± 2.0*
VO_2peak (mL/kg/min)	32.7 ± 1.5	24.9 ± 1.3*
VO_2peak (mL/kg$_{LBM}$/min)	41.7 ± 1.5	40.0 ± 1.8

BMI = body mass index; SEM = standard error of the mean; VO_2peak = peak oxygen consumption.
*$p \leq .001$ normal-weight versus overweight children.

mass index [BMI] > 85%), and 30 subjects had a normal BMI (BMI < 85%). The subjects in the two groups were proportionally matched for gender and age ($\chi^2 \geq 0.61$; $p > .43$). Participants were self-evaluated for pre- and postpubertal status, but Tanner staging to assess pubertal status could not be performed in this study. We realize that puberty impacts the immune response to exercise,[19,20] but it appears that pubertal stage simply modulates the magnitude of the responses (with the exception of TNF-α) rather than changing the directionality of the response.

Subjects with a history of any chronic medical conditions or chronic use of any medications were excluded from participation. The Institutional Review Board at UCI approved the study, and written informed consent, as well as assent, was obtained from all participants and their parents on enrolment.

Anthropometric Measurements

Standard, calibrated scales and stadiometers were used to determine height, body mass, and BMI. Since BMI changes with age during normal growth in children and adolescents, we calculated the BMI percentile for each child using the recently published standards from the Centers for Disease Control and Prevention, National Center for Health Statistics.[28]

Body Composition Assessment by Dual-Energy X-Ray Absorptiometry

Body composition was measured by dual-energy x-ray absorptiometry (DEXA) using the hologic QDR 4500 densitometer (Hologic, Inc., Bedford, MA). Subjects were scanned in light clothing while lying flat on their backs. DEXA scans were performed and analyzed using pediatric software. On the days of each test, the DEXA instrument was calibrated according to the manufacturer's specifications.

Measurement of Fitness

Each subject performed a ramp-type progressive cycle ergometer exercise test (visit 1) to the limit of his or her tolerance. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath by breath, and the anaerobic (lactate) threshold and peak oxygen consumption were calculated using a Sensor Medics (Yorba Linda, CA) metabolic system.

Experimental Exercise Protocol

Exercise consisted of 10 two-minute bouts (visit 2) of constant work-rate cycle ergometry, with a 1-minute rest interval between each bout of exercise. The work rate was individualized for each child and was calculated to be equivalent to the work rate corresponding to 50% of the work rate between the anaerobic threshold and the peak oxygen uptake, as determined noninvasively from the ramp-type test. We have used this protocol in the past to ensure that the exercise input was standardized to physiologic indicators of each subject's exercise capacity.[27] The total duration of the exercise session was 30 minutes (20 minutes of cycling interspersed with 10 minutes of resting).

Blood Sampling and Analysis

All exercise trials occurred during the second visit. The children were instructed not to participate in high-intensity prolonged exercise the day before the visit and not to eat for 3 hours before the exercise trial. These conditions were verified by GCRC staff when they arrived. Blood was sampled from an indwelling catheter placed in the antecubital vein. Separate samples were used for complete blood count, enzyme-linked immunonsorbent assay (ELISA), or flow cytometry assays. The subjects were instructed to sit quietly for 30 minutes prior to baseline draw to ensure that measurable physiologic parameters of stress (eg, heart rate and blood pressure) returned to baseline levels after catheter placement. A baseline blood sample was taken prior to the start of exercise (presampling time point). Subjects then completed the 30-minute exercise session, and a blood sample was obtained immediately following exercise (post-sampling time point). The children then remained in the GCRC area under the supervision of GCRC staff and were allowed to read or watch television for 2 hours after the completion of the exercise. At that time, blood samples were obtained, and the child was released. All serum samples used for ELISAs were aliquoted and stored at −80°C until use. Samples were thawed only once.

Antibodies Used in Flow Cytometry to Detect Surface Markers

On a subset of the sample (16 normal weight and 22 overweight), leukocyte populations were determined using flow cytometry. Surface antigen–specific fluorescent-conjugated monoclonal antibodies CD3 fluorescein isothiocyanate (total T cells), CD4 allophycocyanin (APC) (T helper), CD8 PerCP-Cy5.5 (T cytotoxic), CD19 phycoerythrin (PE) (B cells), and CD16 FITC/56PE; natural killer (NK) cells; and all appropriate isotype controls were purchased from Pharmingen/Becton Dickinson (San Diego, CA).

Surface Antigen Staining

Whole blood was drawn into a vacutainer containing sodium heparin pre- and postexercise. Within 2 hours of blood collection, 100 μL of blood from each sample was added to 12 × 75 mm tubes with specific surface antigen fluorescent-conjugated monoclonal antibodies, mixed well, and incubated in the dark at room temperature for 15 minutes. Two milliliters of 1× FACS lysing solution (Becton Dickinson, San Jose, CA) was added to lyse red blood cells, mixed gently, and incubated for 10 minutes at room temperature in the dark. Two milliliters of 1× wash buffer (Becton Dickinson) was added and centrifuged at 500*g* for 5 minutes. Supernatant was removed without disturbing the cell pellet and were resuspended in 500 μL of 5% paraformaldehyde.

Acquisition and Analysis

Samples were acquired using an FACS Calibur flow cytometer (Becton Dickinson). All surface antibodies were optimized separately for four color fluorescent antibody conjugates. CaliBRITE beads (Becton Dickinson, San Diego, CA) and *FACSComp* (Becton Dickinson) software were used for setting the photomultiplier tube voltages and the fluorescence compensation, as well as checking instrument sensitivity prior to use. A forward scatter threshold was used to acquire 100,000 events for each prepared sample. Flow cytometry data analysis was accomplished using *CellQuest* software (version 3.2.1) (Becton Dickinson). Specific cell populations were delineated using surface markers. Lymphocyte subpopulations were identified by forward and side scatter and separated by antigenic expressions of $CD3^+/CD4^+$ (T-helper lymphocytes), $CD3^+/CD8^+$ (suppressor/cytotoxic T lymphocytes), $CD19^+$ (B lymphocytes), and $CD3^-/CD16^+/CD56^+$NK cells by surface expression.

Inflammatory Cytokines

Serum cytokine levels were determined on the entire sample using the commercially available R&D system Quantikine High Sensitivity ELISA kits (Quantikine, Minneapolis, MN). Intra-assay CV for high sensitivity (HS)-TNF-α was 8.7 to 14.8%, whereas the interassay coefficient of variation (CV) was 16.1 to 22.6%, and the sensitivity was 0.18 pg/mL. HS-IL-6 was found to have an intra-assay CV of 3.8 to 11.1%, interassay CV was 7.1 to 29.5%, and the sensitivity was 0.0094 pg/mL. The IL-1β method had an intra-assay CV of 1.6 to 4.0%, the interassay CV was 5.3 to 9.0%, and the sensitivity was 0.059 pg/mL. Finally, IL-1ra had an intra-assay CV of 3.1 to 6.2%, the inter-assay CV was 4.4 to 6.7%, and the sensitivity was 22 pg/mL.

Statistical Analysis

t-Tests were used to compare the descriptive characteristics between overweight and normal-weight children. The leukocyte and cytokine data were examined for normal distribution using *SAS* procedures (SAS Institute, Cary, NC). The leukocyte data were deemed to have a normal distribution. Thus, two-way repeated measures analysis of variance (ANOVA) was used to assess differences in leukocytes between the normal-weight and overweight groups over the three sampling periods (baseline, immediately postexercise, and 2 hours postexercise). The cytokine data were found not to be normally distributed. These baseline data were first subjected to log transformation and reevaluated for normalcy, and then the two-way ANOVA procedures were used to assess differences between the normal-weight and overweight groups over the three sampling periods. Main effects and interaction effects were evaluated. When a significant ANOVA F value was obtained ($p < .05$), post hoc testing was completed using Tukey honest significant difference procedures. In addition, simple correlations were computed between exercise-induced change in cytokines and body fat or lean body mass. Data are presented as mean ± standard error of the mean.

Results

The anthropometric and fitness characteristics of the study participants are summarized in Table 1. The distribution of ages and the mean age of the two groups were similar. Body weight, BMI, BMI percentiles, body fat, and lean body mass were significantly higher in the overweight children. Fitness, as expressed by peak VO_2 normalized to body weight, was significantly lower in the overweight children. However, when fitness was expressed as peak VO_2 normalized to lean body mass, there were no differences between normal-weight and overweight children. Finally, there were no significant differences between the subset (16 normal weight and 22 overweight) used for the leukocyte analyses and the remainder of the sample ($p > .10$).

Baseline levels, as well as the immediate and 2-hour postexercise responses of WBCs and their subpopulations, are summarized in Table 2. Baseline levels of WBCs, granulocytes, lymphocytes, monocytes, total T cells (CD3), and CD4 cells were significantly higher in the overweight subjects than in the normal-weight subjects. Exercise was associated

Table 2 Mean (± SEM) Resting Leukocytes and Subpopulations of Normal-Weight and Overweight Children: Their Immediate and 2 Hours Postexercise Responses to Intense Exercise

Leukocytes (cells/μL)	*Normal Weight* (n = 16)			*Overweight* (n = 22)		
	Pre	*Immediate Post*	*2 h Post*	*Pre*	*Immediate Post*	*2 h Post*
WBCs*	5,960 ± 355	8,008 ± 387†	7,123 ± 441‡	7,711 ± 480	10,270 ± 593†	9,448 ± 512‡
Granulocytes*	3,325 ± 284	4,380 ± 322†	4,531 ± 323‡	4,523 ± 304	5,967 ± 397†	6,026 ± 408‡
Monocytes*	362 ± 26	529 ± 43†	379 ± 32	472 ± 32	706 ± 56†	570 ± 46‡
Lymphocytes*	2,160 ± 115	3,413 ± 138†	2,212 ± 157	2,718 ± 165	3,493 ± 274†	2,870 ± 150
Total B cells (CD19)	335 ± 40	477 ± 59†	384 ± 50	492 ± 59	546 ± 67	559 ± 50‡
Total T cells* (CD3)	1,438 ± 87	2,075 ± 258†	1,549 ± 116	1,828 ± 110	2,248 ± 166†	1,990 ± 120
CD4 cells*	758 ± 42	656 ± 79	594 ± 51‡	1,031 ± 65	782 ± 69†§	811 ± 62‡
CD8 cells	535 ± 40	504 ± 74	410 ± 40‡	669 ± 59	556 ± 62†	496 ± 44‡
CD4:CD8 ratio†	1.48 ± 0.09	1.40 ± 0.09*	1.52 ± 0.09	1.69 ± 0.13	1.58 ± 0.12†	1.75 ± 0.12
NK cells (CD16/56)	317 ± 46	731 ± 160†	208 ± 28‡	391 ± 30	597 ± 76†	239 ± 22‡
Hematocrit (%)	40.9 ± 1.0	42.1 ± 1.0†	39.9 ± 0.9	39.2 ± 0.8	41.0 ± 0.7†	38.2 ± 0.7

NK = natural killer; SEM = standard error of the mean; WBC = white blood cell.
*$p < .05$: normal-weight versus overweight children.
†$p < .05$: baseline versus immediately postexercise within group.
‡$p < .05$: baseline versus 2 hours postexercise within group.
§$p < .05$: exercise-associated change (immediately postexercise − baseline), normal-weight versus overweight children.

with a significant increase in total WBCs, granulocytes, lymphocytes, monocytes, total T cells, and NK cells for both groups ($p < .05$), but the increases were not significantly different between the groups. The changes in total WBCs and granulocytes were still evident 2 hours after exercise, whereas the changes in NK cells, lymphocytes, and total T cells had abated ($p > .05$; baseline vs 2 hours). In addition, NK cells were found to be below baseline levels 2 hours after exercise for both groups ($p < .05$). For the overweight subjects, monocyte counts were still elevated 2 hours postexercise compared with baseline ($p < .05$) but had returned to baseline levels for the normal-weight subjects ($p > .05$). Exercise was associated with a significant increase in the number of B lymphocytes (CD19) for the normal-weight subjects ($p < .05$), which returned toward baseline 2 hours postexercise ($p > .05$). In contrast, the overweight subjects had a nonsignificant ($p > .05$) increase in B-cell count immediately postexercise, but a significant increase compared with baseline was evident 2 hours postexercise ($p < .05$). Exercise decreased the number of CD4 for both groups, but the decrease was significant only for the overweight group ($p < .05$). CD4 cell counts 2 hours postexercise were significantly lower than baseline for both groups ($p < .05$). CD8 cell responses to exercise and during recovery were similar to those for CD4 cells; they were unchanged with exercise in the normal-weight group but declined in the overweight group ($p < .05$). Further declines were evident in both groups 2 hours postexercise ($p < .05$). The ratio of CD4 to CD8 cells in both groups declined from baseline immediately postexercise ($p < .05$) Two hours postexercise, the ratio had rebounded to slightly above baseline for both groups, but the increase above baseline was not significant ($p > .05$).

Baseline levels and the effects of exercise on circulating cytokines levels are presented in Table 3. Baseline IL-6 levels were significantly higher in the overweight subjects ($p < .001$). Exercise was associated with a significant increase in IL-6 levels in both groups, with further increases over baseline 2 hours postexercise ($p < .001$); however, the changes from baseline were similar for both groups ($p > .05$). There were no significant baseline differences in TNF-α, IL-1β, and IL-1ra between the normal-weight and overweight groups ($p > .17$), and exercise had no significant effect on these cytokines in both groups ($p > .05$). The baseline IL-6 to TNF-α ratio was significantly higher in the overweight group than in the normal-weight group ($p < .05$). Immediate postexercise ratios were slightly, but not significantly, elevated ($p > .05$). The 2-hour postexercise ratios were elevated over baseline ($p < .05$), but there were no differences in the changes in the ratios between the groups ($p > .05$).

Correlations between the subject characteristics and cell counts and cytokines were computed for baseline and the exercise-induced change (immediately postexercise − baseline). As expected, a significant positive correlation were found between BMI percentile or body fat and circulating levels of major cell types, as well as CD3 and CD4 ($r = .48–.34$; $p \leq .05$). Of the cytokines measured, only baseline IL-6 was correlated with BMI percentile ($r = .36$; $p < .05$) and

Table 3 Mean (± SEM) Resting Serum Cytokines of Normal-Weight and Overweight Children: Their Immediate and 2-Hour Postexercise Responses to Intense Exercise

	Normal Weight (n = 28)			*Overweight* (n = 30)		
Cytokines	*Pre*	*Post*	*2 h Post*	*Pre*	*Post*	*2 h Post*
IL-1β (pg/mL)	0.18 ± 0.04	0.21 ± 0.04	0.20 ± 0.03	0.18 ± 0.02	0.17 ± 0.02	0.19 ± 0.02
IL-1ra (pg/mL)	380 ± 52	384 ± 48	398 ± 52	473 ± 61	472 ± 61	498 ± 55
IL-6 (pg/mL)*	1.84 ± 0.37	2.18 ± 0.44†	2.98 ± 0.51‡	3.71 ± 1.02†	4.11 ± 1.04†	4.41 ± 0.93‡
TNF-α (pg/mL)	2.28 ± 0.32	2.33 ± 0.31	2.11 ± 0.44	2.10 ± 0.26	2.13 ± 0.22	2.04 ± 0.26
IL-6:TNF-α ratio*	0.95 ± 0.20	1.02 ± 0.23	1.71 ± 0.39‡	1.98 ± 0.39	2.06 ± 0.34	2.40 ± 0.29‡

IL= interleukin; SEM = standard error of the mean; TNF = tumor necrosis factor.
*$p < .05$: normal-weight versus overweight children.
†$p < .05$: baseline versus immediately postexercise within group.
‡$p < .05$: baseline versus 2 hours postexercise within group.

percent body fat ($r = .34$; $p < .05$). None of the cell or cytokine levels were associated with age ($r < .22$; $p > .17$). The exercise-induced changes in total leukocytes or granulocytes were not related to body composition. However, exercise-induced changes in monocytes and NK cells were directly related to lean body mass ($r = .32–.34$; $p \leq .05$), whereas the changes in CD19 were negatively related to BMI percentile or body fat ($r = -.34–.38$; $p < .04$). Exercise-induced changes in IL-6 were negatively related to BMI percentile or body fat ($r = -.32–.36$; $p \leq .05$).

Discussion

Baseline Differences

One of the striking findings of the present study was that the levels of the inflammatory mediator IL-6, as well as the WBCs and their subpopulations (ie, granulocytes, monocytes, lymphocytes, total T cells, and CD4 cells), were significantly elevated in overweight children and adolescents and were moderately, but significantly, related to their percentage of body fat. Although the overweight children had elevated leukocyte counts, their exercise-induced changes were quite similar to those of normal-weight children. However, their increases in most leukocyte subpopulations persisted for 2 hours after exercise, whereas the responses generally abated in the normal-weight children. Of the cytokines we examined, only IL-6 increased with exercise, and that increase remained 2 hours afterward for both the normal-weight and overweight groups. Although the exercise-induced increase in IL-6 occurred in both normal-weight and overweight children, the magnitude of increase was inversely related to body fat ($r = -.36$), such that there was a tendency for the leaner children to have a greater response than the overweight children.

Our results are consistent with previous reports in the adult population showing that IL-6 levels are increased in obesity and chronic elevation of IL-6 appears to predict the development of insulin resistance and/or non–insulin-dependent diabetes mellitus (NIDDM).[5,7,8] Studies have also found that the circulating levels of the proinflammatory mediator TNF-α are elevated in obese adults and in obese individuals with insulin resistance and NIDDM.[9,29] Moreover, it was shown that chronic elevation of both IL-6 and TNF-α can be associated with endothelial activation in obese adults, providing, therefore, a potential linkage between overweight, low-grade inflammation, and increased cardiovascular risk later in life.[30] In the present study, circulating basal levels of TNF-α were not elevated in our overweight children, suggesting possibly that maturational difference may influence the relationship between adiposity and the proinflammatory response.[19] In support, we computed an multiple regression for baseline TNF-α using body fat and age as the independent variables and found a weak relationship for age. Given that we do not have good pubertal data on our subjects, it is difficult to determine if maturational differences did occur. In contrast to TNF-α, basal circulating IL-6 levels were significantly elevated in our overweight children and adolescents, suggesting that low-grade chronic inflammation may play an important role in the pathogenesis of childhood obesity and the development of its related complications as well.

In addition to the elevated inflammatory cytokine, leukocytes and their subtypes (particularly granulocytes, monocytes, lymphocytes, total T cells, and CD4 cells) were also elevated in our overweight children. These results are consistent with previous results in adults[31,32] and children,[25,33] which demonstrated elevated WBCs and their subsets in the obese population. This is important because an elevated

leukocyte count in adults has been shown to be an independent risk factor for the development of coronary artery disease.[34] The mechanism for this effect is still unknown but may be related to granulocytes and monocytes releasing of TNF-α,[31] free radicals,[35] or procoagulant factors that can exacerbate clot formation and arteriosclerosis.[4,35] Although monocyte counts were higher in the overweight children in the present study, no differences were found in the levels of TNF-α. Nevertheless, our results show that the association of both elevated WBCs and inflammatory cytokines may begin early in overweight children, suggesting that childhood obesity has a potentially intensifying effect on the processes leading to earlier onset of coronary artery disease.

Effect of Exercise

In contrast to our hypothesis, there were no significant differences in the cytokine response to exercise between overweight and normal-weight children. When comparing the metabolic and immunologic effects of exercise in overweight children, whose fitness levels are generally lower than those of normal-weight children, unequal exercise intensities may confound the interpretation of the results. Thus, to ensure that the exercise input in the present study was standardized to each of the participant's exercise capacity, the workload was calculated to be equivalent to 50% of the work rate between the anaerobic threshold and the peak oxygen uptake. Our results show that when exercise was standardized, a similar increase in circulating IL-6 was seen in both groups, and exercise had no significant effect on the other cytokines. The major source for the exercise-related IL-6 increase is the skeletal muscle.[18] Research has also shown that a complex intramuscular signaling process stimulates IL-6 release from the contracting muscle and that this release is associated, although not always, with muscle damage.[22,36] The increase in IL-6 following exercise also depends on exercise duration, intensity, and the muscle mass recruited.[22] Apart from its role in muscle repair, IL-6 appears to assist in glucose homeostasis and lipolysis and to have a regulatory effect on the exercise-related changes of the immune system and the GH–insulin-like growth factor I axis.[37,38] IL-6 released from the muscle during exercise could potentially increase lipolysis in adipose tissue and gluconeogenesis in the liver.[39] Concomitantly, IL-6 inhibits low-level production of TNF-α.[22,39] The elevation of IL-6 may then be beneficial for metabolic and anti-inflammatory reasons. Consistent with this proposed mechanism, we found no significant elevation of TNF-α after exercise, although our IL-6 increased significantly immediately postexercise and remained above rest 2 hours after exercise. In addition, the IL-6 to TNF-α ratio increased marginally with exercise and increased more so in recovery, particularly in the normal-weight group compared with the overweight group (≈80% vs 21%, respectively). The greater increase in the normal-weight children could be related their ability to better regulate glucose and lipid metabolism than overweight children.[27]

Interestingly, exercise had no effect on the other cytokines in both groups. This is in contrast to our previous observation of an increase in IL-1ra and IL-1β following an intense 1-hour wrestling practice in adolescent males.[40] The lack of exercise-associated increase of these inflammatory markers in the present study may be a result of the relatively short exercise duration. Previous studies suggested that in adults there might be a delay of up to several hours between IL-6 and IL-1 or IL-1ra stimulation.[21] Our 2-hour recovery data, however, suggest that if there is a delayed effect of IL-6 on IL-1, then the delay is longer than 2 hours. Timmons and colleagues also suggested that there are differences in immune responses between pubertal stages.[19] Although absolute concentrations of the immune cells and cytokine appear to differ between pubertal stages, in general, the responses are in the same direction, increasing or decreasing in all stages of maturation.[19,20] Interestingly, TNF-α appears to follow a different pattern, being unchanged immediately postexercise in prepubertal children but increasing in adolescents with late-stage puberty. One potential reason why we saw no change in TNF-α was that that average data across developmental stage groups may offset any specific change between the pubertal groups. Once again, since we do not have good pubertal development data on our subjects, this is speculation. This is a limitation of our study.

The effect of exercise on WBCs and subpopulations was studied extensively. The magnitude of the exercise-associated WBC response is also influenced by the type, duration, and intensity of exercise; by the timing of the sample collection; and by the fitness levels of the participants. However, in general, immediately after exercise there is a similar increase in both adults and children in the number of WBCs owing to an increase in granulocytes, monocytes, and lymphocytes.[11,12,41] The mechanism for this increase is not completely clear but probably involves the release of WBCs from marginal pools (liver, lungs, lymph nodes) and is mediated by the secretion of counter-regulatory hormones such as catecholamines, cortisol, GH, and glucagon[11,15,16,42] or possibly to elevated IL-6.[17] To the best of our knowledge, the effect of

exercise on WBC subpopulations in overweight children was never studied. Our results suggest that despite the significant baseline differences in WBCs, granulocytes, monocytes, and lymphocytes, the responses of these immune cells to a standardized exercise input are generally similar between normal-weight and overweight children. The exception appears to be the monocytes and B cells (CD19), which had not returned to baseline 2 hours post-exercise in the overweight youth but returned to baseline in the normal-weight group. The reason for this response is obscure at this time.

The exercise-induced increase in total lymphocytes in the present study was due to an increase in both total T (CD3) and B lymphocytes (CD19), although the helper (CD4) and cytotoxic/suppressor (CD8) declined. This response is consistent with previous reports,[12,14,20,41] although other research found that the exercise-induced increase in lymphocytes was mainly due to an increase in T cells.[43] The increase in both T and, in particular, B cells was less in the overweight children; however, this difference was not statistically significant. NK cells were also elevated immediately after exercise, more so in the normal-weight than in the overweight children (131% vs 53%, respectively). Given that catecholamines stimulate NK cells[15] and there is the suggestion that overweight children have a blunted catecholamine response to exercise,[27] the diminished response of the overweight children may have been related to catecholamines.

The reported effects of exercise on T-lymphocyte subpopulations is not consistent, and CD4 cells were reported to be elevated, unchanged, or decreased after a single exercise bout.[11,19,41] However, it is generally accepted that even if CD4 cells increase during exercise in both adults and children, the increase in CD8 cells is greater and the ratio of CD4 to CD8 cells is decreased.[11,12,19,41] Interestingly, the intense exercise in the present study was associated with decreases in CD4 cells that were greater in the overweight than in the normal-weight group (−24% vs −13%, respectively). Furthermore, the decline was sustained 2 hours postexercise. The CD8 cell count also marginally declined with exercise and continued to decline further 2 hours postexercise. Similar to our results, Boas and colleagues noted that both CD4 and CD8 cells declined 1 hour after exercise.[20] The greater postexercise decline in CD4 and CD8 cells in the overweight youth, coupled with the reduction in circulating NK cells, suggests that fewer cells can respond to mitogens. Thus, acute high-intensity exercise appears to compromise cellular immunity in overweight children compared with normal-weight children.[11,43] Whether the initiation of exercise interventions for overweight children increases the risk of infections is still unknown. Therefore, the effect of exercise, exercise training, improved fitness, and weight reduction on cellular immunity in overweight children still needs to be investigated.

We found that the CD4 to CD8 ratio immediately postexercise was reduced from baseline in both groups and rebounded 2 hours postexercise to slightly above baseline in both groups. Others have shown in adults that the ratio of CD4 to CD8 cells is reduced during and immediately postexercise and rebounded or actually increased over baseline 120 minutes postexercise.[41,44] The meaningfulness of this response is obscure, but the fact that both CD4 and CD8 counts declined suggests that there is an exercise-induced depression of these subtypes of T cells.

In conclusion, our results suggest that although leukocytes, their subpopulations, and IL-6 are elevated at baseline in overweight children, generally, their response to exercise was not different compared with normal-weight children. Overweight children do exhibit subtle exercise-induced changes in the lymphocyte subpopulation (mainly CD4, CD8, and NK cells), suggestive of an acute, probably transient, reduction in immunity in overweight youth. In addition, our results show that short, intense exercise elevates IL-6 without a similar increase in TNF-α, which may have a beneficial effect on metabolism and health status. The duration of these changes and their clinical significance are yet to be determined.

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References

1. Dietz WH. Overweight in childhood and adolescence. N Engl J Med 2004;350:855–7.
2. Berenson GS, Srinivasan SR, Bao W, et al. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 1998;338:1650–6.
3. Mossberg HO. 40-year follow-up of overweight children. Lancet 1989;2:491–3.
4. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. Int J Obes Relat Metab Disord 2003;27 Suppl 3:S25–8.
5. Vozarova B, Weyer C, Hanson K, et al. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 2001;9:414–7.
6. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. Trends Endocrinol Metab 2000;11:327–32.

7. Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286:327–34.
8. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation 2003;107:391–7.
9. Hotamisligil GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. J Intern Med 1999;245: 621–5.
10. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol 2005;45:1563–9.
11. Rowbottom DG, Green KJ. Acute exercise effects on the immune system. Med Sci Sports Exerc 2000;32(7 Suppl): S396–405.
12. Eliakim A, Wolach B, Kodesh E, et al. Cellular and humoral immune response to exercise among gymnasts and untrained girls. Int J Sports Med 1997;18:208–12.
13. Nieman DC, Henson DA, Fagoaga OR, et al. Influence of skinfold sum and peak VO(2) on immune function in children. Int J Obes Relat Metab Disord 2002;26:822–9.
14. Horn PL, Leeman K, Pyne DB, Gore CJ. Expression of CD94 and 56(bright) on natural killer lymphocytes—the influence of exercise. Int J Sports Med 2002;23:595–9.
15. Kapell M, Tvede N, Galbo H, et al. Evidence that the effect of exercise on NK cell activity is mediated by epinephrine. J Appl Physiol 1991;70:2530–5.
16. Kapell M, Poulsen D, Galbo H, Pedersen BK. Effect of elevated plasma noradrenaline concentration on the immune system of humans. Eur J Appl Physiol 1998;79: 93–8.
17. Suwa T, Hogg JC, English D, Van Eeden SF. Interleukin-6 indiuces demargination of intravascular neutrophils and shortens their transit in marrow. Am J Physiol Heart Circ Physiol 2000;279:H2954–60.
18. Steensberg A, Van Hall G, Osada T, et al. Production of interleukin-6 in contracting skeletal muscle can account for the exercise-induced increase in plasma interleukin-6. J Physiol 2000;529(Pt 1):237–42.
19. Timmons BW, Tarnopolsky MA, Snider DP, Bar-Or O. Immunological changes in response to exercise: influence of age, puberty and gender. Med Sci Sports Exerc 2006;38: 293–304.
20. Boas SR, Joswiak ML, Nixon PA, et al. Effects of anaerobic exercise on the immune system in eight- to seventeen-year-old trained and untrained boys. J Pediatr 1996;129: 846–55.
21. Ostrowski K, Rohde T, Asp S, et al. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 1999;515(Pt 1):287–91.
22. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 2002;16:1335–47.
23. Pedersen BK, Ostrowski K, Rohde T, Bruunsqaard H. The cytokine response to strenuous exercise. Can J Physiol Pharmacol 1998;76:505–11.
24. Timmons BW. Immune response to exercise in children: a brief review. In: Farpour N, Stussi C, Kriemler S, editors. Children and Exercise XXIII: Proceedings of the European Group of Pediatrics Work Physiology, 2005 Sept 21-25; Gwatt, Switzerland. p. 37–47.
25. Zaldivar F, McMurray RG, Nemet D, et al. Body fat and circulating leukocytes in children. Int J Obes 2006;30:906–11.
26. Kanaley JA, Weatherup-Dentes MM, Jaynes EB, Hartman ML. Obesity attenuates the growth hormone response to exercise. J Clin Endocrinol Metab 1999;84: 3156–61.
27. Eliakim A, Nemet D, Zaldivar F, et al. Reduced exercise-associated response of the GH-IGF-I axis and catecholamines in obese children and adolescents. J Appl Physiol 2006;100:1630–7.
28. Kuczmarski RJ, Ogden CL, Guo SS, et al. 2000 CDC growth charts for the United States: methods and development. Vital Health Stat 11 2002;(246):1–190.
29. Borst SE. The role of TNF-alpha in insulin resistance. Endocrine 2004;23:177–82.
30. Chan JC, Cheung JC, Stehouwer CD, et al. The central roles of obesity-associated dyslipidaemia, endothelial activation and cytokines in the metabolic syndrome—an analysis by structural equation modelling. Int J Obes Relat Metab Disord 2002;26:994–1008.
31. Kullo IJ, Hensrud DD, Allison TG. Comparison of numbers of circulating blood monocytes in men grouped by body mass index (<25, 25 to <30, > or =30). Am J Cardiol 2002;89:1441–3.
32. Nieman DC, Henson DA, Nehlsen-Cannarella SL, et al. Influence of obesity on immune function. J Am Diet Assoc 1999;99:294–9.
33. Visser M, Bouter LM, McQuillan GM, et al. Low-grade systemic inflammation in overweight children. Pediatrics 2001;107:E13.
34. Grimm RH, Jr, Neaton JD, Ludwig W. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. JAMA 1985;254:1932–7.
35. Avogaro A, deKreutzenberg SV. Mechanisms of endothelial dysfunction in obesity. Clin Chem Acta 2005;360:9–26.
36. Pedersen BK, Steensberg A, Fischer C, et al. Exercise and cytokines with particular focus on muscle-derived IL-6. Exerc Immunol Rev 2001;7:18–31.
37. Pedersen M, Bruunsgaard H, Weis N, et al. Circulating levels of TNF-alpha and IL-6-relation to truncal fat mass and muscle mass in healthy elderly individuals and in patients with type-2 diabetes. Mech Ageing Dev 2003;124: 495–502.
38. Cooper DM, Nemet D, Galassetti P. Exercise, stress, and inflammation in the growing child: from the bench to the playground. Curr Opin Pediatr 2004;16:286–92.
39. Pedersen BK, Steensberg A, Keller P, et al. Muscle-derived interleukin-6: lypolytic, anti-inflammatory and immune regulatory effects. Pflugers Arch 2003;446:9–16.
40. Nemet D, Oh Y, Kim HS, et al. Effect of intense exercise on inflammatory cytokines and growth mediators in adolescent boys. Pediatr 2002;110:681–9.

41. Shek PN, Sabiston BH, Buguet A, Radomski MW. Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. Int J Sports Med 1995;16:466–74.
42. McCarthy DA, Dale MM. The leucocytosis of exercise. A review and model. Sports Med 1988;6:333–63.
43. Christensen RD, Hill HR. Exercise-induced changes in the blood concentration of leukocyte populations in teenage athletes. Am J Pediatr Hematol Oncol 1987;9:140–2.
44. Shinkai S, Shore S, Shek PN, Shephard RJ. Acute exercise and immune function: relationship between lymphocyte activity and changes in subset counts. Int J Sports Med 1992; 13:452–61.