

DR SIMON B COOPER (Orcid ID : 0000-0001-5219-5020)

DR CAROLINE SUNDERLAND (Orcid ID : 0000-0001-7484-1345)

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Cytokine, glycemic and insulinemic responses to an acute bout of games-based activity in adolescents

Karah J. Dring¹, Simon B. Cooper¹, John G. Morris¹, Caroline Sunderland¹,
Gemma A. Foulds², A. Graham Pockley² & Mary E. Nevill¹

1: Department of Sport Science, Sport Health and Performance Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK

2: John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK

Corresponding Author: Dr Simon Cooper

Department of Sport Science,
School of Science and Technology,
Nottingham Trent University,
Clifton Campus

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Nottingham

NG11 8NS

Email: simon.cooper@ntu.ac.uk

Telephone: +0044 (0) 1158 488 058

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Abstract

An acute bout of endurance exercise in adults stimulates a same-day anti-inflammatory response which may affect low-grade chronic inflammation and insulin resistance and benefit cardio-metabolic health. The anti-inflammatory responses to intermittent games-based exercise and to exercise in young people beyond 2-h post-exercise are unknown. Thus, the purpose of the present study is to examine the anti-inflammatory, glycemic and insulinemic response to games-based activity in adolescents. Following ethical approval and familiarization, 39 adolescents (12.3 ± 0.7 y) completed an exercise (E) and rested (R) trial in a counterbalanced, randomized crossover design. Following a standardized breakfast, participants completed 1-h games-based activity. Capillary blood samples were taken at baseline, immediately and 1-h post-exercise and 30, 60 and 120-min following a standardized lunch. A final blood sample was taken the next morning. Data were analyzed using repeated measures ANOVA. IL-6 concentration was higher on day one of the exercise trial (E: 3.4 ± 0.4 , R: 2.7 ± 0.4 pg·mL⁻¹; $p=0.006$), as was the anti-inflammatory IL-6:TNF- α ratio (E: 5.53 ± 0.93 , R: 3.75 ± 0.45 ; $p=0.027$). Levels of the anti-inflammatory cytokine IL-10 increased on day two of the exercise trial (E: 2.11 ± 0.23 , R: 1.66 ± 0.16 pg·mL⁻¹; $p=0.032$). Insulin sensitivity was enhanced on the exercise trial with a reduction in iAUC following the standardized lunch (E: 2310 ± 834 , R: 3122 ± 1443 mUL⁻¹·x120 min; $p<0.001$). Games-based activity stimulated an anti-inflammatory response up to 24 h post-exercise and improved insulin sensitivity in response to a standardized meal in healthy adolescents. These novel findings suggest that games-based activity is an

ecologically valid mode of exercise to elicit beneficial effects on cardio-metabolic risk factors in young people.

Key Words: Games-based activity; Adolescents; Inflammation; Glycemic; Insulinemic; cardio-metabolic health

1. Introduction

Low-grade chronic inflammation is involved in the pathogenesis of several chronic diseases, including cardiovascular disease and type 2 diabetes⁽¹⁻⁴⁾. Although such conditions typically present during adulthood, the development of cardio-metabolic risk factors for these diseases originate during childhood, with low-grade chronic inflammation, atherosclerotic plaques, and insulin resistance observed in pubertal children⁽⁵⁻⁷⁾. Low-grade chronic inflammation is defined as a 2-3 fold increase in systemic concentrations of pro-inflammatory cytokines, including IL-6, IL-1 β , TNF- α and the acute phase protein C-Reactive Protein (CRP)⁽³⁾. The inflammatory response that follows an acute bout of exercise (reflected by increased IL-6 levels that are produced by the contraction of skeletal muscle and the subsequent stimulation of a systemic increase in the concentration of anti-inflammatory cytokines) is a mechanism that stimulates muscle regeneration and reduces low-grade chronic inflammation and insulin resistance in adults^(1,3), with *in vitro* studies reporting that IL-10 inhibits the synthesis of chronic pro-inflammatory mediators TNF- α and IL-1 β ⁽³⁾. Furthermore, in the plantaris muscle of mice increased levels of IL-6 post-exercise increases the expression of glucose transporter-4, which increases glucose uptake⁽⁸⁾. These findings suggest that a rise in IL-6 post-exercise triggers an anti-inflammatory response and enhances insulin sensitivity.

Despite the presence of low-grade chronic inflammation and insulin resistance in young people⁽⁵⁾, information on the inflammatory response to an acute bout of exercise in adolescents is limited. Previous studies examining the release of inflammatory mediators, IL-6 and TNF- α in

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response to an acute bout of endurance exercise have reported increases^(9,10), decreases⁽¹¹⁾, or no changes^(12, 13). However, young people do not typically participate in endurance exercise, with intermittent activity being both enjoyable (important for long-term adherence)⁽¹⁷⁾ and replicative of their physical activity patterns⁽¹⁸⁾. Eccentric exercise is an alternative mode of physical activity that elicits an increase in the release of pro-inflammatory cytokines (IL-6 and IL-1 β) in response to the muscle damage incurred^(14,15,16). Furthermore, games-based activities, such as basketball, are intermittent in nature and replicate the activity patterns of young people whilst also having previously been reported to induce muscle damage and an inflammatory response (increased IL-6 and IL-1 β) in adults⁽¹⁹⁾. Therefore, it is important to determine whether intermittent games-based activity elicits a protective anti-inflammatory response which benefits cardio-metabolic health in young people^(1,3).

Only two studies in young people have assessed the effect of intermittent exercise on inflammatory mediators^(10,20). McMurray et al.⁽²⁰⁾ reported that 10 x 2 min bouts of high intensity intermittent cycling in pubertal adolescents increased the anti-inflammatory IL-6:TNF- α ratio by 80% 2-h post-exercise. Whereas, Scheet et al.⁽¹⁰⁾ observed that 90-min of soccer in pre-pubertal children increased IL-6 (125%) and TNF- α (18%) 2-h post-exercise, yet did not affect anti-inflammatory mediator IL-10. The differences in exercise intensity and duration might explain these discrepant findings. Importantly, these studies have only assessed IL-6, TNF- α and IL-10 for 2-h post-exercise, despite *in vitro* studies reporting that inflammatory mediators (IL-10 and CRP) remain elevated for up to 24-h post-exercise^(1,3). To fully examine the inflammatory response to exercise a complete range of pro-inflammatory (IL-1 β , TNF- α and CRP) and anti-inflammatory (IL-6, IL-10 and IL-6:TNF- α) cytokines should be measured up to 24-h post-exercise.

Another important aspect of cardio-metabolic health is the glycaemic and insulinaemic response following a meal. It has previously been reported that a 45-min bout of aerobic exercise residually enhanced insulin sensitivity in adolescents for up to 17-h, as demonstrated by reduced postprandial glycaemic and insulinaemic responses following a high fat meal⁽²¹⁾. Furthermore, high intensity intermittent and moderate intensity cycling in adolescent boys reduced the glycaemic and insulinaemic responses by 24-29% following an oral glucose tolerance test, in comparison to a rested

control trial⁽²²⁾. However, the impact of exercise on the glycemic and insulinemic response to an ecologically valid meal remains unknown and no studies have examined the association between the inflammatory and glycemic/insulinemic responses post-exercise, despite the anti-inflammatory response being associated with insulin sensitivity⁽²³⁾.

Therefore, the present study investigated if an acute bout of intermittent games-based activity stimulates an anti-inflammatory and metabolic response in young people.

2. Methodology

2.1 Study Design

The institutional ethical advisory committee approved all procedures (approval number SST-417). Participants were recruited from secondary schools and written informed parental consent alongside child assent obtained. The following exclusion criteria were applied (a) a medical history of chronic diseases including, but not limited, to cardiovascular disease, diabetes and hypertension, (b) prescription of regular medication that may affect participation in the study and (c) any factor that would cause an inability to complete the exercise components of the study. A parent/guardian completed a health screen questionnaire on behalf of the participant to ensure there were no medical conditions affecting participation in the study.

The familiarization session preceded the main experimental trial by 7-d. During familiarization, the experimental protocol was explained to participants and they were familiarized with the methods included in the main trials. Participants completed the multi-stage fitness test (MSFT) to predict peak oxygen consumption⁽²⁴⁾. Predicted peak oxygen uptake was calculated using an adolescent specific calculation⁽²⁵⁾ and mean (\pm SD) peak oxygen consumption was $50.3 \pm 4.4 \text{ ml kg}^{-1} \text{ min}^{-1}$.

2.2 Participant Characteristics

Forty-one schoolchildren aged 11-13 y were recruited to the present study. Two participants did not complete the study, as a consequence of which 39 participants (20 males, 19 females) were included in the analysis. During familiarization, body mass (Seca 770 digital scale, Hamburg, Germany), height and sitting height (Leicester Height Measure, Seca, Hamburg, Germany) were measured to determine age at peak height velocity⁽²⁶⁾. Waist circumference was measured at the narrowest point of the torso using a standard tape measure. Four skinfold sites (tricep, subscapular, supraspinale and front thigh) were measured by trained kinanthropometrists in line with ISAK guidelines⁽²⁷⁾. The sum of skinfolds was the preferred measure of body composition, as estimated body fat from skinfolds is associated with large random and systematic error⁽²⁸⁾. The participants' anthropometric characteristics were (mean \pm SD): age 12.3 ± 0.7 y; height 155.7 ± 7.5 cm; body mass: 46.0 ± 9.5 kg; predicted years from peak height velocity: -0.37 ± 1.1 y; waist circumference: 65.4 ± 5.7 cm and sum of skinfolds: 44.9 ± 19.5 mm.

2.3 Main Trials

The study employed a randomized, counterbalanced, crossover design consisting of an exercise and rested (control) trial, separated by 7-d. As one of the main trials incorporated an exercise session, participants were not blinded to the trial condition. The experimental protocol is displayed in figure 1.

Participants recorded their dietary intake for 24-h preceding the first main trial and during evening one of the study; recorded diets were replicated for the subsequent main trial. Participants arrived at school fasted from 9 pm the previous evening on day one and day two of the main trials. Participants were asked to refrain from physical activity 24-h prior to and during all main trials. A telephone call was made to parents/guardians prior to each main trial to ensure compliance.

On arrival at school (8.30 am) participants were fitted with a heart rate monitor (Team Sports System, Firstbeat Technologies Ltd, Finland) which was worn during main trials. A standardized breakfast (cornflakes, milk, toast, margarine) and lunch (chicken sandwich (cheese alternative for vegetarians), baked crisps, apple) were provided, each containing 1.5 g carbohydrate per kg body mass, as used in previous research⁽²⁴⁾. Participants had 15-min to consume each meal.

2.4 Capillary Blood Sample

Capillary blood samples were taken at baseline, immediately post-exercise and 60-min post-exercise (Fig 1). Additional blood samples were taken 30-min, 60-min (2-h post-exercise) and 120-min (3-h post exercise) following a standardized lunch. A final fasted capillary blood sample was taken the following morning (day two) using previously described methods⁽²⁹⁾.

Blood glucose and plasma insulin concentrations were measured in duplicate using commercially available kits (glucose: GOD/PAP method, GL364, Randox, Ireland; insulin: ELISA, Mercodia Ltd, Sweden). The intra-assay coefficient of variation (CV) of the assays, based on eight repeat measurements was 2.3% for blood glucose and 3.2% for plasma insulin concentration. Blood glucose and plasma insulin iAUC following the standardized lunch were calculated^(30,31).

Cytokine (IL-1 β , IL-6, TNF- α and IL-10) concentrations were determined using a flow cytometry-based multiplex bead approach (AimPlexTM multiplex assay, YSL, Pomona, USA) using a Beckman Coulter GalliosTM flow cytometer and KaluzaTM data acquisition and analysis software (Beckman Coulter, London, UK). CRP concentrations were determined using the same approach, but in a separate assay due to a greater sample dilution being required. In line with manufacturer guidelines a minimum of 50 events per bead population were acquired. The intra-assay CV based on eight repeat measurements was in line with the CV of ~16% reported in the literature for these analytes⁽³²⁾ (IL-6: 15.9%; IL-10: 13.2%; TNF- α : 14.7%, IL-1 β : 17.4%; CRP: 10.2%).

A comparison between cytokine concentrations determined from capillary (C) and venous (V) blood was performed on 20 samples obtained from healthy adults. There was no difference for any analytes (IL-6: C 1.41 ± 1.17 , V 1.41 ± 1.49 pg mL^{-1} , $p=0.999$; IL-10: C 0.69 ± 0.30 , V 0.66 ± 0.29 pg mL^{-1} , $p=0.750$; TNF- α : C 1.20 ± 1.79 , V 1.52 ± 2.26 pg mL^{-1} , $p=0.234$; IL-1 β : C 2.66 ± 2.19 , V 1.41 ± 1.43 pg mL^{-1} , $p=0.187$; CRP C 0.32 ± 0.61 , V 0.31 ± 0.56 mg L^{-1} , $p=0.767$). The 95% limits of agreement were calculated⁽³³⁾: IL-6 -0.923 , $+0.823$ pg mL^{-1} ; IL-10 -0.969 , $+0.929$ pg mL^{-1} ; TNF- α -1.208 , $+1.458$ pg mL^{-1} ; IL-1 β -2.102 , $+0.868$ pg mL^{-1} ; and CRP -0.471 , $+0.434$ mg L^{-1} .

2.5 Exercise Protocol

The exercise trial incorporated a 60-min basketball session, which commenced 1-h after breakfast on day 1 (Fig 1). An experienced basketball coach delivered the sessions to groups of 10 participants in a school sports hall. The basketball session consisted of a warm-up (5-min of jogging on the court followed by dynamic stretches), skill-based drills (30-min passing, dribbling and shooting drills) and small-sided games (25 min) to finish. Immediately post-exercise, participants returned to the classroom and rested quietly for the remainder of the day. During the control trial, participants rested in the classroom throughout the day.

2.6 Statistical Analysis

A power calculation was performed using GPower 3.1.9.2 based on the previously reported effects of exercise on IL-6 in young people⁽³⁴⁾ and an estimated effect size of 0.3 (two-tailed significance), the power analysis yielded a required total sample size of 38.

Data were assessed for normality using the Shapiro-Wilk test and all dependent variables were normally distributed (all $p > 0.05$). Inflammatory cytokine, blood glucose and plasma insulin concentration data were analyzed in SPSS (Version 24, SPSS Inc, Chicago, USA) via three-way (trial*time*sex) Analysis of Variance (ANOVA) with repeated measures for trial and time. Separate

ANOVAs were conducted for day one and between resting measures on day one and day two. Where significant interactions were observed, post-hoc pairwise comparisons were performed using a Bonferonni correction. Blood glucose iAUC, plasma insulin iAUC and heart rate were compared using a paired samples t-test. Where significant effects existed for main effect of trial and trial by time interactions, effect sizes were calculated as Cohen's d. For all analysis significance was accepted as $p < 0.05$ and data are presented as mean \pm S.E.M..

3. Results

3.1 Heart Rate

Mean heart rate during the basketball was 157 ± 11 beats min^{-1} ($76 \pm 5\%$ of maximum heart rate achieved during the MSFT; HRmax) and maximum recorded mean heart rate was 197 ± 9 beats min^{-1} ($96 \pm 4\%$ HRmax). Consequentially, mean heart rate was higher during the exercise trial than the control trial (exercise: 104 ± 14 beats min^{-1} , $51 \pm 7\%$ HRmax; resting: 90 ± 10 beats min^{-1} , $44 \pm 5\%$ HRmax; $t_{(38)} = -7.2$, $p < 0.001$).

3.2 Inflammatory and Metabolic Responses

At baseline, there was no difference in inflammatory cytokine, blood glucose or plasma insulin concentration between the exercise and control trials (all $p > 0.05$). When considering the effect of sex, there were no differences in inflammatory cytokine concentrations between the boys and girls at baseline or post-exercise (all $p > 0.05$).

3.2.1 Inflammatory Variables

The response of the inflammatory variables can be found in table 1. Overall, IL-6 concentration was higher during day one of the exercise trial when compared with the control trial (exercise: 3.4 ± 0.4 , resting: 2.7 ± 0.4 pg mL^{-1} ; main effect trial, $F_{(1,35)}=8.7$, $p=0.006$; $d=0.3$). Furthermore, IL-6 concentration increased across time during both trials on day one (main effect time, $F_{(1,35)}=11.3$, $p<0.001$). No trial*time interaction effect was observed for IL-6 concentration on day one ($p=0.604$), nor were there any differences 24-h post-exercise (main effect trial, $p=0.422$; trial*time interaction, $p=0.852$).

Overall, IL-10 concentration did not differ between the exercise and control trial on day one (main effect trial, $p=0.569$); nor were there changes across time (main effect time, $p=0.151$), nor a trial*time interaction ($p=0.161$). However, when considering the response of IL-10 24-h post-exercise, there was a trial*time interaction ($F_{(1,38)}=5.9$, $p=0.020$; $d=0.4$) whereby IL-10 concentration was higher on day two of the exercise trial than the control trial ($t_{(34)}=-2.2$, $p=0.032$).

Overall, there were no differences in TNF- α concentration between the exercise and control trials on day one (main effect trial, $p=0.400$), nor were there changes across time (main effect time, $p=0.197$). There was a trial*time interaction for TNF- α concentration during day one ($F_{(4,108)}=2.5$, $p=0.048$), whereby 2-h post-exercise there was a tendency for TNF- α concentration to increase during the exercise trial in contrast to the control trial ($t_{(27)}=2.3$, $p=0.076$, $d=0.2$). When considering the response of TNF- α 24-h post-exercise, there was no difference between trials (main effect trial, $p=0.680$; trial*time interaction, $p=0.083$).

Overall, IL-1 β concentration did not differ between the exercise and control trials on day one (main effect trial, $p=0.220$), nor were there any differences in IL-1 β concentration across time (main effect time, $p=0.647$). Furthermore, the pattern of change in IL-1 β concentration was similar between trials during day one (trial*time interaction, $p=0.952$). When considering the response of IL-1 β 24-h post-exercise, there was no difference between trials (main effect trial, $p=0.068$; trial*time interaction, $p=0.621$).

Overall, CRP concentration did not differ between the exercise and control trials on day one (main effect trial, $p=0.967$), nor were there any differences in CRP concentration across time (main effect time, $p=0.190$). The pattern of change in CRP concentration was similar between trials during day one (trial*time interaction, $p=0.593$). When considering the response of CRP 24-h post-exercise, there was no difference between trials (main effect trial, $p=0.716$; trial*time interaction, $p=0.116$).

Overall, the IL-6:TNF- α ratio was higher during day one of the exercise trial when compared with the control trial (exercise: 5.53 ± 0.93 , resting 3.75 ± 0.45 ; main effect trial, $F_{(1, 24)}=5.5$, $p=0.027$; $d=0.5$). Furthermore, the IL-6:TNF- α ratio increased across time during both trials on day one (main effect time, $F_{(4, 96)}=3.3$, $p=0.043$). There was no trial*time interaction effect observed for the IL-6:TNF- α ratio on day one ($p=0.764$), nor was there a difference between trials 24-h post-exercise (main effect trial, $p=0.827$; trial*time interaction, $p=0.348$).

3.2.2 Metabolic Variables

The response of the metabolic variables can be found in table 2. Overall blood glucose concentration did not differ between the exercise and control trial (main effect trial, $p=0.087$), yet did differ over time (main effect time, $F_{(6,210)}=61.2$, $p<0.001$). Furthermore, the pattern of change in blood glucose concentration differed between trials (trial*time interaction, $F_{(6,210)}=8.8$, $p<0.001$); whereby blood glucose concentration was higher immediately post-exercise during the exercise trial compared to the control trial ($t_{(35)}=3.1$, $p<0.001$, $d=0.9$). Blood glucose concentration was also lower 1-h post-exercise on the exercise trial compared to the control trial ($t_{(35)}=2.3$, $p<0.001$, $d=0.8$). No differences were evident at any other time point on day one (all $p>0.05$). On day two, fasting blood glucose concentration was lower for the exercise trial compared to the control trial ($t_{(35)}=3.3$, $p=0.027$, $d=0.6$). When considering the effect of sex, blood glucose concentration was higher at baseline and immediately post-exercise in females compared with males (trial*sex interaction, $F_{(1,35)}=6.5$, $p=0.016$).

Blood glucose iAUC did not differ between the exercise and the control trial (main effect trial, $p=0.084$), nor was there an effect of sex on blood glucose iAUC following the standardized lunch (trial*sex interaction, $p=0.083$).

Overall, plasma insulin concentration was lower during the exercise trial than the control trial (exercise: 20.8 ± 2.5 , resting: 24.2 ± 1.7 $\text{mU}\cdot\text{L}^{-1}$; main effect trial, $F_{(1,23)}=6.7$, $p=0.016$, $d=0.3$) and changed across time (main effect time, $F_{(6, 138)}=55.9$, $p<0.001$). The pattern of change in plasma insulin concentration differed between trials (trial*time interaction, $F_{(6,138)}=7.9$, $p<0.001$). Specifically, during the exercise trial plasma insulin concentration reached a higher peak than the control trial immediately post-exercise ($t_{(23)}=-1.33$, $p=0.011$, $d=0.5$), whereas plasma insulin concentration 1-h post-exercise was lower on the exercise trial when compared to the control trial ($t_{(23)}=0.29$, $p=0.039$, $d=0.3$). Plasma insulin concentration was lower during the exercise trial compared to the control trial 30-min post-lunch ($t_{(23)}=1.35$, $p<0.001$, $d=0.7$) and 60-min post-lunch ($t_{(23)}=0.61$, $p=0.048$, $d=0.4$). When considering the effect of sex, the response to exercise did not differ between boys and girls (trial*sex interaction, $p=0.082$).

Plasma insulin iAUC following the consumption of the standardized lunch was lower during the exercise trial compared to the control trial (exercise: 2310 ± 834 , resting: 3122 ± 1443 $\text{mU}\cdot\text{L}^{-1}\times 120$ min, $t_{(24)}=3.0$, $p<0.001$, $d=0.7$), but this effect was not different between the sexes (trial*sex interaction, $p=0.170$).

HOMA-IR was calculated for the fasted blood samples on day one and day two, with no difference between trials (main effect trial, $p=0.136$), or between day one and day two (main effect time, $p=0.519$). Furthermore, the change in HOMA-IR between day one and day two was similar between trials (trial*time interaction, $p=0.439$).

4. Discussion

The primary finding of the present study was that an acute bout of intermittent games-based activity elicited an anti-inflammatory response with a 132% increase in IL-6 concentration and a 200% rise in the anti-inflammatory IL-6:TNF- α ratio 3-h post-exercise when compared with the rested trial. Furthermore, there was a 27% increase in concentration of anti-inflammatory cytokine IL-10 24-h post-exercise in comparison with the rested trial. The pro-inflammatory cytokine IL-1 β and acute phase protein CRP were unaffected by the 60-min bout of games-based activity, whereas the concentration of TNF- α increased following exercise. In addition, the insulinemic response to a standardized lunch was reduced by 35% following the games-based exercise when compared with the control trial. This is the first study to examine a range of inflammatory cytokines and the glycaemic and insulinemic responses up to 24-h following games-based activity in adolescents.

The observed response of IL-6 in the present study is consistent with the findings of the only other study which has examined games-based activity in young people, in which a 91% increase was observed 1-h post-exercise in pre-pubertal boys⁽¹⁰⁾. The present study is novel as it is the first to report that the IL-6 concentration continues to increase in healthy adolescents 3-h post-exercise, whereas previous studies suggested IL-6 concentration increased transiently and returned to resting levels 1-h post-exercise⁽¹⁾. The increase in IL-6 concentration stimulated a 2-fold increase in the anti-inflammatory IL-6:TNF- α ratio 3-h post-exercise and a 27% increase in IL-10 concentration 24-h post-exercise. The 200% increase in the IL-6:TNF- α ratio was greater than the 80% increase following 10 x 2 min bouts of high intensity intermittent cycling in adolescents⁽²⁰⁾, whilst the present study is the first to report an increase IL-10 concentration 24-h post-exercise in young people. The greater inflammatory response observed in the present study may relate to the longer duration of the exercise session compared to previous studies. Alternatively, this might relate to the mode of exercise undertaken, as basketball has an intense eccentric component, which in adults induces muscle damage that stimulates an inflammatory response of similar magnitude to that observed in the present study⁽¹⁹⁾. However, these suggestions are speculative and future research should examine the optimum duration and intensity of games-based activity for eliciting an inflammatory response.

The increase in IL-6 concentration post-exercise has both pro- and anti-inflammatory role in exercise induced inflammation⁽³⁾, as indicated by the 31% increase in pro-inflammatory cytokine TNF- α 2-h post-exercise. The increase in TNF- α concentration in the present study is consistent with the ~10–30% increase reported in young people following moderate-to-vigorous exercise^(9,20,34). Although a chronic increase in TNF- α is suggested to increase cardio-metabolic disease risk in adults⁽³⁶⁾, the transient increase in TNF- α following exercise in adolescents in the present study may also elicit cardio-metabolic health benefits. Previous research has suggested that following damage to skeletal muscle during moderate-to-vigorous exercise, the transient increase in pro-inflammatory cytokine TNF- α advances muscle regeneration and augments glucose uptake with the increased expression of GLUT-1⁽³⁷⁾, potentially contributing to the enhanced insulin sensitivity associated with regular participation in exercise.

IL-1 β and CRP were the only inflammatory markers in the present study to be unaffected by the acute bout of games-based activity. The lack of an effect of exercise on IL-1 β concentration is consistent with one previous study that reported no change following 90-min games-based activity in pre-pubertal boys⁽¹⁰⁾. The present study is the first, to the authors' knowledge, to assess the response of CRP following an acute bout of exercise in adolescents. However, one previous study in adults reported a small increase in CRP concentration the day following a marathon race⁽³⁾. It is therefore possible that longer duration bouts of exercise lead to greater increases in the systemic concentration of inflammatory mediators. Further research is required to determine the relationship between exercise intensity, duration and the subsequent inflammatory response. However, it is important to note that the milieu of cytokine responses observed in the present study is likely to arise from the release of IL-6 as a result of the contraction of skeletal muscle during the games-based activity.

In the present study, the glycemic response to a standardized lunch was similar between trials, with no difference in blood glucose iAUC observed. Yet, postprandial plasma insulin iAUC was 35% lower and peak plasma insulin was 61% lower following the games-based activity. An acute bout of high intensity intermittent exercise (lasting ~ 22-min) has previously resulted in a 29% reduction in postprandial blood glucose iAUC and a 24% reduction in plasma insulin iAUC in adolescent boys⁽²²⁾.

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It is important to note that the present study is the first to report an exercise-induced reduction in peak plasma insulin concentration following an ecologically valid meal and a reduction in insulin iAUC as a result of games-based activity which was of greater magnitude than in previous studies using different types of exercise⁽²²⁾.

The greater enhancement in insulin sensitivity may relate to the training status and higher peak oxygen uptake of participants in the present study^(21,22) which may have enabled exercise at higher absolute intensities. Although it has previously been proposed that the capacity for insulin sensitivity to change following exercise is reduced in well-trained participants⁽²²⁾, the present study suggests that, if adolescents having a greater level of fitness sustain overall higher absolute exercise intensities, then they experience a greater enhancement in insulin sensitivity post-exercise. Nonetheless, the enhanced insulin sensitivity observed in the present study reduces the risk of developing chronic diseases such as type 2 diabetes, thereby highlighting the importance of games-based activity for adolescent health.

Finally, the intermittent games-based activity employed in the present study is considered an enjoyable mode of exercise for adolescents⁽¹⁷⁾ and can be undertaken during the school day, thereby facilitating participation for all young people. These issues of appropriateness and accessibility of physical activity are particularly important given that only 23% of adolescents currently meet the recommended guidelines of 60-min moderate-to-vigorous physical activity per day⁽¹⁸⁾.

Perspectives

The present study shows that an acute bout of games-based activity in adolescents elicits anti-inflammatory effects, as evidenced by the increase in systemic concentrations of anti-inflammatory cytokines (IL-6, IL-10) and a higher anti-inflammatory IL-6:TNF- α ratio, alongside a reduced insulinemic response to a standardized lunch. These findings demonstrate a beneficial effect across these cardio-metabolic disease risk factors and have important implications for the health of young people, especially given that the anti-inflammatory effects are evident up to 24-h post-exercise. If

such exercise was repeated regularly then it would elicit beneficial effects on cardio-metabolic health in adolescents. These findings will inform those responsible for designing and implementing physical activity interventions in schools. Such interventions are particularly important given that less than one in four young people currently meet physical activity guidelines⁽¹⁸⁾ and adherence may be enhanced with games-based activity. Future research should aim to further quantify the optimum intensity and duration of exercise for cardio-metabolic health in young people and identify effective interventions for the implementation of this in practice.

References

1. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* 2011;11(9):607-615.
2. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M, Chrousos GP, Sidossis LS. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin and inflammatory markers in overweight and obese girls. *Metabolism* 2005;54:1472-1479.
3. Petersen AMW, Pedersen BK. The anti-inflammatory effects of exercise. *J Appl Physiol* 2005;98(4):1154-1162.
4. Sarzynski MA, Sternfield B, Carnethon MR, Sidney S, Quesenberry CP, Haskell WL, Jacobs DR, Lewis CE, Schreiner PJ, Williams OD. Association of 20-year changes in cardiorespiratory fitness with incident dyslipidemia between years 20 and 25 in the CARDIA Fitness Study. *Circulation*. 2013;127(12):A038.
5. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. *N Engl J Med* 1998;338:1650-1656.
6. Ehtisham S, Barrett TG, Shaw NJ. Type 2 diabetes mellitus in UK children – an emerging

problem. *Diabetic Med* 2000;17(12):867-871.

7. Warnberg J, Ascension M. Low-grade inflammation and the metabolic syndrome in children and adolescents. *Curr Opin Lipidol* 2008;19(1):11-15.
8. Ikeda SI, Tamura Y, Kakehi S, Sanada H, Kawamori R, Watada H. Exercise-induced increase in IL-6 level enhances GLUT4 expression and insulin sensitivity in mouse skeletal muscle. *Biochem Biophys Res Commun* 2016;473(4):947-952.
9. Nemet D, Youngman O, Kim HS, Hill MA, Cooper DM. Effect of intense exercise on inflammatory cytokines and growth mediators in adolescent boys. *Pediatrics* 2002;110(4):681-689.
10. Scheet TP, Mills PJ, Ziegler MG, Stoppani J, Cooper DM. Effect of exercise on cytokines and growth mediators in pre-pubertal children. *Pediatr Res*. 1999;46:429-434.
11. Nemet D, Rose-Grotton CM, Mills PJ, Cooper DM. Effect of water polo practice on cytokines, growth mediators and leukocytes in girls. *Med Sci Sports and Exerc* 2003;35(2):356-363.
12. 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(2):293-304.
13. Paltoglou G, Schoina M, Valsamakis G, Salakos N, Avlonti A, Chatzinikolaou A, Margeli A, Skevaki C, Papagianni M, Kanaka-Gantenbein C, Papassotiriou I, Chrousos GP, Fatouros IG, Mastorakos G. Interrelations among the adipocytokines leptin and adiponectin, oxidative stress and aseptic inflammation markers in early-pubertal normal-weight and obese boys. *Endocrine* 2017;55:925-933.
14. Jamurtas AZ, Garyfallopoulou A, Theodorou AA, Zalavras A, Paschalis V, Deli CK, Nikolaidis MG, Fatouros IG, Koutedakis Y. A single bout of downhill running transiently increases HOMA-IR without altering adipokine response in healthy adult women. *Eur J Appl Physiol* 2012;113:2925-2932
15. Paschalis V, Nikolaidis MG, Giakis G, Theodorou AA, Sakellariou GK, Fatouros IG, Koutedakis Y, Jamurtas AZ. Beneficial changes in energy expenditure and lipid profile after eccentric exercise in overweight and lean women. *Scand J Med Sci Sports* 2010;20:e103-

e111.

16. Paschalis V, Nikolaidis MG, Theodorou AA, Panayiotou G, Fatouros IG, Koutedakis Y, Jamurtas AZ. A weekly bout of eccentric exercise is sufficient to induce health-promoting effects. *Med Sci Sports Exerc* 2011;43(1):64-73.
17. Howe CA, Freedson PS, Feldman HA, Osganian SK. Energy expenditure and enjoyment of common children's games in a stimulated free-play environment. *J Pediatr* 2010;157(6):936-942.
18. Rowland AV, Pilgrim EL, Eston RG. Patterns of habitual activity across weekdays and weekend days in 9-11 year old children. *Prev Med* 2008;46(4):317-324.
19. Chatzinikolaou A, Draganidis D, Avloniti A, Karipidis A, Jamurtas AZ, Skevaki CL, Tsoukas D, Sovatzidis A, Theodorou A, Kambas A, Papassotiriou I, Taxildaris K, Fatouros I. The microcycle of inflammation and performance changes after a basketball match. *J Spor Sci* 2014;32(9):870-882.
20. McMurray RG, Zaldivar F, Galasetti P. Cellular immunity and inflammatory mediator responses to intense exercise in overweight children and adolescents. *J Investig Med* 2007;55(3):120-129.
21. Short KR, Pratt LV, Teague AM, Man CD, Cobelli C. Postprandial improvement in insulin sensitivity after a single exercise session in adolescents with low aerobic fitness and physical activity. *Pediatr Diabetes* 2013;14(2):129-137.
22. Cockcroft EJ, Williams CA, Tomlinson OW, Vlachopoulos D, Jackman SR, Armstrong N, Barker AR. High intensity interval exercise is an effective alternative to moderate intensity exercise for improving glucose tolerance and insulin sensitivity in adolescent boys. *J Sci Med Spor* 2014;18(6):720-724.
23. Straczkowski M, Kowalska I, Nikolajuk A, Krukowska A, Gorska M. Plasma interleukin-10 concentration is positively related to insulin sensitivity in young healthy individuals. *Diabetes Care* 2005;28(8):2036-2037.
24. Ramsbottom R, Brewer J, Williams C. A progressive shuttle run test to estimate maximal oxygen uptake. *Br J Sports Med* 1988;22(4):141-144.

25. Barnett A, Chan LYS, Bruce IC. A preliminary study of the 20-m multistage shuttle run as a predictor of peak VO_2 in Hong Kong Chinese students. *Pediatr Exerc Sci* 1993;5(1):42–50.
26. Moore SA, McKay HA, Macdonald H, Nettlefold L. Enhancing a somatic maturity prediction model. *Med Sci Sports Exerc* 2015;47(8):1755-1764.
27. The International Society for the Advancement of Kinanthropometry. International standards for anthropometric assessment. National Library of Australia. 2001;19-63.
28. Reilly JJ, Wilson J, Durmin V. Determination of body composition from skinfold thickness: a validation study. *Arch Dis Child* 1995;73(4):305-310.
29. Cooper SB, Bandelow S, Nute ML, Morris JG, Nevill ME. Breakfast glycaemic index and cognitive function in adolescent school children. *Br J Nutr* 2012;107:1823-1832.
30. Brouns F, Bjorck I, Frayn KN, Gibbs AL. Glycaemic index methodology. *Nutr Res Rev* 2005;18:145-171.
31. Wolever TM, Jenkins DJ. The use of the glycemic index in predicting the blood glucose response to mixed meals. *Am J Clin Nutri.* 1986;43(1):167-172.
32. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;i:307-310.
33. De Jager W, Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin Vaccine Immunol* 2003;10(1):133-139.
34. Nemet D, Eliakim A, Mills PJ, Meckal Y. Immunological and growth mediator response to cross-country training in adolescent females. *J Pediatr Endocrinol Metab* 2009;22(11):995-1008.
35. Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab* 2003;285(2):E433-437.
36. Green DJ, Maiorana A, O'Driscoll G, Taylor R. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* 2004;561(1):1-25.
37. Tidball JG. Inflammatory processes in muscle injury and repair. *Am J Physiol Regul Integr Comp Physiol* 2005;288(2):R345-353.

Additional Information

Conflicts of Interest

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Table 1. Summary of the inflammatory responses following 60-min high intensity, intermittent, games-based activity and during the rested control trial. Levels of inflammatory mediators reported as Mean \pm S.E.M. * Significant difference between trials, $p < 0.05$.

	Rest	Post Exercise	1 h Post Exercise	2 h Post Exercise	3 h Post Exercise	Day 2 Rest
IL-6 (pgmL ⁻¹)	2.07 \pm 0.34	2.46 \pm 0.35	3.51 \pm 0.56	4.28 \pm 0.49	4.81 \pm 0.84	2.30 \pm 0.45
Exercise						
Control	1.98 \pm 0.33	2.06 \pm 0.35	2.88 \pm 0.50	3.19 \pm 0.70	3.77 \pm 0.42	2.14 \pm 0.33
IL-10 (pgmL ⁻¹)	1.45 \pm 0.14	1.63 \pm 0.19	1.43 \pm 0.14	1.74 \pm 0.15	1.75 \pm 0.19	2.11 \pm 0.23*
Exercise						
Control	1.80 \pm 0.17	1.73 \pm 0.18	1.44 \pm 0.12	1.60 \pm 0.14	1.72 \pm 0.13	1.66 \pm 0.16
TNF- α (pgmL ⁻¹)	1.08 \pm 0.21	1.00 \pm 0.19	0.98 \pm 0.20	1.28 \pm 0.33*	1.17 \pm 0.24	1.18 \pm 0.26
Exercise						
Control	1.17 \pm 0.19	0.99 \pm 0.14	0.93 \pm 0.14	0.98 \pm 0.17	0.96 \pm 0.14	1.02 \pm 0.15
IL-1 β (pgmL ⁻¹)	3.13 \pm 0.29	2.95 \pm 0.31	3.05 \pm 0.31	3.08 \pm 0.35	3.02 \pm 0.35	3.17 \pm 0.34
Exercise						
Control	2.85 \pm 0.23	2.68 \pm 0.22	2.85 \pm 0.25	3.01 \pm 0.37	2.94 \pm 0.27	2.73 \pm 0.23
CRP (mgL ⁻¹)	0.24 \pm 0.06	0.25 \pm 0.06	0.20 \pm 0.04	0.21 \pm 0.06	0.23 \pm 0.06	0.25 \pm 0.08
Exercise						
Control	0.26 \pm 0.05	0.25 \pm 0.05	0.22 \pm 0.05	0.20 \pm 0.04	0.19 \pm 0.04	0.17 \pm 0.04
IL-6: TNF-a	2.38 \pm 0.30	5.21 \pm 1.80	5.48 \pm 0.90	7.25 \pm 1.83	7.27 \pm 2.61	2.66 \pm 0.37
Exercise						
Control	2.19 \pm 0.28	3.06 \pm 0.50	3.95 \pm 0.85	4.58 \pm 0.68	4.94 \pm 0.81	3.15 \pm 0.85

Table 2. Summary of the glycemic and insulinemic responses following 60-min high intensity, intermittent games based activity and during the rested control trial. Glycemic and insulinemic responses reported as Mean \pm S.E.M. * Significant difference between trials, $p < 0.05$.

	Rest	Post Exercise	1 h Post Exercise	30 min Post Lunch	60 min Post Lunch	120 min Post Lunch	Day 2
Blood Glucose (mmol·L ⁻¹)							
Exercise	4.38 \pm 0.06	5.82 \pm 0.16 *	4.25 \pm 0.07 *	5.89 \pm 0.13	5.01 \pm 0.07	4.93 \pm 0.08	4.38 \pm 0.06
Control	4.54 \pm 0.08	5.07 \pm 0.10	4.74 \pm 0.13	6.07 \pm 0.17	5.09 \pm 0.12	4.90 \pm 0.08	4.72 \pm 0.12
Plasma Insulin (mU·L ⁻¹)							
Exercise	7.26 \pm 0.86	35.95 \pm 4.03 *	7.83 \pm 1.60 *	36.64 \pm 3.90 *	28.84 \pm 2.31 *	20.11 \pm 2.05	7.22 \pm 0.72
Control	8.17 \pm 0.94	25.98 \pm 3.39	10.50 \pm 1.98	59.01 \pm 7.84	33.26 \pm 2.81	26.28 \pm 3.19	8.70 \pm 1.18
HOMA-IR							
Exercise	1.55 \pm 0.63	-	-	-	-	-	1.40 \pm 0.75
Control	1.64 \pm 0.86	-	-	-	-	-	1.59 \pm 0.93



Fig. 1: Experimental Protocol