Author(s): Haapala, Eero A.; Kuronen, Emmi; Ihalainen, Johanna K.; Lintu, Niina; Leppänen, Marja H.; Tompuri, Tuomo; Atalay, Mustafa; Schwab, Ursula; Lakka, Timo A.

Title: Cross-sectional associations between physical fitness and biomarkers of inflammation in children : the PANIC study

Year: 2023

Version: Published version

Please cite the original version:

Haapala, E. A., Kuronen, E., Ihalainen, J. K., Lintu, N., Leppänen, M. H., Tompuri, T., Atalay, M., Schwab, U., & Lakka, T. A. (2023). Cross-sectional associations between physical fitness and biomarkers of inflammation in children : the PANIC study. Scandinavian Journal of Medicine and Science in Sports, 33(6), 1000-1009. https://doi.org/10.1111/sms.14337

Received: 29 June 2022 | Revised: 25 January 2023 | Accepted: 6 February 2023

DOI: 10.1111/sms.14337

ORIGINAL ARTICLE

WILEY

Cross-sectional associations between physical fitness and biomarkers of inflammation in children—The PANIC study

Eero A. Haapala[1,2] | **Emmi Kuronen**[1] | **Johanna K. Ihalainen**[1] | **Niina Lintu**[2] | **Marja H. Leppänen**[1,3] | **Tuomo Tompuri**[2,4] | **Mustafa Atalay**[2] | **Ursula Schwab**[5,6] | **Timo A. Lakka**[2,4,7]

[1]Sports & Exercise Medicine, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland

[2]Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio, Finland

[3]Folkhälsan Research Center, Helsinki, Finland

[4]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland

[5]Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

[6]Department of Medicine, Endocrinology and Clinical Nutrition, Kuopio University Hospital, Kuopio, Finland

[7]Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland

Correspondence
Eero A Haapala, Sports & Exercise Medicine, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland.
Email: eero.a.haapala@jyu.fi

Funding information
City of Kuopio; Diabetestutkimussäätiö; Finnish Innovation Fund Sitra; Juho Vainion Säätiö; Kela; Lastentautien Tutkimussäätiö; Opetus- ja Kulttuuriministeriö; Paavo Nurmen Säätiö; Research Committee of the Kuopio University Hospital Catchment Area (State Research Funding); Sosiaali- ja Terveysministeriö; Suomen Kulttuurirahasto; Sydäntautien tutkimussäätiö; Yrjö Jahnssonin Säätiö

Background: Systemic low-grade inflammation has been proposed as an underlying pathophysiological mechanism for cardiometabolic diseases. We investigated the associations of physical fitness with a systemic low-grade inflammatory state in a population sample of children.

Methods: Altogether 391 children aged 6–9 years were examined. Cardiorespiratory fitness (maximal power output, W_{max}) was assessed by a maximal cycle ergometer test and neuromuscular fitness by hand grip strength, sit-up, standing long jump, 50-meter shuttle run, static balance, sit-and-reach, and box and block tests. Body fat percentage (BF%) and lean mass (LM) were assessed by dual-energy X-ray absorptiometry (DXA). High sensitivity C-reactive protein (hs-CRP), leptin, leptin receptor, high molecular weight adiponectin (HMW-adiponectin), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and glycoprotein acetyls (GlycA) were assessed from fasting blood samples. The modified inflammatory score (IS) was calculated using the population-specific *z*-scores and formula (^{z}hs-CRP + ^{z}leptin + ^{z}IL-6 + ^{z}TNF-α + ^{z}GlycA)—^{z}leptin receptor—^{z}HMW-adiponectin. The data were analyzed using linear regression analyses.

Results: Higher W_{max}/kg of body mass (β = −0.416, 95% CI = −0.514 to −0.318), higher number of completed sit-ups (β = −0.147, 95% CI = −0.244 to −0.049), a longer distance jumped in the standing long jump test (β = −0.270,

Eero A. Haapala and Emmi Kuronen shared first authorship.

Section III: Health, Disease & Physical Activity. Section editor: Mark Hamer.

Clinical trial registration: ClinicalTrials.gov NCT01803776.

95% CI = −0.371 to −0.169), and a shorter time in the 50-meter shuttle run test (β = 0.123, 95% CI = 0.022 to 0.223) were associated with lower IS. None of these associations remained statistically significant after adjustment for BF%.

Conclusions: Higher physical fitness is associated with a more favorable inflammatory biomarker profile in children. However, the associations were explained by BF%.

KEYWORDS

biomarkers, fitness, inflammation, obesity, pediatrics, physical fitness

1 | INTRODUCTION

Increased prevalence of pediatric obesity has led to higher rates of low-grade inflammation already in childhood.[1] Persistent low-grade inflammation evokes insulin resistance and endothelial dysfunction and accelerates atherosclerotic progression thereby increasing the risk of cardiometabolic diseases.[2,3] While elevated body fat content has been found to be the strongest determinant of low-grade inflammation,[4] higher physical fitness may counteract the harmful impact of adiposity on low-grade inflammation.[5,6] However, there is limited evidence on the associations of physical fitness with biomarkers of inflammation in children.

Previous studies have reported inverse associations of cardiorespiratory fitness with biomarkers of inflammation among children.[7–10] However, the measures of cardiorespiratory fitness used in these studies, such as the 20-meter shuttle run and peak oxygen uptake scaled by body mass (BM), are heavily influenced by body fat content, which leads to underestimation of cardiorespiratory capacity in heavier children and adolescents.[11–13] This is an important methodological consideration because increased levels of adipose tissue release pro-inflammatory adipokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[3] and other biomarkers of inflammation, such as leptin and adiponectin.[4] Accordingly, some,[14–17] but not all,[8,18] previous studies in children and adolescents have denoted that the associations between cardiorespiratory fitness and the biomarkers of inflammation attenuated after controlling for measures of body fat content. However, an adjustment may not completely remove the effect of body fat content when a measure of cardiorespiratory fitness also includes a compound of body fat content.[19] Furthermore, different measures of adiposity used in previous studies, such as body fat percentage (BF%) assessed by dual-energy x-ray absorptiometry (DXA), body mass index (BMI), and waist circumference, also provide different information on the amount and localization of the adipose tissue.[20] Accompanied by the methodological issues related to the measures of cardiorespiratory fitness, different methods used to estimate body fat content increase the uncertainty in the observed associations.

To overcome the limitations of field tests that provide indirect measures of cardiorespiratory fitness and peak oxygen uptake divided by BM, cardiorespiratory fitness has been recommended to be scaled by lean mass (LM) or fat-free mass to provide a more accurate measure of cardiorespiratory capacity.[11,21] Applying this approach, Hosick et al.[16] found a weaker association of peak oxygen uptake scaled by fat-free mass than that scaled by BM with leptin in a small sample of adolescents. Unfortunately, they investigated the associations of cardiorespiratory fitness only with leptin.

While most studies on the associations between physical fitness and biomarkers of inflammation have focused on cardiorespiratory fitness, some studies have also reported that higher hand grip strength and better standing long jump performance are related to lower serum high-sensitivity C-reactive protein (hs-CRP) and leptin concentrations in children and adolescents.[10,18,22] Nevertheless, BF% has been found to be a strong mediator in these associations.[7,22] Performance in the 40-meter agility shuttle run test, a measure of neuromuscular fitness, has also been inversely associated with serum IL-6, leptin, and adiponectin but not to hs-CRP and TNF-α.[10] The amount of muscle and fat mass and the level of physical activity differently influence the performance in different physical fitness tests, potentially altering the associations of physical fitness with biomarkers of inflammation.[23–25] However, the evidence on the associations of different measures of physical fitness and biomarkers of inflammation is scarce and elusive, leaving a significant knowledge gap.

Previous studies have used only a limited number of measures of physical fitness and biomarkers of inflammation. Studying the associations of a broader range of measures of physical fitness with overall systemic low-grade inflammatory state and individual biomarkers of inflammation is important because the different measures of physical fitness may have different associations with low-grade inflammation and because it is not clear which biomarkers best describe low-grade inflammation.[26]

Furthermore, it remains unknown whether the different measures of body fat content similarly affect the associations. Therefore, we investigated the associations of various measures of cardiorespiratory and neuromuscular fitness with modified inflammatory score (IS), representing an overall systemic low-grade inflammatory state, and several individual biomarkers of inflammation in children. We also studied whether BF%, BMI-standard deviation score (BMI-SDS), or waist-to-height ratio similarly alter these associations.

2 | METHODS

2.1 | Study design and participants

The present data are from the Physical Activity and Nutrition in Children (PANIC) study, which is an 8-year physical activity and dietary intervention study and a long-term follow-up study in a population sample of children from the city of Kuopio, Finland.[27] The Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland, approved the study protocol in 2006 (Statement 69/2006). The parents or caregivers of the children gave their written informed consent, and the children provided their assent to participation. The PANIC study has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2008.

Altogether 736 children 6–9 years of age from primary schools in Kuopio were invited to participate in the baseline examination in 2007–2009. A total of 512 children, who represented 70% of those invited, participated in the baseline examinations. Six children were excluded from the study at baseline because of physical disabilities that could hamper participation in the intervention or no time or motivation to attend the study. The participants did not differ in sex distribution, age, or BMI-SDS from all children who started the first grade in 2007–2009 based on data from the standard school health examinations performed for all Finnish children before the first grade (data not shown). Complete data on variables used in the analyses dealing with the associations of the measures of physical fitness with inflammatory biomarkers were available for 391 children (189 girls, 202 boys).

2.2 | Assessment of physical fitness

We assessed cardiorespiratory fitness by a maximal exercise test using an electromagnetically braked Ergoselect 200 K® cycle ergometer coupled with a pediatric saddle module (Ergoline).[28] The exercise test protocol included a 2.5-min anticipatory period with the child sitting on the ergometer; a 3-min warm-up period with a workload of 5 W; a 1-min steady-state period with a workload of 20 W; an exercise period with an increase in the workload of 1 watt per 6 s until exhaustion, and a 4-min recovery period with a workload of 5 W. The children were asked to keep the cadence stable and within 70–80 revolutions per minute. Exhaustion was defined as the inability to maintain a cadence above 65 revolutions per minute regardless of vigorous verbal exhortation. The exercise test was considered maximal by an experienced physician (TT) supervising the test if objective and subjective criteria (heart rate > 85% of predicted, sweating, flushing, inability to continue exercise test regardless of strong verbal encouragement) indicated maximal effort and maximal cardiovascular capacity.[28] Maximal power output (W_{max}) measured at the end of the exercise test divided by BM or LM was used as measures of cardiorespiratory fitness. We used W_{max} as a measure of cardiorespiratory fitness because we did not perform respiratory gas analyses at baseline and it has been demonstrated to be a good surrogate measure of cardiorespiratory fitness in children.[29]

Speed and agility were assessed by the 50-meter shuttle run test.[30] The children were asked to run 5 meters from a starting line to another line as fast as possible, to turn on the line, to run back to the starting line, and to continue until five shuttles were completed. The test score was the running time in seconds, with a longer time indicating poorer performance.

Static balance was assessed by the modified flamingo balance test. The children were asked to stand barefoot on one self-chosen leg with eyes closed for 30 s. The test score was the number of floor touches with free foot or eye openings over 30 s, a higher number of floor touches, and eye openings, indicating poorer static balance.

Manual dexterity and upper limb movement speed were assessed by the box and block test. The children were asked to pick up small wooden cubes (2.5 cm per side) one by one with the dominant hand from one side of a wooden box (53.7 × 25.4 × 8 cm) and to move as many cubes as possible to the other side of the box over 60 s. The task was repeated with the nondominant hand. The test score was the total number of cubes moved to the other side of the box over 120 s, and a smaller number of cubes moved, indicating poorer manual dexterity.

Hand grip strength was assessed by the Martin vigorimeter (Martin). The children were asked to keep the elbow close to the body with the arm flexed at 90° and to squeeze a rubber bulb as hard as possible three times with each hand. The mean of the best result from each hand was used in the analyses. Hand grip strength was expressed in kilopascals (kPa). Handgrip strength in kPa was scaled by LM.

Lower limb explosive strength was assessed by the standing long jump test.[30] The children were asked to stand with their feet together, jump as far as possible, and land on both feet. The test score was the longest jump of three attempts in centimeters.

The sit-up test was used to assess abdominal muscle strength and endurance.[30] The children were asked to lie down with knees flexed at 90°, feet positioned on the ground, and arms behind the neck. The children were told to do as many sit-ups as possible in 30 s with the elbows coming up to touch the knees with the assistant holding their feet on the floor. The test score was the number of technically correct sit-ups completed in 30 s.

2.3 | Assessment of the biomarkers of inflammation

Venous blood samples were taken from the children having fasted for 12 h. Blood was immediately centrifuged and stored at a temperature of −75°C until biochemical analyses. Plasma hs-CRP was measured using an enhanced immunoturbidimetric assay with the CRP (Latex) High Sensitive Assay reagent (Roche Diagnostics GmbH) and the limit of quantitation of 0.3 mg/L. Plasma leptin concentration was measured by a competitive radioimmunoassay (Multigamma 1261–001, PerkinElmer Wallac Oy). Commercially available ELISA kits were employed for the measurement of plasma IL-6 and TNF-α (Sanquin Reagents) and leptin receptor concentrations (Human Leptin Receptor ELISA, BioVendor LM a. s.). Serum HMW-adiponectin concentration was analyzed using an ELISA kit after specific proteolytic digestion of other multimeric adiponectin forms (Millipore). The Nightingale high-throughput nuclear magnetic resonance (NMR) metabolomics platform was used to quantify plasma glycoprotein acetyls (GlycA).[31,32] We used modified IS as our primary outcome describing the overall systemic low-grade inflammatory state.[33] The modified IS was constructed by summing up biomarkers considered pro-inflammatory (hs-CRP, leptin, IL-6, TNF-α, GlycA) and subtracting biomarkers considered anti-inflammatory (leptin receptor, HMW-adiponectin).[33] We computed the modified IS using the population-specific *z*-scores and formula (^{z}hs-CRP + ^{z}leptin + ^{z}IL-6 + ^{z}TNF-α + ^{z}GlycA)—^{z}leptin receptor—^{z}HMW-adiponectin. We used standardized IS with a mean of 0 and a standard deviation of 1.

2.4 | Assessment of body size and composition

Body weight was measured twice with the children having fasted for 12 h, emptied the bladder, and standing in light underwear using a weight scale integrated into a calibrated InBody® 720 bioelectrical impedance device (Biospace) to an accuracy of 0.1 kg. The mean of these two values was used in the analyses. Body height was measured three times with the children standing in the Frankfurt plane without shoes using a wall-mounted stadiometer to an accuracy of 0.1 cm. The mean of the nearest two values was used in the analyses. BMI was calculated by dividing weight (kg) by height (m) squared. BMI-SDS was calculated based on Finnish reference data.[34] The prevalence of overweight and obesity was defined using the cut-off values provided by Cole et al.[35] Fat mass, BF%, and LM were measured by the Lunar® DXA device (GE Medical Systems) using a standardized protocol.[36] Waist circumference was measured three times after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest with an unstretchable measuring tape to an accuracy of 0.1 cm. The waist-to-height ratio was calculated as waist circumference (cm) divided by body height (cm).

2.5 | Statistical methods

Statistical analyses were performed using the SPSS statistical software, version 27.0 (IBM corp.). The characteristics of children between boys and girls were compared using the Student's *t*-test for normally distributed continuous variables, the Mann–Whitney's *U*-test for continuous variables with skewed distributions, or the χ^2-test for categorical variables. The associations between the measures of physical fitness and biomarkers of inflammation were investigated using linear regression analyses adjusted for age and sex. These data were further adjusted for BF%, BMI-SDS, or waist-to-height ratio, which were entered into models separately. To study the modifying effect of sex on the associations between the measures of physical fitness and biomarkers of inflammation, we used general linear models including a sex × physical fitness interaction term in the models. The data are presented as standardized regression coefficient and their 95% confidence intervals. We conventionally considered standardized regression coefficients of 0.10–0.29, 0.30–0.49, and ≥0.50 to describe small, moderate, and strong magnitude of the associations, respectively.[37]

3 | RESULTS

3.1 | Basic characteristics

Girls were shorter, had higher fat mass and BF%, and had less LM than boys (Table 1). Girls also achieved a

lower absolute and relative W_{max}, and had lower absolute hand grip strength, had a poorer performance in the standing long jump and 50-metre shuttle run tests, and a better performance in the Flamingo balance and box and block tests than boys. Furthermore, girls had higher SI, hs-CRP, leptin, and leptin receptor and lower IL-6 levels than boys.

3.2 | Associations of adiposity with biomarkers of inflammation

Higher BF%, BMI-SDS, and waist-to-height ratio were associated with higher IS (Table 2), hs-CRP, leptin, IL-6, and GlycA after adjustment of age and sex (Table S4). Higher BF%, BMI-SDS, and waist-to-height ratio were

TABLE 1 Basic characteristics.

	All ($n = 391$)	Girls ($n = 189$)	Boys ($n = 202$)	p-Value
Age (years)	7.6 (0.4)	7.6 (0.4)	7.7 (0.4)	0.417
Height (cm)	128.7 (5.6)	127.7 (5.8)	129.7 (5.2)	**<0.001**
Weight (kg)*	26.0 (5.8)	25.6 (5.9)	26.6 (5.5)	0.090
BMI-SDS	−0.18 (1.09)	−0.15 (1.08)	−0.22 (1.10)	0.530
Waist circumference (cm)	56.7 (5.7)	56.1 (6.0)	57.2 (5.4)	0.068
Waist-to-height ratio	0.44 (0.04)	0.44 (0.04)	0.44 (0.04)	0.734
Fat mass (kg)*	4.8 (3.8)	5.5 (4.0)	4.2 (3.6)	**<0.001**
BF%*	18.7 (11.3)	20.8 (9.8)	15.3 (10.2)	**<0.001**
Lean body mass (kg)	20.6 (2.4)	19.5 (2.2)	21.6 (2.2)	**<0.001**
Prevalence of overweight and obesity (%)	13.0%	14.8%	11.4%	0.314
Measures of physical fitness				
Maximal power output (W)	76.6 (15.3)	70.2 (13.2)	82.6 (14.7)	**<0.001**
Relative power output (W/kg of BM)	2.9 (0.5)	2.7 (0.5)	3.1 (0.5)	**<0.001**
Relative power output (W/kg of LM)	3.7 (0.5)	3.6 (0.5)	3.8 (0.5)	**<0.001**
Hand grip strength (kPa)	47.5 (8.6)	45.6 (8.5)	49.4 (8.2)	**<0.001**
Relative hand grip strength (kPa/kg of LM)	2.3 (0.4)	2.3 (0.4)	2.3 (0.4)	0.185
Sit-ups (n/30 s)*	11.0 (6.0)	11.0 (6.0)	11.0 (6.0)	0.851
Standing long jump (cm)	126.1 (16.6)	121.2 (15.6)	130.7 (16.1)	**<0.001**
50-meter shuttle run (s)	24.00 (2.1)	24.3 (2.0)	23.7 (2.1)	**0.003**
Flamingo-balance (errors/30 s)*	3.0 (3.0)	3.0 (4.0)	4.0 (4.0)	**<0.001**
Sit-and-reach (cm)	−3.2 (7.9)	0.04 (7.3)	−6.2 (7.2)	**<0.001**
Box & block (n of cubes/60 s)	101.8 (12.8)	104.4 (12.6)	99.4 (12.5)	**<0.001**
Biomarkers of inflammation				
Modified IS	0.0 (1.0)	0.15 (1.0)	−0.14 (1.0)	**0.004**
hs-CRP (mg/l)*	0.3 (0.3)	0.3 (0.4)	0.3 (0.2)	**0.008**
Leptin (ng/ml)*	3.7 (3.5)	4.5 (4.2)	3.2 (2.2)	**<0.001**
Leptin receptor (ng/ml)	40.6 (11.1)	37.5 (10.1)	43.4 (11.2)	**<0.001**
HMW-adiponectin (μg/ml)*	8.5 (5.3)	8.0 (5.4)	8.8 (5.3)	0.579
IL-6 (pg/ml)*	0.9 (0.8)	0.8 (0.7)	1.00 (0.9)	**0.005**
TNF-α (pg/ml)*	14.8 (28.5)	13.3 (26.4)	16.7 (31.2)	0.200
GlycA (mmol/l)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.544

Note: p-Value for the differences between girls and boys from Student's t-test, Mann–Whitney U-test, or χ^2-test.

Statistically significant differences are bolded.

Abbreviations: BF%, Body fat percentage; BM, body mass; BMI, body mass index; BMI-SDS, body mass index-standard deviation score; GlycA, Glycoprotein acetyls; HMW, high-molecular weight; hs, high-sensitivity; IS, inflammatory score; LM, lean mass; TNF-α, tumor necrosis factor alpha.

*The data are means and standard deviations or medians and interquartile ranges.

TABLE 2 Associations of the measures of body fat content with the IS.

	IS
BF%	**0.675 (0.594 to 0.755)**
BMI-SDS	**0.573 (0.492 to 0.653)**
Waist-to-height ratio	**0.587 (0.507 to 0.666)**

Note: The data are standardized regression coefficients and their 95% confidence intervals adjusted for age and sex. BMI-SDS. body mass index standard deviation score. Statistically significant associations are bolded.

Abbreviations: BF%, Body fat percentage; BMI-SDS, body mass index-standard deviation score; IS, inflammatory score.

also associated with lower leptin receptor concentration. Corresponding unstandardized regression coefficients and accompanying 95% confidence intervals are presented in Tables S1 and S2.

3.3 | Associations of measures of physical fitness with biomarkers of inflammation

Higher W_{max}/kg of BM, a higher number of sit-ups completed, a longer distance jumped in the standing long jump test, and a faster time in the 50-meter shuttle run test were associated with lower IS (Table 3).

Higher W_{max}/kg of BM was associated with lower hs-CRP, leptin, IL-6, and GlycA and higher leptin receptor concentrations after adjustment for age and sex (Table S5). Higher W_{max}/kg of LM was associated with lower leptin concentration and better sit-up performance was associated with lower leptin and IL-6 and higher leptin receptor concentrations. Better standing long jump performance was associated with lower hs-CRP, leptin, and GlycA and higher leptin receptor concentrations. A shorter time in the 50-meter shuttle run test was associated with lower leptin and HMW-adiponectin and higher leptin receptor concentrations.

We also detected that higher W_{max}/kg of BM was associated with lower GlycA concentration in girls ($\beta = -0.275$, 95% CI = −0.414 to −0.135, $p < 0.001$), but not in boys ($\beta = -0.098$, 95% CI = −0.237 to 0.041, $p = 0.167$, $p = 0.046$ for interaction). Furthermore, W_{max}/kg of LM had a statistically non-significant direct association with IL-6 in girls ($\beta = 0.075$, 95% CI = −0.075 to 0.225, $p = 0.325$), but higher W_{max}/kg of LM was associated with lower IL-6 concentration in boys ($\beta = -0.152$, 95% CI = −0.292 to −0.012, $p = 0.033$, $p = 0.022$ for interaction).

TABLE 3 Associations of measures of physical fitness with the inflammatory score.

	Adjusted for age and sex	Adjusted for age, sex, and BF%	Adjusted for age, sex, and BMI-SDS	Adjusted for age, sex, and waist-to-height ratio
Relative peak power output (W/kg of BM)	**−0.416 (−0.514 to −0.318)**	0.066 (−0.043 to 0.176)	**−0.147 (−0.246 to −0.048)**	**−0.124 (−0.223 to −0.024)**
Relative peak power output (W/kg or LM)	−0.038 (−0.141 to 0.066)	0.046 (−0.034 to 0.126)	−0.031 (−0.116 to 0.053)	0.008 (−0.076 to 0.092)
Hand grip strength (kPa/LM kg)	−0.055 (−0.154 to 0.044)	−0.016 (−0.092 to 0.060)	−0.011 (−0.092 to 0.070)	−0.019 (−0.099 to −0.061)
Sit-ups (*n*/30 s)	**−0.147 (−0.244 to −0.049)**	0.020 (−0.058 to 0.099)	−0.049 (−0.131 to 0.032)	−0.027 (−0.109 to 0.054)
Standing long jump (cm)	**−0.270 (−0.371 to −0.169)**	0.026 (−0.063 to 0.115)	**−0.120 (−0.207 to −0.032)**	**−0.091 (−0.178 to −0.003)**
50-meter shuttle run (s)	**0.123 (0.022 to 0.223)**	−0.065 (−0.146 to 0.016)	0.050 (−0.033 to 0.133)	0.011 (−0.072 to 0.094)
Flamingo balance (errors/30 s)	−0.001 (−0.103 to 0.101)	**−0.082 (−0.161 to −0.003)**	−0.028 (−0.111 to 0.056)	−0.040 (−0.123 to 0.042)
Sit-and-reach (cm)	−0.088 (−0.195 to 0.020)	−0.039 (−0.122 to 0.044)	−0.080 (−0.168 to 0.008)	−0.048 (−0.135 to 0.039)
Box & block (n of cubes/60 s)	−0.034 (−0.136 to 0.068)	0.036 (−0.042 to 0.115)	0.031 (−0.052 to 0.115)	0.033 (−0.050 to 0.115)

Note: The data are standardized regression coefficients and their 95% confidence intervals. Statistically significant associations are bolded.

Abbreviations: BF%, body fat percentage; BMI-SDS, body mass index standard deviation score; IS, inflammatory score; LM, lean mass; W, watts.

Corresponding unstandardized regression coefficients and accompanying 95% confidence intervals are presented in Table S3.

3.4 | The effects of the measures of body fat content on the associations of measures of physical fitness with an overall inflammatory state

None of the associations between the measures of physical fitness and IS remained statistically significant after adjustment for BF% (Table 3). However, a larger number of errors in the flamingo balance test was associated with lower IS only after adjustment for BF%. Higher W_{max}/kg of BM and a longer distance jumped in the standing long jump test remained statistically significantly associated with lower IS after adjustment for BMI-SDS or waist-to-height ratio. The effects of the measures of body fat content on the associations between measures of physical fitness and individual biomarkers of inflammation are presented in Tables S6–S8.

4 | DISCUSSION

Higher levels of physical fitness, as indicated by W_{max} scaled by BM and performance in the standing long jump, sit-up, and 50-meter shuttle run tests, were associated with and lower IS and lower levels of several individual pro-inflammatory biomarkers in children. W_{max} scaled by BM exhibited moderate to strong associations with IS, leptin, and leptin receptors and weak associations with other biomarkers of inflammation. Moreover, the magnitude of the associations of W_{max} scaled by LM and the measures of neuromuscular fitness with biomarkers of inflammation was relatively small. We also observed a strong direct association between body fat content and inflammation. Furthermore, the associations between the measures of physical fitness and inflammation were largely explained by BF%. Therefore, our results suggest that higher physical fitness is associated with a more favorable overall systemic low-grade inflammatory state and lower levels of individual pro-inflammatory biomarkers in children, but that the associations are explained by the differences in BF% between children with lower and higher levels of physical fitness and inflammation.

The findings of our study on the inverse association between cardiorespiratory fitness and low-grade systemic inflammation, such as overall systemic low-grade inflammatory state, hs-CRP, and leptin, are in line with the results of previous studies.[10,18] Similarly to some,[10,38] but not all,[18,39] earlier studies, we also observed an inverse association of cardiorespiratory fitness scaled by BM with IL-6. Cardiorespiratory fitness scaled by BM was also inversely associated with GlycA, a biomarker of systemic low-grade inflammation that has been directly associated with the risk of incident cardiometabolic diseases and premature death.[40,41] However, when cardiorespiratory fitness was scaled by LM, only the inverse association between cardiorespiratory fitness and leptin remained statistically significant. This finding is convergent with the results of Hosick et al.[16] who demonstrated in their small study in adolescents that the inverse association between cardiorespiratory fitness scaled by fat-free mass and leptin was statistically significant, but was considerably weaker than the association between cardiorespiratory fitness scaled for BM and leptin. Therefore, because scaling cardiorespiratory fitness by LM reduces its dependence on body size and composition,[21,42] our results together with previous findings suggest that the associations of cardiorespiratory fitness scaled by or dependent on supporting BM with biomarkers of inflammation are confounded by adiposity.

Previous studies have reported that better neuromuscular fitness, as indicated mainly by higher handgrip strength, is related to lower hs-CRP and leptin.[7,10,18] Moreover, Delgado-Alfonso et al.[10] observed that better standing long jump and 40-meter shuttle run test performance were associated with lower clustered inflammatory biomarker scores in children and adolescents. Some studies have also found inverse associations of hand grip strength with IL-6[10] and TNF-α,[18] but the evidence on the associations of measures of neuromuscular fitness with these biomarkers of inflammation remains equivocal. Accordingly, we found versatile associations of measures of neuromuscular fitness with the biomarkers of inflammation, the associations being most consistent with IS and leptin. Specifically, the measures of neuromuscular fitness requiring moving or supporting BM were inversely related to leptin supporting the results of previous studies.[18] A better performance in a weight-bearing standing long-jump test was also associated with lower IS, hs-CRP, and GlycA suggesting that standing long-jump performance could be a useful measure to estimate low-grade inflammation status in children. Nevertheless, non-weight-bearing measures of neuromuscular fitness were not associated with biomarkers of inflammation suggesting that indicators of physical fitness without weight-bearing or a component related to adiposity are not strongly related to biomarkers of inflammation.

We also observed that better a 50-meter shuttle run test performance was associated with lower adiponectin. Because adiponectin is considered an anti-inflammatory adipokine and low adiponectin have been related to an increased risk of type 2 diabetes,[3] the inverse association is intuitively surprising. However, previous studies in youth have also reported an inverse association between physical

fitness and adiponectin.[7,10,38] Moreover, we have previously reported that physical activity is inversely associated with adiponectin[32] and a 50-meter shuttle run test time,[43] suggesting that the mechanism behind the association between performance in the 50-meter shuttle run test and adiponectin could be similar to that of physical activity. Nevertheless, intervention studies have indicated that exercise increases adiponectin.[44] Thus, the inverse cross-sectional associations of fitness and adiponectin with decreased adiponectin levels may reflect better insulin sensitivity or affinity in fitter children compared to those with lower fitness levels.[45]

In the present study, all but one of the associations between the measures of physical fitness and the biomarkers of inflammation were diminished after adjustment for BF%. However, the associations of measures of physical fitness with several biomarkers of inflammation remained statistically significant after adjustment for waist-to-height ratio and particularly for BMI-SDS. Our results, however, are not uniform with all previous findings. For example, Ruiz et al.[15] found that the associations of physical fitness with hs-CRP and complement component 3 disappeared after adjustment for BMI, waist circumference, or the sum of skinfolds, whereas Steene-Johannessen and et al.[18] and Delgado-Alfonso et al.[10] revealed that the adjustment for waist circumference weakened but did not completely diminish the associations of physical fitness with hs-CRP and leptin. Furthermore, Brand et al.[7] found that the associations between measures of physical fitness and biomarkers of inflammation, excluding that between muscle strength and hs-CRP, diminished after adjustment for BF% measured by DXA. These equivocal findings may be explained by differences in the characteristics of populations examined and in the levels of biomarkers of inflammation. For example, the mean levels of pro-inflammatory biomarkers were lower in our cohort compared to the participants in the studies by Steene-Johannessen et al.[18] and Delgado-Alfonso et al.[10] Another possible reason for the different effects of controlling for measures of body fat content may be that BF% measured by DXA is a more direct and sensitive measure of endocrinologically active adipose tissue than BMI-SDS and waist-to-height ratio.[20] Nevertheless, our results suggest that different measures of body fatness should not be used interchangeably to control for adiposity in the associations between the measures of physical fitness and the biomarkers of inflammation.

We observed sex differences in the associations between measures of physical fitness, particularly cardiorespiratory fitness, and biomarkers of inflammation. For example, W_{max} scaled by BM was inversely associated with GlycA only in girls, and W_{max} scaled by LM was inversely related to IL-6 only in boys. Few previous studies have reported sex differences in the associations between measures of physical fitness and biomarkers of inflammation among children. Isasi et al.[14] found an inverse association between cardiorespiratory fitness with hs-CRP in boys but not in girls. Nevertheless, Steene-Johannessen et al.[18] reported that the correlations between the measures of physical fitness and biomarkers of inflammation were mainly similar between boys and girls. A possible explanation for the conflicting results is differences in fat mass, physical fitness, or the levels of biomarkers of inflammation between girls and boys. However, most of the associations we detected in the present study were independent of sex, indicating that sex does not markedly modify the associations between measures of physical fitness and biomarkers of inflammation in children.

The strengths of the present study include the valid and reproducible measurements of physical fitness and several different biomarkers of inflammation in a population sample of children. Because there is no consensus on the biomarkers of inflammation that best describe systemic low-grade inflammation,[26] we used a modified IS as a measure of overall systemic low-grade inflammatory state and seven individual biomarkers of inflammation, including a NMR derived GlycA, to provide a more comprehensive view on the associations between measures of physical fitness and biomarkers of inflammation. We also could measure body composition using whole-body DXA. We did not directly assess maximal oxygen uptake which is considered the gold standard for measuring cardiorespiratory fitness. However, W_{max} determined from an exercise test until exhaustion has been found to be a valid measure of cardiorespiratory fitness in children.[29] Furthermore, we did not consider diet quality in the present analyses because we have previously reported that it is not associated with the biomarkers of inflammation in children.[32] Finally, our study was cross-sectional which limits our ability to make causal inferences.

In conclusion, our results suggest that higher physical fitness is associated with a more favorable overall systemic low-grade inflammatory state in children. However, BF% largely explained most of these associations. Our results also suggest that various measures of body fat content, such as BF%, BMI-SDS, and waist-to-height ratio, cannot be used interchangeably to control for adiposity in the associations between measures of physical fitness and biomarkers of inflammation. Therefore, forthcoming studies should accurately analyze and describe the interwoven associations of various measures of physical fitness and body fatness with biomarkers of inflammation in children.

5 | PERSPECTIVE

A prolonged exposure to low-grade systemic inflammation often precedes endothelial dysfunction, insulin resistance, and the development of clinical cardiometabolic

diseases. In contrast, physical fitness has been considered a powerful marker of cardiometabolic health since childhood, and children and adolescents are encouraged to enhance their physical fitness to lower the future risk of cardiometabolic diseases. Therefore, our finding that children with higher levels of physical fitness have favorable inflammatory biomarker profile could have significant public health relevance. Nevertheless, we also observed that the associations between the measures of physical fitness and the biomarkers of inflammation were largely explained by adiposity, suggesting that children with higher levels of physical fitness and favorable inflammatory biomarker profile also have less adipose tissue than other children. Our results along with others suggest that supporting the maintenance of healthy weight in childhood would be a good investment in the prevention of systemic low-grade inflammation and accompanying cardiometabolic diseases.

ACKNOWLEDGMENTS

We are grateful to all children and their parents and caregivers who have participated in the PANIC study. We are also indebted to all members of the PANIC research team for their invaluable contribution to the acquisition of the data throughout the study. The PANIC study has been supported financially by grants from the Ministry of Education and Culture of Finland, the Ministry of Social Affairs and Health of Finland, the Research Committee of the Kuopio University Hospital Catchment Area (State Research Funding), Finnish Innovation Fund Sitra, Social Insurance Institution of Finland, Finnish Cultural Foundation, Foundation for Pediatric Research, Diabetes Research Foundation in Finland, Finnish Foundation for Cardiovascular Research, Juho Vainio Foundation, Paavo Nurmi Foundation, Yrjö Jahnsson Foundation, and the city of Kuopio.

FUNDING INFORMATION

The PANIC study has been supported financially by grants from the Ministry of Education and Culture of Finland, the Ministry of Social Affairs and Health of Finland, the Research Committee of the Kuopio University Hospital Catchment Area (State Research Funding), Finnish Innovation Fund Sitra, Social Insurance Institution of Finland, Finnish Cultural Foundation, Foundation for Pediatric Research, Diabetes Research Foundation in Finland, Finnish Foundation for Cardiovascular Research, Juho Vainio Foundation, Paavo Nurmi Foundation, Yrjö Jahnsson Foundation, and the city of Kuopio.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

DATA AVAILABILITY STATEMENT

Information about the PANIC study and the data used in the present paper is available at www.panicstudy.fi/en/etusivu. The data are not publicly available due to research ethical reasons and because the owner of the data is the University of Eastern Finland and not the research group. However, the corresponding author can provide further information on the PANIC study and the PANIC data on a reasonable request.

ORCID

Johanna K. Ihalainen https://orcid.org/0000-0001-9428-4689
Mustafa Atalay https://orcid.org/0000-0001-8999-1426

REFERENCES

1. Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. *Pediatrics*. 2010;125:e801-e809.
2. Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. *Mediators Inflamm*. 2009;2008:109502.
3. Fasshauer M, Blüher M. Adipokines in health and disease. *Trends Pharmacol Sci*. 2015;36:461-470.
4. Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat Rev Endocrinol*. 2019;15:507-524.
5. Lang JJ, Belanger K, Poitras V, Janssen I, Tomkinson GR, Tremblay MS. Systematic review of the relationship between 20m shuttle run performance and health indicators among children and youth. *J Sci Med Sport*. 2018;21:383-397.
6. de Lima TR, Martins PC, Moreno YMF, et al. Muscular fitness and Cardiometabolic variables in children and adolescents: a systematic review. *Sports Med*. 2022;52:1555-1575.
7. Brand C, Gaya ACA, Dias AF, et al. The role of adiposity in the relationship between physical fitness with cardiometabolic risk factors, adipocytokines and inflammation in children. *Sport Sci Health*. 2021;17:127-136.
8. González-Gil EM, Santaliestra-Pasías AM, Buck C, et al. Improving cardiorespiratory fitness protects against inflammation in children: the IDEFICS study. *Pediatr Res*. 2022;91:681-689.
9. Andersen LB, Müller K, Eiberg S, et al. Cytokines and clustered cardiovascular risk factors in children. *Metabolism*. 2010;59:561-566.
10. Delgado-Alfonso A, Pérez-Bey A, Conde-Caveda J, et al. Independent and combined associations of physical fitness components with inflammatory biomarkers in children and adolescents. *Pediatr Res*. 2018;84:704-712.
11. Welsman J, Armstrong N. Interpreting aerobic fitness in youth: the fallacy of ratio scaling. *Pediatr Exerc Sci*. 2019;31:184-190.
12. Armstrong N, Welsman J. Fact and fiction in youth cardiorespiratory fitness. *Int J Phys Educ Fit Sports*. 2019;8:8-13.
13. Tanner JM. Fallacy of per-weight and per-surface area standards, and their relation to spurious correlation. *J Appl Physiol*. 1949;2:1-15.
14. Isasi CR, Deckelbaum RJ, Tracy RP, Starc TJ, Berglund L, Shea S. Physical fitness and C-reactive protein level in children and

young adults: the Columbia University BioMarkers study. *Pediatrics*. 2003;111:332-338.
15. Ruiz JR, Ortega FB, Warnberg J, Sjöström M. Associations of low-grade inflammation with physical activity, fitness and fatness in prepubertal children; the European youth heart study. *Int J Obes (Lond)*. 2007;31:1545-1551.
16. Hosick PA, McMurray RG, Cooper DM. The relationships between leptin and measures of fitness and fatness are dependent upon obesity status in youth. *Pediatr Exerc Sci*. 2010;22:195-204.
17. Isasi CR, Strizich GM, Kaplan R, et al. The association of cardiorespiratory fitness with cardiometabolic factors, markers of inflammation, and endothelial dysfunction in Latino youth: findings from the Hispanic community Children's health study/study of Latino youth. *Ann Epidemiol*. 2018;28:583-589.e3.
18. Steene-Johannessen J, Kolle E, Andersen LB, Anderssen SA. Adiposity, aerobic fitness, muscle fitness, and markers of inflammation in children. *Med Sci Sports Exerc*. 2013;45:714-721.
19. Shaibi GQ, Cruz ML, Ball GDC, et al. Cardiovascular fitness and the metabolic syndrome in overweight Latino youths. *Med Sci Sports Exerc*. 2005;37:922-928.
20. Cornier MA, Després JP, Davis N, et al. Assessing adiposity. *Circulation*. 2011;124:1996-2019.
21. Loftin M, Sothern M, Abe T, Bonis M. Expression of VO2peak in children and youth, with special reference to Allometric scaling. *Sports Med*. 2016;46:1451-1460.
22. Artero EG, España-Romero V, Jiménez-Pavón D, et al. Muscular fitness, fatness and inflammatory biomarkers in adolescents. *Pediatr Obes*. 2014;9:391-400.
23. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived Interleukin-6. *Physiol Rev*. 2008;88:1379-1406.
24. 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;11:607-615.
25. Luoto R, Ruuskanen O, Ihalainen JK, et al. Inflammatory biomarkers in elite cross-country skiers after a competition season: a case–control study. *J Sci Sport Exerc*. 2022. doi:10.1007/s42978-022-00186-w
26. Minihane AM, Vinoy S, Russell WR, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr*. 2015;114:999-1012.
27. Eloranta AM, Lindi V, Schwab U, et al. Dietary factors and their associations with socioeconomic background in Finnish girls and boys 6–8 years of age: the PANIC study. *Eur J Clin Nutr*. 2011;65:1211-1218.
28. Tompuri T, Lintu N, Savonen K, et al. Measures of cardiorespiratory fitness in relation to measures of body size and composition among children. *Clin Physiol Funct Imaging*. 2015;35:469-477.
29. Dencker M, Thorsson O, Karlsson MK, Lindén C, Wollmer P, Andersen LB. Maximal oxygen uptake versus maximal power output in children. *J Sports Sci*. 2008;26:1397-1402.
30. European Council. *EUROFIT: Handbook for the EUROFIT Tests of Physical Fitness*. Council of Europe; 1988.
31. Soininen P, Kangas AJ, Würtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet*. 2015;8:192-206.
32. Haapala EA, Väistö J, Ihalainen JK, et al. Associations of physical activity, sedentary time, and diet quality with biomarkers of inflammation in children. *Eur J Sport Sci*. 2022;22:906-915.
33. de Faria AP, Ritter AMV, Gasparetti CS, et al. A proposed inflammatory score of circulating cytokines/Adipokines associated with resistant hypertension, but dependent on obesity parameters. *Arq Bras Cardiol*. 2019;112:383-389.
34. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med*. 2011;43:235-248.
35. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. 2000;320:1240-1243.
36. Tompuri TT, Lakka TA, Hakulinen M, et al. Assessment of body composition by dual-energy X-ray absorptiometry, bioimpedance analysis and anthropometrics in children: the physical activity and nutrition in children study. *Clin Physiol Funct Imaging*. 2015;35:21-33.
37. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. L. Erlbaum Associates; 1988:567.
38. Bugge A, El-Naaman B, McMurray RG, et al. Inflammatory markers and clustered cardiovascular disease risk factors in Danish adolescents. *Horm Res Paediatr*. 2012;78:288-296.
39. Martinez-Gomez D, Gomez-Martinez S, Ruiz JR, et al. Objectively-measured and self-reported physical activity and fitness in relation to inflammatory markers in European adolescents: the HELENA study. *Atherosclerosis*. 2012;221:260-267.
40. Connelly MA, Otvos JD, Shalaurova I, Playford MP, Mehta NN. GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk. *J Transl Med*. 2017;15:219.
41. Kettunen J, Ritchie SC, Anufrieva O, et al. Biomarker glycoprotein acetyls is associated with the risk of a wide Spectrum of incident diseases and stratifies mortality risk in angiography patients. *Circ Genomic Precis Med*. 2018;11:e002234.
42. Haapala EA, Gao Y, Lintu N, et al. Associations between cardiorespiratory fitness, motor competence, and adiposity in children. *Transl Sports Med*. 2021;4:56-64.
43. Haapala EA, Väistö J, Lintu N, et al. Adiposity, physical activity, and neuromuscular performance in children. *J Sports Sci*. 2016;34:1699-1706.
44. Simpson KA, Singh MAF. Effects of exercise on adiponectin: a systematic review. *Obesity*. 2008;16:241-256.
45. Su H, Lau WB, Ma XL. Hypoadiponectinaemia in diabetes mellitus type 2: molecular mechanisms and clinical significance. *Clin Exp Pharmacol Physiol*. 2011;38:897-904.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Haapala EA, Kuronen E, Ihalainen JK, et al. Cross-sectional associations between physical fitness and biomarkers of inflammation in children—The PANIC study. *Scand J Med Sci Sports*. 2023;33:1000-1009. doi:10.1111/sms.14337