European Journal of Sport Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tejs20

Associations of physical activity, sedentary time, and diet quality with biomarkers of inflammation in children

Eero A. Haapala, Juuso Väistö, Johanna K. Ihalainen, Claudia Tomaselli González, Marja H. Leppänen, Aapo Veijalainen, Taisa Sallinen, Aino-Maija Eloranta, Ulf Ekelund, Ursula Schwab, Soren Brage, Mustafa Atalay & Timo A. Lakka

To cite this article: Eero A. Haapala, Juuso Väistö, Johanna K. Ihalainen, Claudia Tomaselli González, Marja H. Leppänen, Aapo Veijalainen, Taisa Sallinen, Aino-Maija Eloranta, Ulf Ekelund, Ursula Schwab, Soren Brage, Mustafa Atalay & Timo A. Lakka (2021): Associations of physical activity, sedentary time, and diet quality with biomarkers of inflammation in children, European Journal of Sport Science, DOI: 10.1080/17461391.2021.1892830

To link to this article: https://doi.org/10.1080/17461391.2021.1892830

Published online: 14 Mar 2021.

EUROPEAN JOURNAL OF SPORT SCIENCE
https://doi.org/10.1080/17461391.2021.1892830

Associations of physical activity, sedentary time, and diet quality with biomarkers of inflammation in children

Eero A. Haapala [a,b], Juuso Väistö [b], Johanna K. Ihalainen [a,c], Claudia Tomaselli González[b], Marja H. Leppänen [a,d], Aapo Veijalainen [b], Taisa Sallinen [b], Aino-Maija Eloranta [e], Ulf Ekelund [f], Ursula Schwab [e,g], Soren Brage [h], Mustafa Atalay[b] and Timo A. Lakka [b,i,j]

[a]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [b]Institute of Biomedicine, School of Medicine, University of Eastern Finland, Kuopio, Finland; [c]Swedish Winter Sports Research Centre, Mid Sweden University, Sweden; [d]Folkhälsan Research Center, Helsinki, Finland; [e]Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland; [f]Norwegian School of Sports Science, Oslo, Norway; [g]Department of Medicine, Endocrinology and Clinical Nutrition, Kuopio University Hospital, Kuopio, Finland; [h]MRC Epidemiology Unit, University of Cambridge, Cambridge, UK; [i]Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland; [j]Foundation for Research in Health Exercise and Nutrition, Kuopio Research Institute of Exercise Medicine, Kuopio, Finland

ABSTRACT

We investigated the associations of physical activity (PA), sedentary time (ST), and diet quality with biomarkers of inflammation in 390 children (192 girls, 198 boys) aged 6–8 years. PA energy expenditure (PAEE), light PA, moderate PA (MPA), vigorous PA (VPA), moderate-to-vigorous PA (MVPA), and ST were assessed by combined movement and heart rate sensor. Finnish Children Healthy Eating Index was calculated using data from 4 d food records. Body fat percentage (BF %) was measured by dual-energy X-ray absorptiometry. High-sensitivity C-reactive protein (Hs-CRP), leptin, interleukin-6 (IL-6), adiponectin, tumour necrosis factor-α, and glycoprotein acetyls were measured from fasting blood samples. PAEE, MPA, VPA, and MVPA were inversely associated with hs-CRP (β=−191 to −139, 95% CI=−0.294 to −0.024), leptin (β=−0.409 to −0.301, 95% CI=−0.499 to −0.107), IL-6 (β=−0.136 to −0.104, 95% CI=−0.240 to −0.001) and PAEE, MPA, and MVPA were inversely associated with glycoprotein acetyls (β=−0.117 to −0.103, 95% CI=−0.213 to −0.001). ST was directly associated with hs-CRP (β=0.170, 95% CI=0.070–0.269), leptin (β=0.355, 95% CI=0.265–0.445), and IL-6 (β=0.105, 95% CI=0.005–0.205). VPA was inversely associated with hs-CRP, leptin, and IL-6 in children with higher BF% (β=−0.344 to −0.181, 95% CI=−0.477 to −0.033) but not among children with lower BF% (β=−0.007–0.033, 95% CI=−0.183–0.184). In conclusion, PA was inversely and ST directly associated with circulating levels of biomarkers of inflammation among children. Furthermore, we observed that PA was inversely associated with these biomarkers for inflammation in children with a higher BF%.

Abbreviations: BF%: Body fat percentage; BMI: Body mass index; BMI-SDS: Body mass index standard deviation score; FCHEI: Finnish Children Healthy Eating Index; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6; MET: Metabolic Equivalent of Task; PA: Physical activity; PANIC: Physical Activity and Nutrition in Children Study; ST: Sedentary time; TNF-α: Tumour necrosis factor α.

Highlights

- Systemic inflammation, as indicated by increased circulating concentrations of biomarkers for inflammation, may be important in causal pathways leading to insulin resistance, sub-clinical atherosclerosis, and eventually clinical manifestations of cardiovascular diseases.
- Higher levels of physical activity and lower levels of sedentary time were associated with more favourable inflammatory profile.
- Body fat percentage modified these associations and especially vigorous intensity physical activity was inversely associated with biomarkers of inflammation on children with higher body fat percentage but not in children with lower body fat percentage.

KEYWORDS
Inflammation; biomarkers; youth; exercise; nutrition; obesity

CONTACT Eero A. Haapala eero.a.haapala@jyu.fi Faculty of Sport and Health Sciences, University of Jyväskylä, PO Box 35, room VIV 247, Jyväskylä FI-40014, Finland

Introduction

The prevalence of paediatric obesity is increasing in most parts of the world, predisposing children to increased risk for type 2 diabetes and cardiovascular diseases already in childhood (Juonala et al., 2011). Furthermore, few children meet the recommended levels of physical activity (PA) (Cooper et al., 2015; Guinhouya, Samouda, & de Beaufort, 2013) and adhere to the dietary recommendations (Banfield et al., 2016; Eloranta et al., 2011). Physical inactivity (Wahid et al., 2016) and poor diet quality (Rodríguez-Monforte, Flores-Mateo, & Sánchez, 2015) have been found to increase the risk of type 2 diabetes and cardiovascular diseases in adults. Importantly, pathological process leading to these cardiometabolic diseases begin already in childhood (Steinberger et al., 2016). Traditional cardiometabolic risk factors, such as overweight, insulin resistance, and dyslipidaemia, have been associated with increased risk of type 2 diabetes and sub-clinical atherosclerosis and cardiovascular diseases in adulthood (Baigent et al., 2020; Magnussen et al., 2010). However, recent evidence suggests that systemic inflammation, as indicated by increased circulating concentrations of biomarkers for inflammation, may be important in causal pathways leading to insulin resistance, sub-clinical atherosclerosis, and eventually clinical manifestations of cardiovascular diseases (Gleeson et al., 2011).

Obese children have been found to have higher circulating levels of biomarkers of low-grade inflammation than their normal-weight peers (Lund et al., 2020). Furthermore, higher levels of PA and better diet quality, as indicated by a higher consumption of fruits, vegetables, berries, vegetable oils, and fish and low consumption of foods containing high amount of sugar, have been associated with lower levels of traditional cardiometabolic risk factors already in children (Eloranta et al., 2016; Poitras et al., 2016). Even if some of the associations of PA and diet quality with biomarkers for inflammation may be modified by fat mass, it has been suggested that increased PA and improved diet quality are independently associated with decreased systemic inflammation. There are few studies on the associations of PA and diet quality with biomarkers for inflammation in general populations of children and the observed associations have been weak (Poitras et al., 2016). Furthermore, screen time, commonly defined as time spent in TV viewing, and sedentary time (ST) assessed by accelerometers, has been directly associated with circulating levels of high-sensitivity C-reactive protein (hs-CRP) (Carson & Janssen, 2011; Gabel et al., 2016) but not with interleukin 6 (IL-6), adiponectin, or tumour necrosis factor-α (TNF-α) in children and adolescents (Gabel et al., 2016). The results of intervention studies in obese children suggest that exercise training has a potential to reduce circulating levels of hs-CRP (Han et al., 2019), leptin, and IL-6 (Sirico et al., 2018) and increase circulating levels of adiponectin (García-Hermoso et al., 2017; Sirico et al., 2018). Evidence on the effects of exercise training on individual biomarkers of inflammation is still mixed and most of the studies addressing this issue have been performed among overweight or obese children (García-Hermoso et al., 2017; Han et al., 2019; Sirico et al., 2018). These contradictory observations may be due to differences in exercise intensity or in the reduction of fat mass in response to exercise training (García-Hermoso et al., 2017).

Healthy dietary patterns have been associated with decreased circulating levels of hs-CRP in adults (Norde, Collese, Giovannucci, & Rogero, 2021), but evidence on the role of diet quality indices in systemic low-grade inflammation in children is limited and mixed. Liese et al. found no associations of diet quality indices with biomarkers for inflammation, such as hs-CRP and IL-6, in youth with type 1 diabetes mellitus (Liese et al., 2018). Nevertheless, Saneei et al. observed that a dietary intervention reduced hs-CRP, but had no effect on IL-6, adiponectin, or TNF-a in adolescents with metabolic syndrome (Saneei, Hashemipour, Kelishadi, & Esmaillzadeh, 2014). Moreover, some studies have reported a weak if any effect of a combined exercise and diet intervention on hs-CRP and TNF-α (Blüher et al., 2014). Nevertheless, two-week intensive combined diet and exercise intervention decreased circulating levels of leptin, IL-6, and TNF-α and to increase adiponectin in normal weight and obese children and youth aged 8–17 years independent of changes in waist circumference, body weight, or body mass index (BMI) (Roberts, Izadpanah, Angadi, & Barnard, 2013). Interestingly, dietary intervention or a combined dietary and exercise intervention increased serum adiponectin but a moderate intensity exercise intervention alone decreased it over 12 weeks among obese children (Shalitin et al., 2009). In addition, a combined exercise, diet, and behavioural intervention has been found to increase adiponectin and decrease hs-CRP but not IL-6 and leptin in obese children (Nemet, Oren, Pantanowitz, & Eliakim, 2013). There is also some evidence that diet quality modifies the association between screen time and biomarkers for inflammation in adolescents (Arouca et al., 2019). However, little is known about the possible modifying effects of diet quality or body fat percentage (BF%) on the associations of PA with biomarkers for inflammation in general populations of children. Furthermore, there are no previous studies on the associations of PA and diet quality with glycoprotein acetyls in children. Increased level of glycoprotein acetyls, assessed by nuclear magnetic resonance (NMR) spectroscopy, is a composite biomarker

of systemic inflammation that have been independently associated with increased risk of type 2 diabetes, cardiovascular diseases, cardiovascular mortality (Connelly et al., 2017; Kettunen et al., 2018).

We investigated the associations of ST, screen time, PA at different intensities, and diet quality with biomarkers for inflammation, including hs-CRP, leptin, IL-6, adiponectin, TNF-α, and glycoprotein acetyls, in a population sample of children. We also investigated the modifying effect of BF% on these associations. Finally, we studied the modifying effect of diet quality on the associations of ST, screen time, and PA at different intensities with these biomarkers of inflammation.

Methods

Study design and study 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 (Eloranta et al., 2011). The Research Ethics Committee of the Hospital District of Northern Savo 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 6–8 years of age from primary schools of 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 standard deviation score (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 on the associations of ST, media time, PA, and diet quality with inflammatory biomarkers were available for 390 children (192 girls, 198 boys).

Assessment of body size, body composition, and pubertal status

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, Seoul, South Korea) to an accuracy of 0.1 kg. The mean of these two values was used in the analyses. Stature 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 stature (m) squared. BMI-SDS was calculated based on Finnish reference data (Saari et al., 2011). The prevalence of overweight and obesity was defined using the cut-off values provided by Cole et al. (2000). Body fat percentage (BF%) was measured by the Lunar® dual-energy X-ray absorptiometry device (GE Medical Systems, Madison, WI, USA) using a standardised protocol.

A research physician assessed pubertal status using a 5-stage scale described by Marshall and Tanner (1969, 1970). Boys were defined as having entered clinical puberty if their testicular volume assessed by an orchidometer was ≥4 mL (stage ≥2). Girls were defined having entered clinical puberty if their breast development had started (stage ≥2).

Assessment of biomarkers for inflammation

Venous blood samples were taken 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, Mannheim, Germany) 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, Turku, Finland). Commercially available ELISA kits were employed for the measurement of plasma IL-6 and TNF-α concentrations (Sanquin Reagents, Amsterdam, The Netherlands). Serum high-molecular-weight adiponectin concentration was analysed using an ELISA kit after a specific proteolytic digestion of other multimeric adiponectin forms (Millipore, Billerica, MA, USA). The Nightingale high-throughput NMR metabolomics platform was used to quantify plasma glycoprotein acetyls (Soininen et al., 2015).

Assessment of sedentary time and physical activity

ST and PA were assessed using a combined heart rate and movement sensor (Actiheart®, CamNtech Ltd., Papworth, UK) for a minimum of four consecutive days without

interruption, including two weekdays and two weekend days, analysed in 60 s epochs (Brage et al., 2005; Collings et al., 2017). The combined heart rate and movement sensor was attached to the child's chest with two standard eletrocardiogram electrodes (Bio Protech Inc, Wonju, South Korea). The children were asked to wear the monitor continuously, including sleep and water-based activities, and not to change their usual behaviour during the monitoring period. Data on heart rate were cleaned and individually calibrated with parameters from the maximal exercise test and combined with movement sensor data to derive PA energy expenditure (PAEE). Instantaneous physical activity energy expenditure (PAEE), i.e. PA intensity, was estimated using branched equation modelling as explained, in detail earlier (Brage et al., 2007) and summarised as daily PA volume ($kJ \cdot day^{-1} \cdot /kg^{-1}$) and time spent at certain levels of standard metabolic equivalents of task (METs) in minutes per day, weighting all hours of the day equally to reduce diurnal bias caused by imbalances in wear-time. Initially, the summarised data included 25 narrowly defined intensity categories. For the present analyses, we re-categorised these intensity categories into a broader format of sedentary time (≤1.5 METs), light PA (1.5-4 METs), moderate PA (>4-7 METs), vigorous PA (>7 METs) and MVPA (>4 METs), which have been commonly applied in investigations of PA among children and youth. In order to estimate the time spent sedentary whilst awake, we subtracted average daily sleep duration from total ST. We only included children who had sufficient valid data, i.e. a recording period of at least 48 h of wear data with the additional requirement that enough data were included from all four quadrants of a 24 h day to avoid bias from over-representation of specific parts of days (Brage et al., 2013). This resulted in at least 12 h of wear data from morning (3 am – 9 am), noon (9 am – 3 pm), afternoon / evening (3 pm – 9 pm), and night (9 pm – 3 am). Screen time was assessed by the PANIC Physical Activity Questionnaire filled out at home by the parents or caregivers of the children. Screen time included watching TV and videos, using a computer and playing video games, and using a mobile phone and playing mobile games. Screen time was calculated by summing up times spent in each type of screen time and was expressed in hours per week weighted by the numbers of weekdays and weekend days.

Assessment of diet quality

Food consumption and nutrient intake were assessed by food records administered by the parents on four predefined consecutive days, including two weekdays and two weekend days (99.5% of participants) or three week- days and one weekend day (0.5% of participants), as described previously (Eloranta et al., 2011). The food records were analysed using the Micro Nutrica dietary analysis software, Version 2.5 (The Social Insurance Institution of Finland). We used the Finnish Children Healthy Eating Index (FCHEI) as a measure of overall diet quality (Kyttälä et al., 2014). FCHEI summarises the consumption of vegetables, fruit, and berries; vegetable oils and vegetable oil-based margarine; foods containing high amounts of sugar; fish; and low-fat (<1%) milk based on deciles of these dietary variables in the study population. A higher scores indicate a better diet quality.

Other assessments

Parental education was used as a measure of socioeconomic status. The parents were asked to report in a questionnaire their completed or ongoing educational degrees (vocational school or less, polytechnic or university). The degree of the more educated parent was used in the analyses.

Statistical methods

Statistical analyses were performed by the SPSS statistical software, version 25.0 (IBM corp. Armonk, NY, USA). 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 of ST, screen time, PA, and diet quality with biomarkers for inflammation were investigated using linear regression analyses adjusted for age and sex. The data were further adjusted for BF% and parental education. To study the modifying effect of BF% and FCHEI, we dichotomised BF% using sex-specific medians and FCHEI using a sample-specific median. The modifying effect of BF% on the associations of ST, media time, PA, and diet quality with biomarkers for inflammation and modifying effect of FCHEI on the associations of ST, media time, and PA with biomarkers for inflammation were investigated using general linear models adjusted for age and sex.

Results

Characteristics of children

Girls were shorter and lighter and had higher BF% than boys (Table 1). Girls also accumulated less screen time, MPA, and VPA, and had lower PAEE than boys. Moreover, girls had higher leptin and lower IL-6 and TNF-α than boys.

Table 1. Characteristics of children.

	All	Girls	Boys	*P*
Age (years)	7.6 (0.4)	7.6 (0.4)	7.7 (0.4)	0.171
Stature (cm)	128.8 (5.5)	127.6 (5.5)	129.9 (5.3)	<0.001
Weight (kg)	25.9 (5.5)[1]	25.4 (5.8)	26.8 (5.5)	0.011
Body mass index standard deviation score	−0.21 (1.1)	−0.22 (1.1)	−0.19 (1.1)	0.802
Body fat percentage (%)	18.9 (10.2)[1]	20.3 (9.3)	15.9 (9.8)	<0.001
Prevalence of overweight and obesity	8.2 / 3.6	9.4 / 3.6	7.1 / 3.5	0.705
Proportion of children entered to clinical puberty (%)	2.1	2.6	1.5	0.448
Sedentary time (min/d)	205 (171)[1]	211 (182)	203 (156)	0.117
Media time (min/d)	94.3 (62.1)[1]	86.8 (55.7)	98.6 (60.0)	<0.001
Light physical activity (min/d)	510 (106)	518 (110)	503 (102)	0.170
Moderate physical activity (min/d)	81.4 (57.9)[1]	73.0 (56.9)	86.7 (66.8)	<0.001
Vigorous physical activity (min/d)	17.0 (27.5)[1]	12.0 (18.2)	23.0 (38.5)	<0.001
Moderate-to-vigorous physical activity (min/d)	102 (83.4)[1]	88.6 (73.4)	128 (92.7)	<0.001
Physical Activity Energy Expenditure (kJ/kg/d)	97.5 (31.8)	89.3 (28.3)	105 (33.1)	<0.001
Finnish Children Healthy Eating Index	23.1 (7.1)	23.5 (6.5)	22.7 (7.7)	0.294
hS-CRP (mg/L)	0.3 (0.3)[1]	0.29 (0.33)	0.29 (0.22)	0.052
Leptin (ng/mL)	3.7 (3.1)[1]	4.3 (3.8)	3.1 (2.3)	<0.001
Interleukin (IL)−6 (pg/mL)	0.9 (0.9)[1]	0.8 (0.7)	1.1 (0.9)	0.005
High-molecular-weight adiponectin (µg/mL)	8.1 (5.5)[1]	7.8 (5.5)	8.4 (5.5)	0.363
Tumour necrosis factor (TNF)-a (pg/mL)	15.8 (31.8)[1]	13.4 (27.5)	17.8 (36.3)	0.037
Glycoprotein acetyls (mmol/L)	0.76 (0.11)	0.76 (0.11)	0.76 (0.11)	0.759

Data are from the Student *t*-test or Mann-Whitney U test for continuous variables and from the Chi-square test for categorical variables and are displayed as means (SD), medians (IQR), or percentages (%).

Associations of sedentary time, physical activity, and diet quality with biomarkers for inflammation

ST was directly and MPA, VPA, MVPA, and PAEE inversely associated with hs-CRP and IL-6 after adjustment for age and sex (Table 2). ST was directly and LPA, MPA, VPA, MVPA, and PAEE inversely associated with leptin after adjustment for age and sex. MPA, MVPA, and PAEE were inversely associated with glycoprotein acetyls after adjustment for age and sex. However, none of these associations were statistically significant after further adjustment for BF% (p=0.084–0.895). FCHEI was not associated with biomarkers for inflammation after adjustment for age and sex. BF% was directly associated with hs-CRP, leptin, IL-6, and glycoprotein acetyls. Further adjustment for parental education had no effect on these associations.

Modifying effect of body fat percentage on the associations of sedentary time, physical activity, and diet quality with biomarkers for inflammation

ST time directly associated with hs-CRP in children with higher BF% (β=0.160, 95% CI=0.018–0.301, p=0.027) but not among those with lower BF%

Table 2. Associations of sedentary time, physical activity, and diet quality with biomarkers for inflammation in children.

	Hs-CRP	Leptin	Interleukin-6	Adiponectin	Tumour necrosis factor-α	Glycoprotein acetyls
Media time	0.062 (−0.040–0.164)	−0.012 (−0.109–0.086)	0.014 (−0.088–0.115)	−0.046 (−0.148–0.056)	0.033 (−0.069–0.134)	−0.057 (−0.159–0.045)
ST	**0.170 (0.070–0.269)****	**0.355 (0.265–0.445)*****	**0.105 (0.005–0.205)***	−0.030 (−0.131–0.070)	−0.033 (−0.133–0.068)	0.100 (0.000–0.201)
LPA	−0.070 (−0.171–0.030)	**−0.201 (−0.295 to −0.107)*****	−0.036 (−0.136–0.064)	0.040 (−0.060–0.140)	0.061 (−0.039–0.161)	−0.041 (−0.141–0.059)
MPA	**−0.168 (−0.269 to −0.067)****	**−0.301 (−0.395 to −0.208)*****	**−0.114 (−0.216 to −0.013)***	0.004 (−0.099–0.106)	−0.030 (−0.132–0.072)	**−0.103 (−0.205 to −0.001)***
VPA	**−0.139 (−0.233 to −0.024)***	**−0.318 (−0.414 to −0.222)*****	**−0.107 (−0.211 to −0.002)***	−0.053 (−0.158–0.052)	−0.061 (−0.166–0.045)	−0.080 (−0.186–0.132)
MVPA	**−0.191 (−0.294 to −0.088)*****	**−0.372 (−0.465 to −0.279)*****	**−0.136 (−0.240 to −0.033)***	−0.015 (−0.120–0.090)	−0.047 (−0.152–0.057)	**−0.117 (−0.221 to −0.013)***
PAEE	**−0.184 (−0.286 to −0.083)*****	**−0.409 (−0.499 to −0.318)*****	**−0.104 (−0.206 to −0.001)***	−0.022 (−0.128–0.081)	−0.017 (−0.120–0.086)	**−0.110 (−0.213 to −0.007)***
FCHEI	0.043 (−0.057–0.144)	0.000 (−0.096–0.097)	0.032 (−0.068–0.133)	−0.010 (−0.110–0.091)	0.015 (−0.085–0.116)	0.045 (−0.055–0.146)
BF%	**0.382 (0.285–0.480)*****	**0.851 (0.797–0.904)*****	**0.131 (0.027–0.235)***	−0.043 (−0.148–0.062)	−0.013 (−0.118–0.091)	**0.302 (0.201–0.403)*****

Notes: Data are standardised regression coefficient and their 95% confidence intervals from multivariate linear regression analyses adjusted for age and sex. ST, sedentary time; LPA, light physical activity; MPA, moderate intensity physical activity; VPA, vigorous intensity physical activity; PAEE, physical activity energy expenditure; MVPA, moderate-to-vigorous physical activity; FCHEI, Finnish Children Healthy Eating Index; BF%, body fat percentage.
*p<0.05; **p<0.01; ***p<0.001.

(β=0.071, 95% CI=−0.072–0.214, p=0.329, p=0.055 for interaction).

VPA was inversely associated with hs-CRP (p=0.003 for interaction), leptin (p=0.034 for interaction), IL-6 (p=0.036 for interaction), and glycoprotein acetyls (p=0.027 for interaction) in children with higher BF% but not in children with lower BF% (Table 3). MVPA was inversely associated with hs-CRP in children with higher BF% but not among those with lower BF% (p=0.019 for interaction). PAEE was inversely associated with CRP in children with higher BF% but not among those with lower BF% (p=0.019 for interaction) (Table 3). PAEE was also inversely associated with adiponectin in children with lower BF% (β=−0.160, 95% CI=−0.308 to −0.012, p=0.034) but not among those with higher BF% (β=0.088, 95% CI=−0.057–0.233, p=0.231, p=0.016 for interaction).

Modifying effect of diet quality on the associations of sedentary time, physical activity, and diet quality with inflammatory biomarkers

MPA was inversely associated with hs-CRP in children with a lower FCHEI (β=−0.254, 95% CI=−0.397 to −0.112, p=0.001) but not among those with a higher FCHEI (β=−0.084, 95% CI=−0.229–0.061, p=0.253, p=0.036 for interaction) after adjustment for age and sex. MVPA was inversely associated with hs-CRP in children with a lower FCHEI (β=−0.283, 95% CI=−0.427 to −0.139, p<0.001) but not among those with a higher FCHEI (β=−0.092, 95% CI=−0.240–0.056, p=0.221, p=0.034 for interaction). PAEE was inversely associated with hs-CRP in children with a lower FCHEI (β=−0.256, 95% CI=−0.400 to −0.111, p=0.001) but not among those with a higher FCHEI (β=−0.107, 95% CI=−0.253–0.038, p=0.147, p=0.053 for interaction). However, the associations of MPA (β=−0.121, 95% CI=−0.259–0.018, p=0.088), MVPA (β=−0.117, 95% CI=−0.264–0.029, p=0.117), and PAEE (β=−0.057, 95% CI=−0.208–0.094, p=0.456) with hs-CRP in children with lower FCHEI attenuated markedly and were no longer statistically significant after further adjustment for BF%

Discussion

We found that BF% was strongly and directly associated with circulating levels of biomarkers for inflammation and it also largely explained the associations of ST, MPA, VPA, MVPA, and PAEE with these biomarkers for inflammation in children 6–8 years of age. We also observed that higher levels of ST and lower levels of PA were associated with higher circulating levels of biomarkers for inflammation in children with a higher BF% but not among those with a lower BF%. Moreover, we found that PA was inversely associated with circulating levels of biomarkers for inflammation in children with poorer diet quality but not among those with better diet quality. These inverse associations were mainly observed between PA at least moderate intensity and hs-CRP, leptin, IL-6, and glycoprotein acetyls.

In contrast to the results of some previous studies in children (Poitras et al., 2016), we found inverse associations of PA with hs-CRP, leptin, IL-6 and direct associations of ST with hs-CRP, leptin, and IL-6 in children. Individual biomarkers of inflammation often exhibit a high intra-individual variability (Connelly et al., 2017). Therefore, our findings on the inverse associations of MPA, MVPA, and PAEE with glycoprotein acetyls derived from NMR metabolomics, a composite indicator of systemic inflammation providing novel and more stable (Connelly et al., 2017) evidence on the inverse association between PA and systemic inflammation. However, all of these associations in our study were largely explained by BF%. Our results are supported by evidence suggesting that reductions in body fat mass mediates the positive effects of exercise training on

Table 3. Associations of physical activity with biomarkers for inflammation in children with higher or lower body fat percentage.

	Hs-CRP		Leptin		Interleukin-6		Glycoprotein acetyls	
	Body fat percentage		Body fat percentage		Body fat percentage		Body fat percentage	
	<median	≥median	<median	≥median	<median	≥median	<median	≥median
VPA	0.031 (−0.122–0.184)	**−0.186 (−0.332 to −0.039)***	−0.007 (−0.146–0.131)	**−0.344 (−0.477 to −0.212)*****	0.033 (−0.183–0.117)	**−0.181 (−0.328 to −0.033)***	0.060 (−0.092–0.213)	−0.143 (−0.291–0.005)
MVPA	−0.070 (−0.221–0.080)	**−0.215 (−0.360 to −0.069)****						
PAEE	−0.058 (−0.209–0.092)	**−0.193 (−0.336 to −0.050)****						

Note: Data are standardised regression coefficient and their 95% confidence intervals from multivariate linear regression analyses adjusted for age and sex. VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity; PAEE, physical activity energy expenditure.
*p<0.05; **p<0.01; ***p<0.001.

biomarkers for inflammation (García-Hermoso et al., 2017). Nevertheless, MVPA was inversely associated with hs-CRP, IL-6, and adiponectin and ST was directly associated with adiponectin independent of adiposity in Danish children (Nielsen et al., 2016). Furthermore, in contrast to the results of some previous studies (Dong et al., 2017), we found no association between screen time and biomarkers for inflammation. However, we observed that ST was directly associated with biomarkers for inflammation although these associations were mostly explained by BF%. The inconsistent findings may be explained by differences in maturation status and PA levels between study populations and methods used to assess screen time and ST. Furthermore, it is possible that increased ST, not screen time per se, is associated with increased systemic inflammation through increased adiposity. Therefore, these results together suggest that increased levels of PA have potential to decrease systemic inflammation, but it is possible that the associations are mediated by decreased adiposity.

We found that PA at least at moderate intensity was associated with biomarkers for inflammation only in children with a higher BF%. These results agree with our previous observations suggesting that higher levels of MVPA and lower levels of ST were associated with lower insulin resistance only in children with higher levels of adiposity (Haapala et al., 2020) suggesting that increasing PA could decrease systemic inflammation and insulin resistance particularly among overweight and obese children. In contrast to the results of some intervention studies in youth showing that exercise training increased adiponectin in obese children (García-Hermoso et al., 2017), we observed an inverse association between PAEE and adiponectin in children with lower BF% and no statistically significant association in children with higher BF%. Furthermore, Nielssen et al. (2016) suggested that the negative association between MVPA and adiponectin could be due to lower insulin resistance in children with higher levels of MVPA as they also reported an inverse association between insulin resistance and adiponectin (Nielsen et al., 2016). Similarly, Su et al. (2011) reported that individuals with lower levels of insulin and body fat mass also had lower adiponectin than those with higher levels of insulin and fat mass. We have previously reported that children with higher levels of MVPA or PAEE and lower levels of adiposity have lower insulin resistance than other children (Haapala et al., 2020). It is, therefore, possible that the inverse association between PAEE and adiponectin in the present study and between MVPA and adiponectin in the study by Nielssen et al. is due to adaptive mechanisms driven by insulin resistance (Nielsen et al., 2016). We have observed that PAEE, from all measure of PA in our study, has the strongest association with insulin resistance (Haapala et al., 2020; Väistö et al., 2019) and BF% (Väistö et al., 2019), which may explain why PAEE also had the strongest association with adiponectin in the present study.

We found that BF% was the strongest determinant of biomarkers for inflammation and even if the mechanisms of low-grade inflammation are not fully understood, it has been shown that excess adiposity leads to secretion of several cytokines and adipokines that activate the immune system. Gleeson et al. suggested that both the reduction in visceral fat mass and the anti-inflammatory environment induced by each exercise session might elicit long-term anti-inflammatory effects (Gleeson et al., 2011).

In line with the results of previous observational studies in children, we did not find statistically significant associations between diet quality and biomarkers for inflammation (Liese et al., 2018). One reason for not observing such associations is that children in our population sample were relatively healthy and most of them were normal weight and physically active. However, we found that higher levels of PA were associated with lower levels of systemic low-grade inflammation in children with poorer diet quality. These results suggest that poor diet quality together with low PA levels may increase systemic low-grade inflammation in children, although diet quality did not have independent associations with biomarkers for inflammation. Nevertheless, the associations of PA at different intensity levels with hs-CRP in children with poorer diet quality were partly explained by BF% suggesting that adiposity may also mediate the combined association of diet quality and PA with biomarkers for inflammation.

The strengths of the present study include a relatively large and representative sample of children with valid and reproducible assessment of free-living PA and ST by individually calibrated combined movement and heart rate sensing, diet quality by 4-day dietary records, body composition using whole-body dual-energy X-ray absorptiometry, and several biomarkers for inflammation in a population sample of children. However, we used 60 s epochs in the assessment of ST and PA because of limited memory capacity of the monitor. This may have influenced especially the amount of VPA in the current study (Gao et al., 2018) and weaken the associations of VPA with biomarkers for inflammation. Our study was cross sectional which limits our ability to make causal inferences. Furthermore, our study included mainly Caucasian children and therefore our results cannot be directly generalised to the

samples of different ethnical backgrounds. Systemic low-grade inflammation and its relationship to the pathogenesis of cardiometabolic diseases is multifaceted, and no single optimal biomarker for it exists. Although we studied the associations ST, PA, and diet quality with several possible biomarkers for inflammation, there are many other pro- or anti-inflammatory factors that could have been measured. Finally, the applicability and clinical relevance of these biomarkers for inflammation in identifying children at increased risk of cardiometabolic diseases remains unclear.

In conclusion, we found that higher levels of MPA, VPA, MVPA, and PAEE and lower levels of ST were associated with lower circulating levels of biomarkers of inflammation among children, but these associations were largely explained by BF%. Furthermore, we observed that PA was inversely associated with these biomarkers for inflammation in children with a higher BF%. In addition, our study provides some evidence that higher levels of PA could improve circulating biomarkers of inflammation in children with poorer diet quality. More research on the long-term effects of PA and diet as well as their synergistic effects on biomarkers for inflammation is warranted.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The PANIC Study has financially been supported by Ministry of Education and Culture of Finland, Ministry of Social Affairs and Health of Finland, 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 Paediatric Research, Diabetes Research Foundation in Finland, Finnish Foundation for Cardiovascular Research, Juho Vainion Säätiö Vainio Foundation, Paavo Nurmen Säätiö Nurmi Foundation, Yrjö Jahnssonin Säätiö Jahnsson Foundation, and the city of Kuopio. Moreover, the PhD students and postdoctoral researchers of The PANIC Study have been supported by Program for Clinical Research and Program for Health Sciences of Doctoral School of University of Eastern Finland, Finnish Doctoral Programs in Public Health, Päivikki and Sakari Sohlberg Foundation, Paulo Foundation, Jalmari and Rauha Ahokas Foundation, Aarne and Aili Turunen Foundation, Finnish Medical Foundation, Jenny and Antti Wihuri Foundation, Kuopio Naturalists' Society, Olvi Foundation, and the city of Kuopio. SB was supported by UK Medical Research Council (MC_UU_12015/3) and the NIHR Biomedical Research Centre Cambridge [IS-BRC-1215-20014]. The sponsors had no role in designing the study, the collection, analysis, or interpretation of the data, the writing of the report, or the decision to submit the manuscript for publication.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, [EAH], upon reasonable request.

Author contributions

EAH analysed the data. EAH and JKI drafted the manuscripts. JV, AV, TS, A-ME, UE, US, SB, MA, and TAL collected and processed the data for analyses. MHL, AV contributed to planning the manuscript and interpreting the results and reviewed the manuscript. All authors provided significant intellectual contribution to the manuscript.

ORCID

Eero A. Haapala http://orcid.org/0000-0001-5096-851X
Juuso Väistö http://orcid.org/0000-0001-7026-5934
Johanna K. Ihalainen http://orcid.org/0000-0001-9428-4689
Marja H. Leppänen http://orcid.org/0000-0001-6933-8809
Aapo Veijalainen http://orcid.org/0000-0002-6838-5061
Taisa Sallinen http://orcid.org/0000-0003-2769-6338
Aino-Maija Eloranta http://orcid.org/0000-0002-0290-8245
Ulf Ekelund http://orcid.org/0000-0003-2115-9267
Ursula Schwab http://orcid.org/0000-0003-1838-7525
Soren Brage http://orcid.org/0000-0002-1265-7355
Timo A. Lakka http://orcid.org/0000-0002-9199-2871

References

Arouca, A. B., Santaliestra-Pasías, A. M., Moreno, L. A., Marcos, A., Widhalm, K., Molnár, D., … Michels, N. (2019). Diet as a moderator in the association of sedentary behaviors with inflammatory biomarkers among adolescents in the HELENA study. *European Journal of Nutrition*, *58*, 2051–2065. doi:10.1007/s00394-018-1764-4

Baigent, C., Catapano, A. L., Koskinas, K. C., Casula, M., Badimon, L., Chapman, M. J., … Patel, R. S. (2020). 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *European Heart Journal*, *41*, 111–188.

Banfield, E. C., Liu, Y., Davis, J. S., Chang, S., & Frazier-Wood A. C. (2016). Poor adherence to US dietary Guidelines for children and adolescents in the national Health and Nutrition examination survey population. *Journal of the Academy of Nutrition and Dietetics*, *116*, 21–27. doi:10.1016/j.jand.2015.08.010

Blüher, S., Panagiotou, G., Petroff, D., Markert, J., Wagner, A., Klemm, T., … Mantzoros, C. S. (2014). Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. *Obesity*, *22*, 1701–1708. doi:10.1002/oby.20739

Brage, S., Brage, N., Franks, P. W., Brage, S., Brage, N., Franks, P. W., … Wareham, N. J. (2005). Reliability and validity of the combined heart rate and movement sensor actiheart. *European Journal of Clinical Nutrition*, *59*, 561–570. doi:10.1038/sj.ejcn.1602118

Brage, S., Ekelund, U., Brage, N., Hennings, M. A., Froberg, K. … Franks, P. W., (2007). Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity. *Journal of Applied Physiology*, *103*, 682–692. doi:10.1152/japplphysiol.00092.2006

Brage, S., Westgate, K., Wijndaele, K.,Godinho, J., Griffing, S., & Wareham, N. (2013). *Evaluation of a method for minimising diurnal information bias in objective sensor data*. Int Conf Amb Mon Phys Act Mov, (Conference Proceeding).

Carson, V., & Janssen, I. (2011). Volume, patterns, and types of sedentary behavior and cardio-metabolic health in children and adolescents: A cross-sectional study. *BMC Public Health*, *11*, 274. doi:10.1186/1471-2458-11-274

Cole, T. J., Bellizzi, M. C., Flegal, K. M., & Dietz, W. H. (2000). Establishing a standard definition for child overweight and obesity worldwide: International survey. *BMJ*, *320*, 1240. doi:10.1136/bmj.320.7244.1240

Collings, P. J., Westgate, K., Väistö, J., Wijndaele, K., Atkin, A. J., Haapala, E. A. … Lintu, N. (2017). Cross-Sectional associations of objectively-measured physical activity and sedentary time with Body composition and cardiorespiratory fitness in mid-childhood: The PANIC study. *Sports Medicine*, *47*, 769–780. doi:10.1007/s40279-016-0606-x

Connelly, M. A., Otvos, J. D., Shalaurova, I., Playford, M. P., & Mehta, N. N. (2017). Glyca, a novel biomarker of systemic inflammation and cardiovascular disease risk. *Journal of Translational Medicine*, *15*, 219. doi:10.1186/s12967-017-1321-6

Cooper, A. R., Goodman, A., Page, A. S., Sherar, L. B., Esliger, D. W., van Sluijs, E. M. F., … Ekelund, U. (2015). Objectively measured physical activity and sedentary time in youth: The International children's accelerometry database (ICAD). *International Journal of Behavioral Nutrition and Physical Activity*, *12*, 113. doi:10.1186/s12966-015-0274-5

Dong, F., Howard, A. G., Herring, A. H., Thompson, A. L., Adair, L. S., Popkin, B. M … Gordon-Larsen, P. (2017). Longitudinal associations of away-from-home eating, snacking, screen time, and physical activity behaviors with cardiometabolic risk factors among Chinese children and their parents. *The American Journal of Clinical Nutrition*, *106*, 168–178. doi:10.3945/ajcn.116.146217

Eloranta, A. M., Lindi, V., Schwab, U., Kiiskinen, S., Kalinkin, M., Lakka, H. M., … Lakka, T. A. (2011). Dietary factors and their associations with socioeconomic background in Finnish girls and boys 6–8 years of age: The PANIC study. *European Journal of Clinical Nutrition*, *65*, 1211–1218. doi:10.1038/ejcn.2011.113

Eloranta, A. M., Schwab, U., Venäläinen, T., Kiiskinen, S., Lakka, H.M., Laaksonen D.E. … Lindi, V. (2016). Dietary quality indices in relation to cardiometabolic risk among Finnish children aged 6–8 years – The PANIC study. *Nutrition, Metabolism and Cardiovascular Diseases*, *26*, 833–841. doi:10.1016/j.numecd.2016.05.005

Gabel, L., Ridgers, N. D., Gatta, P. A. D., Arundell L., Cerin E., Robinson S. … Salmon, J. (2016). Associations of sedentary time patterns and TV viewing time with inflammatory and endothelial function biomarkers in children. *Pediatric Obesity*, *11*, 194–201. doi:10.1111/ijpo.12045

Gao, Y., Melin, M., Mäkäräinen, K., Rantalainen, T., Pesola, A. J., Laukkanen, A. … Finni, T. (2018). Children's physical activity and sedentary time compared using assessments of accelerometry counts and muscle activity level. *PeerJ*, *6*, e5437. doi:10.7717/peerj.5437

García-Hermoso, A., Ceballos-Ceballos, R. J. M., Poblete-Aro, C. E., Hackney, A. C., Mota, J., & Ramírez-Vélez, R. (2017). Exercise, adipokines and pediatric obesity: A meta-analysis of randomized controlled trials. *International Journal of Obesity*, *41*, 475–482. doi:10.1038/ijo.2016.230

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., & Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. *Nature Reviews Immunology*, *11*, 607–615. doi:10.1038/nri3041

Guinhouya, B. C., Samouda, H., & de Beaufort, C. (2013). Level of physical activity among children and adolescents in Europe: A review of physical activity assessed objectively by accelerometry. *Public Health*, *127*, 301–311. doi:10.1016/j.puhe.2013.01.020

Haapala, E. A., Wiklund, P., Lintu, N., Tompuri, T., Väistö, J., Finni, T. … Lakka, T. A. (2020). Cardiorespiratory Fitness, physical activity, and insulin resistance in children. *Medicine & Science in Sports & Exercise*, *52*, 1144–1152. doi:10.1249/MSS.0000000000002216

Han, Y., Liu, Y., Zhao, Z., Zhen, S., Chen, J., Ding, N. … Wen, D. (2019). Does physical activity-based intervention improve systemic proinflammatory cytokine levels in overweight or obese children and adolescents? Insights from a meta-analysis of Randomized control trials. *Obesity Facts*, *12*, 653–668.

Juonala, M., Magnussen, C. G., Berenson, G. S., Venn, A., Burns, T. L., Sabin, M. A. … Raitakari, O. T. (2011). Childhood adiposity, adult adiposity, and cardiovascular risk factors. *New England Journal of Medicine*, *365*, 1876–1885.

Kettunen, J., Ritchie Scott, C., Olga, A., Lyytikäinen, L-P., Hernesniemi, J., Karhunen, P. J. … Würtz, P. (2018). Biomarker glycoprotein acetyls is associated with the risk of a wide spectrum of incident diseases and stratifies mortality risk in angiography patients. *Circulation: Genomic and Precision Medicine*, *11*, e002234.

Kyttälä, P., Erkkola, M., Lehtinen-Jacks, S., Ovaskainen, M-L., Uusitalo, L., Veijola, R … Virtanen, S. M. (2014). Finnish Children Healthy Eating Index (FCHEI) and its associations with family and child characteristics in pre-school children. *Public Health Nutrition*, *17*, 2519–2527. doi:10.1017/S1368980013002772

Liese, A. D., Ma, X., Ma, X., Mittleman, M. A., The, N. S., Standiford, D. A … Puett, R. C. (2018). Dietary quality and markers of inflammation: No association in youth with type 1 diabetes in the SEARCH for diabetes in youth study. *Journal of Diabetes and its Complications*, *32*, 179–184. doi:10.1016/j.jdiacomp.2017.10.015

Lund, A. V., Thostrup, A. H., Frithioff-Bøjsøe, C., Lausten-Thomsen, U., Hedley, P. L., Pedersen, O … Holm, J-C. (2020). Low-grade inflammation independently associates with cardiometabolic risk in children with overweight/obesity. *Nutrition, Metabolism and Cardiovascular Diseases*, *30*, 1544–1553.

Magnussen, C. G., Koskinen, J., Chen, W., Thomson, R., Schmidt, M. D., Srinivasan, S. R. … Raitakari, O. T. (2010). Pediatric metabolic syndrome predicts adulthood metabolic syndrome, subclinical atherosclerosis, and type 2 diabetes mellitus but is no better than body mass index alone. *Circulation*, *122*, 1604–1611.

Marshall, W. A., & Tanner, J. M. (1969). Variations in pattern of pubertal changes in girls. *Archives of Disease in Childhood*, *44*, 291–303.

Marshall, W. A., & Tanner, J. M. (1970). Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood*, *45*, 13–23.

Nemet, D., Oren, S., Pantanowitz, M., & Eliakim, A. (2013). Effects of a multidisciplinary Childhood obesity treatment intervention on adipocytokines, inflammatory and growth mediators. *Hormone Research in Paediatrics*, *79*, 325–332.

Nielsen, M. S., Quist, J. S., Chaput, J.-P., Dalskov, S-M., Damsgaard, C. T., Ritz, C … Hjorth, M. F. (2016). Physical activity, sedentary time, and sleep and the association with inflammatory markers and adiponectin in 8- to 11-year-Old Danish children. *Journal of Physical Activity and Health*, *13*, 733–739. doi:10.1123/jpah.2015-0123

Norde, M. M., Collese, T. S., Giovannucci, E., & Rogero, M. M. (2021). A posteriori dietary patterns and their association with systemic low-grade inflammation in adults: A systematic review and meta-analysis. *Nutrition Reviews*. *79*, 331–350.

Poitras, V. J., Gray, C. E., Borghese, M. M., Carson, V., Chaput, J-P., Janssen, I., … Tremblay, M. S. (2016). Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition, and Metabolism*, *41*, S197–S239. doi:10.1139/apnm-2015-0663

Roberts, C. K., Izadpanah, A., Angadi, S. S., & Barnard, R. J. (2013). Effects of an intensive short-term diet and exercise intervention: Comparison between normal-weight and obese children. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *305*, R552–R557. doi:10.1152/ajpregu.00131.2013

Rodríguez-Monforte, M., Flores-Mateo, G., & Sánchez, E. (2015). Dietary patterns and CVD: A systematic review and meta-analysis of observational studies. *British Journal of Nutrition*, *114*, 1341–1359. doi:10.1017/S0007114515003177

Saari, A., Sankilampi, U., Hannila, M.-L., Kiviniemi, V., Kesseli, K., & Dunkel L. (2011). 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. *Annals of Medicine*, *43*, 235–248. doi:10.3109/07853890.2010.515603

Saneei, P., Hashemipour, M., Kelishadi, R., & Esmaillzadeh, A. (2014). The dietary approaches to stop hypertension (DASH) diet affects inflammation in Childhood metabolic syndrome: A Randomized cross-over clinical trial. *Annals of Nutrition and Metabolism*, *64*, 20–27.

Shalitin, S., Ashkenazi-Hoffnung, L., Yackobovitch-Gavan, M., Nagelberg, N., Karni, Y., Hershkovitz, E., … Phillip M. (2009). Effects of a twelve-week Randomized intervention of exercise and/or diet on weight loss and weight maintenance, and other metabolic parameters in obese preadolescent children. *Hormone Research*, *72*, 287–301.

Sirico, F., Bianco, A., D'Alicandro, G., Castaldo, C., Montagnani, S., Spera, R., … Nurzynska, D. (2018). Effects of physical exercise on adiponectin, leptin, and Inflammatory Markers in Childhood Obesity: Systematic review and meta-analysis. *Childhood Obesity*, *14*, 207–217. doi:10.1089/chi.2017.0269

Soininen, P., Kangas, A. J., Würtz, P., Suna, T., & Ala-Korpela, M. (2015). Quantitative Serum Nuclear magnetic resonance metabolomics in cardiovascular Epidemiology and genetics. *Circulation: Cardiovascular Genetics*, *8*, 192–206. doi:10.1161/CIRCGENETICS.114.000216

Steinberger, J., Daniels, S. R., Hagberg, N., Isasi, C. R., Kelly, A. S., Lloyd-Jones, D. … Zachariah, J. P. (2016). Cardiovascular Health promotion in children: Challenges and opportunities for 2020 and beyond: A scientific Statement from the American heart association. *Circulation*, *134*, e236–e255. doi:10.1161/CIR.0000000000000441

Su, H., Lau, W. B., & Ma, X.-L. (2011). Hypoadiponectinaemia in diabetes mellitus type 2: Molecular mechanisms and clinical significance. *Clinical and Experimental Pharmacology and Physiology*, *38*, 897–904. doi:10.1111/j.1440-1681.2011.05606.x

Väistö, J., Haapala, E. A., Viitasalo, A., Schnurr, T. M., Kilpeläinen, T. O., Karjalainen, P., … Lakka, T. A. (2019). Longitudinal associations of physical activity and sedentary time with cardiometabolic risk factors in children. *Scandinavian Journal of Medicine & Science in Sports*, *29*, 113–123. doi:10.1111/sms.13315

Wahid, A., Manek, N., Nichols, N., Kelly, P., Foster, C., Webster, P. … Scarborough, P. (2016). Quantifying the association between physical activity and cardiovascular disease and diabetes: A Systematic review and meta-analysis. *Journal of the American Heart Association*, *5*, e002495. doi:10.1161/JAHA.115.002495