ORIGINAL ARTICLE

Effects of prolonged sitting and physical activity breaks on measures of arterial stiffness and cortisol in adolescents

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Abstract

Aim: In adults, prolonged periods of sitting have been linked to acute negative effects on vascular structure and function. The aim of this study was to evaluate the acute effects of physical activity (PA) breaks during prolonged sitting on arterial stiffness, cortisol and psychological factors in adolescents.

Methods: Adolescents underwent different short (3-min) breaks starting every 20 min, during 80 min of sitting on three separate days. Breaks were (A) social seated breaks (SOC), (B) low-intensity simple resistance activity PA breaks (SRA) and (C) moderate-intensity step-up PA breaks (STEP). The arterial stiffness measures were augmentation index (AIx), AIx@75 and pulse wave velocity (PWV). Cortisol was measured from saliva. Psychological factors were self-reported.

Results: Eleven girls and six boys (average age 13.6 ± 0.7 years) participated, with average baseline heart rates of 72 ± 11 bpm, systolic/diastolic blood pressure $111 \pm 7/64 \pm 6$ mmHg and cortisol 10.9 ± 5.8 nmoL/L. PWV, cortisol and psychological factors did not change after any of the conditions. AIx@75 increased significantly $(4.9 \pm 8.7 – 9.2 \pm 13.2)$ after the STEP intervention compared with SOC and SRA (time × condition $p < 0.05$).

Conclusion: Arterial stiffness increased after prolonged sitting with frequent, short step-up activity breaks. The results indicate potential important intensity-dependent effects of physical activity on vascular regulation in youth.

KEYWORDS
adolescents, AIx, activity breaks, arterial stiffness, cortisol, PWV

1 | INTRODUCTION

Swedish adolescents spend on average 10.3 h per day sedentary and approximately one in three meet the World Health Organisation's recommendations for physical activity (1). Lack of sufficient physical activity has been linked to altered vascular structure and function in observational studies of children and adolescents (2). In addition, the sedentary time has been shown to be associated with increases in arterial stiffness (3). The negative impact of prolonged sitting on vascular health among adolescents precedes hypertension, and prevention may benefit cardiovascular health later in life (4).

In adults, prolonged periods of sitting have been shown in experimental settings to acutely affect vascular resistance measures such as pulse wave velocity (PWV) and augmentation index (AIx) (5). The stiffening of the arteries increases the pulse wave reflection from peripheral points in the arterial tree, over time resulting in an increased risk of cardiovascular- and/or cerebrovascular diseases (6). Less data on such effects are available on children and adolescents, although uninterrupted sitting for 3 h in young girls has been shown to reduce vascular function, measured by flow-mediated dilatation (7). Effects seen in adults are most pronounced in individuals with challenged metabolic or vascular health (8). As children and adolescents are generally healthier, it is questionable if findings in adults can be extrapolated to younger populations.

Regular physical activity can improve cortisol levels (9) and mood (10), but whether different intensities of acute physical activity breaks in healthy adolescents have similar effects is not fully known.

The aim of the present study was to evaluate the acute effects of light- or moderate-intensity physical activity breaks and seated social breaks during 80 min of prolonged sitting, on arterial stiffness measures, cortisol levels and psychological factors in adolescents.

The specific hypotheses were:

1. Arterial stiffness (measured as AIx (%), AIx@75 and PWV) will be reduced after the physically active break conditions, and these changes will be different from the prolonged sitting condition, where no changes will occur, or an increase in arterial stiffness will be observed.
2. Cortisol-measured stress and other psychological factors, such as self-reported mood, alertness and sleepiness, will change in a favourable direction after the physical activity break conditions, and these changes will be significantly different from those in the prolonged sitting condition, where there will be no change in psychological factors.

1.1 | Participants and methods

This study is part of a larger project called 'Physical activity for healthy brain functions in school youth'. The design of the present study has previously been published in a protocol paper (11). Ethical approval was obtained by the Swedish Ethical Review Authority, Stockholm, Sweden, Dnr 2020–02597. The design of the study

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Key notes

- In healthy adolescents, breaking up prolonged sitting by 3 min of physical activity starting every 20 min, resulted in increased arterial stiffness when the breaks were of moderate intensity but remained unchanged after low-intensity physical activity breaks or uninterrupted sitting.
- Cortisol and psychological factors were not affected.
- Before implementing activity breaks in schools, long-term effects of repeated physical activity breaks should be investigated on vascular health in youth.

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was registered on: www.clinicaltrials.gov, trial registration number: NCT04552626.

1.2 | Recruitment process

Schools that have previously participated in another substudy of the larger project were contacted for the present study. After agreement by teachers and the schools' principals, consent forms were sent home to be signed by both parents. The interested adolescents were then invited to the laboratory at the Swedish School of Sport and Health Sciences (GIH) with their parents for a familiarisation session or the schools were visited by researchers.

1.3 | Participants

Adolescents aged 12–15 years (7th or 8th graders), without ongoing infections or medications that might affect circulatory status were included in the study. Participants were also required to comprehend the study information and instructions. All participants agreed to participate and were informed that they could terminate their participation at any time. As compensation for participating, they received a 600 SEK (~50 Euros) gift card.

1.4 | Study design

We performed a crossover study, where the participants were required to come to the GIH laboratory on three separate occasions for three different conditions. Randomisation of the conditions was computer-generated. On test-days, the participants arrived at the laboratory at 07.30 in a fasting state. To measure heart rate during conditions they were equipped with a chest heart rate ActivPAL monitor (11). They sat at desks, working on school assignments for 80 min with different types of 3-min breaks starting every 20 min (i.e. 17 min of sitting followed by 3 min of activity, repeated four times). The 3-min breaks consisted of (A) social breaks

(SOC), involving a chat between a research staff member and the participant while seated; (B) low-intensity physical activity breaks with simple resistance activity (SRA), including body weight half-squats, calf raises, gluteal contractions and knee raises with standardised video instructions; and (C) physical activity breaks with step-up activity (STEP), performed at moderate intensity at a predetermined pace at around 110 beats per min (bpm). There was a minimum washout period of 1 week between the visits. To standardise the 24 h prior to the test-days, the participants were advised to abstain from heavy physical activity and recorded their activity by wearing an accelerometer. They were also asked to record sleep and dietary intake 24 h prior to the test-day in a diary, to consume the same dinner before each of the test-days and to abstain from caffeine after dinner the nights before experiments. To standardise test-days and minimise physical activity, participants were transported to the laboratory by taxi and a standardised breakfast was served.

1.5 | Arterial stiffness, blood pressure and resting heart rate

Before and 30 min after the prolonged sitting, arterial stiffness was measured as augmentation index (AIx) and pulse wave velocity (PWV) using a SphygmoCor XCEL system (AtCor Medical, Sydney, NSW, Australia) (12). The technology to measure AIx is a noninvasive, reproducible and accurate method that uses the radial pressure waveform to calculate the aortic pressure and waveform. The AIx is derived from the aortic waveform, and augmentation pressure (AP) is defined as the difference between the first and the second systolic peak. In this study AIx is expressed as AIx mmHg (%), (augmentation pressure (AP)/pulse pressure (PP) *100) and as AIx@75 normalised to a heart rate of 75 bpm.

First, the subjects rested for 2 min in a supine position. The arm cuff was positioned on the left arm. Blood pressure, resting heart rate and AIx were automatically determined by the equipment. Then, the leg cuff was positioned on the thigh. The distances from the suprasternal notch to the carotid site, from the suprasternal notch to the femoral site (top of the leg cuff) and from the femoral pulse to the top of the leg cuff were measured using a measuring tape. If the femoral pulse was difficult to detect, the participants were asked to find the pulse themselves and then indicate the location to the data collector. These distances and the previously measured blood pressure were subsequently inputted into the SphygmoCor software to accurately calculate carotid-femoral PWV using the formula: PWV (m/s) = distance between measurement location (m)/transit time (s). Three high-fidelity pressure waveforms were recorded from the carotid site using a carotid tonometer. Simultaneously, the leg cuff captured the blood pressure waveforms at the femoral site. The average of the three high-quality recordings was used to determine PWV (m/s). Pre- and post-heart rate and blood pressure were retrieved at the same time as the measures of arterial stiffness, whereas heart rate was additionally captured during the breaks.

1.6 | Cortisol

Physiological indication of stress was measured using salivary cortisol concentrations. Saliva samples were collected at 0, 30, 45 and 60 min after waking up in the morning, as well as 45 min before and after each of the three 80-min interventions. The participants placed a swab in their mouth and were instructed to chew for 1 min. The swab was then placed in a sample tube. The participants brought the morning samples to the lab. After being collected, the sample tubes were centrifuged for 10 min at 4°C at 2800 rpm and subsequently frozen at minus 80°C. The concentrations were measured using the ELISA kit Abcam, ab154996. First, the reagents were prepared, and then, standards, controls and samples were added to the wells. Secondly, after 1 h of incubation with the cortisol-HRP conjugate, the wells were washed. Thirdly, after washing, TMB substrate was added, and the samples were incubated for 15 min before the addition of the stop solution. The absorbance was measured at 450 nm, and the concentrations (ng/ml) were calculated using the standard curve (two-parameter, single exponential decay equation). Finally, the concentrations were converted to nmol/l.

1.7 | Psychological factors: Mood, alertness and sleepiness

Before the arterial stiffness measurement, mood, alertness and sleepiness were assessed. Mood was assessed using the Positive and Negative Affect Scale (PANAS), which includes 10 positive and 10 negative effects. The participants rated on a 5-point scale (very slightly/not at all; a little; moderately; quite a bit; extremely) the extent to which they experienced the mood at the present moment. Alertness was measured using a simple 10-cm visual analogue scale (VAS), going from 'not at all' to 'completely alert'. Sleepiness was measured using the Karolinska Sleepiness Questionnaire (KSS), where the participants rated on a 9-point scale their current level of sleepiness going from 'extremely alert' to 'very sleepy, great effort to keep awake and fighting sleep'.

1.8 | Statistical analysis

Statistical calculations were performed using Excel (2008 for Windows; Microsoft Co) and IBM SPSS Statistics, version 26 for Windows (IBM Corp). The Kolmogorov–Smirnov and the Shapiro–Wilk tests were used to check the variables for normal distributions, and data were presented as means and standard deviations (SD). Differences between baseline values and values after the different conditions were calculated. Linear mixed-effects models were used to compare pre- and postvalues for the different conditions and to test time × condition interactions. In addition, Tukey's honest significant post-hoc tests were used. Pearson's correlation coefficient (r) was used to study correlations between variables. The level of significance was expressed as $p < 0.05$.

2 | RESULTS

There were 17 study participants (11 girls). There were no significant differences between the sexes in any of the parameters presented in Tables 1 and 2.

Average heart rate (bpm) during the three experimental conditions differed (mean $\pm$ SD) for SOC: 85.1 ± 11.7 bpm, SRA: 114.5 ± 15.9 bpm and STEP: 157.2 ± 14.9 bpm, ($p < 0.05$). No lasting effects of the interventions were noted on heart rate, and systolic and diastolic blood pressure, as all these measures returned to pre-condition values after cessation of the exercise (Table 2).

A significant time $\times$ condition interaction effect was noted for AIx@75 after the STEP intervention compared with after SOC and SRA (Figure 1). Contrary to our first hypothesis, post-hoc analyses revealed a significant increase after STEP condition ($F = 5.57$, df = 2, partial eta = 0.28 and $p < 0.001$), with no significant changes for SOC or SRA. Also, in contrast to our first hypothesis, no time $\times$ condition interaction effects were found for AIx or PWV (Figures 2 and 3). No correlation was observed between baseline heart rate and the baseline arterial stiffness measures. Baseline systolic and diastolic blood pressure were also not correlated to the baseline arterial stiffness measures AIx, AIx@75 or PWV (all $p > 0.05$).

Regarding the second hypothesis, none of the conditions resulted in alterations in cortisol levels (Figure 4). There were also no differences in the morning cortisol levels (0, 30, 45 and 60 min) or between the morning and precondition levels Tables 2 and 3.

Mood, alertness and sleepiness, measured with PANAS, VAS and KSS, respectively, showed no significant time $\times$ condition interactions (all interactions $p > 0.05$) (Table 3).

3 | DISCUSSION

The results from the present study demonstrate that the arterial stiffness measures of augmentation index (AIx %), AIx@75 and PWV did not change after prolonged sitting with social breaks or breaks with simple resistance activity. However, after repeated, short step-up activity breaks, i.e., moderate endurance activity, AIx@75 significantly increased compared with baseline values, which was significantly different from the other two conditions. Cortisol levels remained close to baseline values and none of the self-reported psychological factors were altered by any of the conditions. The increased AIx@75 is in line with results from a systematic review and meta-analysis showing increased AIx@75 immediately after different exercise modes in healthy adults, 18–45 years old (13).

3.1 | Arterial stiffness

In the protocol paper, it was hypothesised that arterial stiffness measures AIx, AIx@75 and PWV would be reduced after the physical activity breaks compared to after prolonged sitting (11). However, we saw an increase in arterial stiffness, specifically in AIx@75. The lack of an effect on AIx and PWV could be explained by the fact that PWV is the gold standard for arterial stiffness measurement most frequently employed in adults, elderly persons and youth at risk for metabolic disorders (4), while AIx is considered as a more sensitive marker of vascular health in healthy, normal-weight, younger persons (13,14). Reduced AIx@75 is generally interpreted as a sign of lowered stiffness in arteries, and the expected effect of regular endurance training is a reduction in AIx@75. In adults, chronic endurance exercise has been shown to reduce the arterial stiffness measures of AIx@75 and PWV after 4 weeks of endurance training (15). However, different responses after acute endurance and resistance exercise and after regular training have been previously described in adult men (16,17). In the study by Pierce et al., the influence of different types of acute exercise on postexercise arterial stiffness in healthy adult males (26.7 ± 7.2 years) was evaluated (17). AIx@75 increased after both aerobic and resistance exercises but increased even more after resistance exercise. In their study, the resistance exercise consisted of 30 min of moderate to hard resistance exercise as compared to, in this study, 3-min breaks consisting of light resistance exercise repeated four times during an 80-min sitting period. It was concluded that exercise influences the pressure wave reflection after a brief bout of resistance exercise (17). Our study provides the first evidence suggesting that this response to acute exercise can occur from acute endurance activity in adolescents. Interestingly, the results from Table S1 and Table S2 indicate that the response on the step-up activity breaks might differ between boys and girls. It has previously been shown that activity patterns during school days differ between sexes (18). The possible sex differences in the acute effects of physical activity breaks on arterial stiffness deserve further investigation.

One previously described explanation for increased AIx@75 was that acute exercise stimulates the sympathetic nervous system increasing the wave reflection. It has been demonstrated that adrenergic stimulation of the sympathetic nervous system and the

|	All ($n = 17$)	Girls ($n = 11$)	Boys ($n = 6$)
Age (year)	13.6 ± 0.7	13.9 ± 0.2	13.2 ± 0.2
Height (cm)	160.9 ± 8.8	162.7 ± 7.5	157.4 ± 10.6
Weight (kg)	48.7 ± 7.2	49.8 ± 5.9	47.1 ± 9.4
Body mass index (BMI) (kg/m^2)	18.9 ± 2.1	18.8 ± 1.8	18.9 ± 2.8

TABLE 1 Basic characteristics for the 17 study participants, 11 girls and 6 boys. Data are expressed as means and standard deviations (SD).

TABLE 2 Results on heart rate (HR), blood pressure (BP) and arterial stiffness measures for the 17 study participants pre- and 30-min postconditions, cortisol was measured 45-min postconditions. Data are expressed as means and standard deviations (SD).

	Pre-SOC	Post-SOC	Pre-SRA	Post-SRA	Pre-STEP	Post-STEP
HR (beats/min)	72.0±11.5	69.0±9.1	72.3±10.8	69.8±13.9	70.2±10.2	73.0±10.2
Systolic BP (mmHg)	110.7±6.6	107.9±6.3	112.4±6.3	108.1±7.0	109.9±8.0	106.8±6.7
Diastolic BP (mmHg)	63.1±5.4	61.9±6.1	64.5±7.0	62.4±6.7	62.9±6.0	60.4±6.7
Cortisol (nmol/l)	9.8±4.5	9.6±5.8	13.2±6.9	11.2±7.4	9.7±5.9	11.2±8.1
AIx mmHg (%)	7.8±10.9	7.7±10.7	6.3±10.9	6.2±11.6	5.4±10.2	9.9±15.4
AIx@75 (%)	6.1±8.2	4.6±10.1	5.4±9.5	4.3±8.1	3.1±8.4	9.2±13.2*
PWV (m/s)	4.6±0.9	4,4±0.8	4.5±0.8	4.4±0.4	4.4±0.9	4.3±0.9

Note: Augmentation index (AIx), AIx normalised to heart rate 75 beats/min (AIx@75) and pulse wave velocity (PWV), * = $p < 0.05$. Missings: AIx Pre-SRA = 1; AIx@75 pre-SRA = 1; AIx@75 post-SRA = 1; Cortisol pre- and post-STEP = 2. All missing values were from female participants.

FIGURE 1 AIx@75bpm (%) pre and post the three conditions, social break (SOC), simple resistance activity break (SRA) and step-up activity break (STEP). A significant time×condition interaction was noted ($F = 5.56$, $df = 2$, $p < 0.01$, partial eta = 0.28).
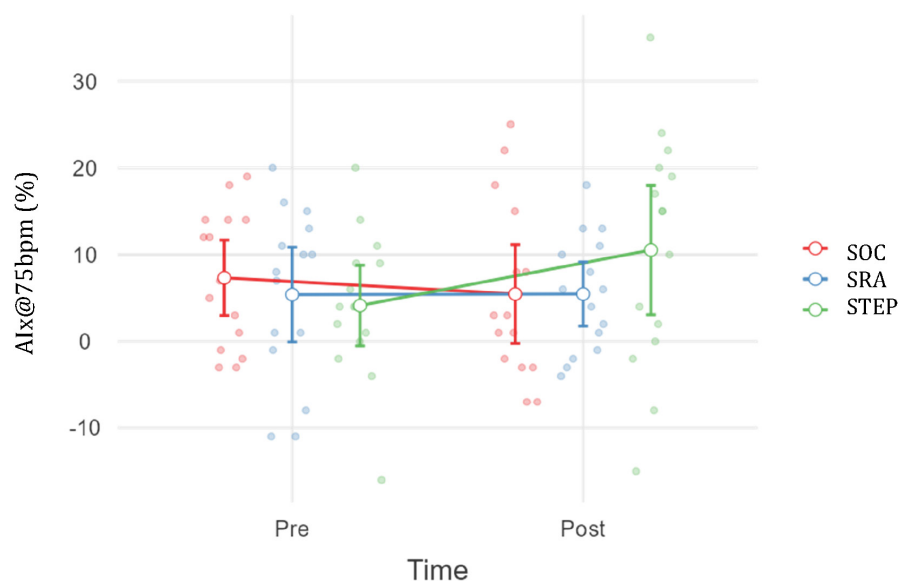
3.2 | PWV

PWV has been shown to be higher in overweight and obese children compared with normal-weight children (20). The participants in this study were normal weight, and the average PWV before conditions in the present study was 4.5 m/s for girls and 4.3 m/s for boys. In a study from 14 centres across Europe ($n = 1847$), PWV for adolescents was 3.6 m/s for girls and 3.7 m/s for boys (21). PWV values from the present study were slightly higher. Age and height are known to influence arterial stiffness measures, but neither were correlated with PWV in the present study. A potential weakness in the present study is that metabolic risk factors such as insulin resistance and lipid profiles were not investigated. This should be added in future studies evaluating the long-term effects of activity breaks. Furthermore, this study was originally designed to examine changes in cerebral circulation (11), thus the present outcomes may not have been sufficiently powered to see an effect.

3.3 | Cortisol

In the present study, the sympathetic activity was measured as cortisol in saliva. There are many advantages of measuring cortisol in the saliva. It is a noninvasive method, allowing measurement of the biological active concentration, and the levels reflect the serum cortisol levels (22). The measured values correspond well with previously measured reference values for adolescents, such as by Törnhage et al., who compared 10- to 12-year-olds and, reported 11.5 nmoL/L in boys and 10.9 nmoL/L in girls (23).

The interventions did not alter salivary cortisol concentrations, as indicated by the nonsignificant time×condition interactions in the present study. The reasons for this are not fully understood. While SRA conditions were performed at low intensity, the activities in the step-up condition were at least moderate, as indicated by the heart rate response of 70%–80% of the maximal heart rate. This was expected to elicit the hypothalamic–pituitary–adrenal (HPA) axis to
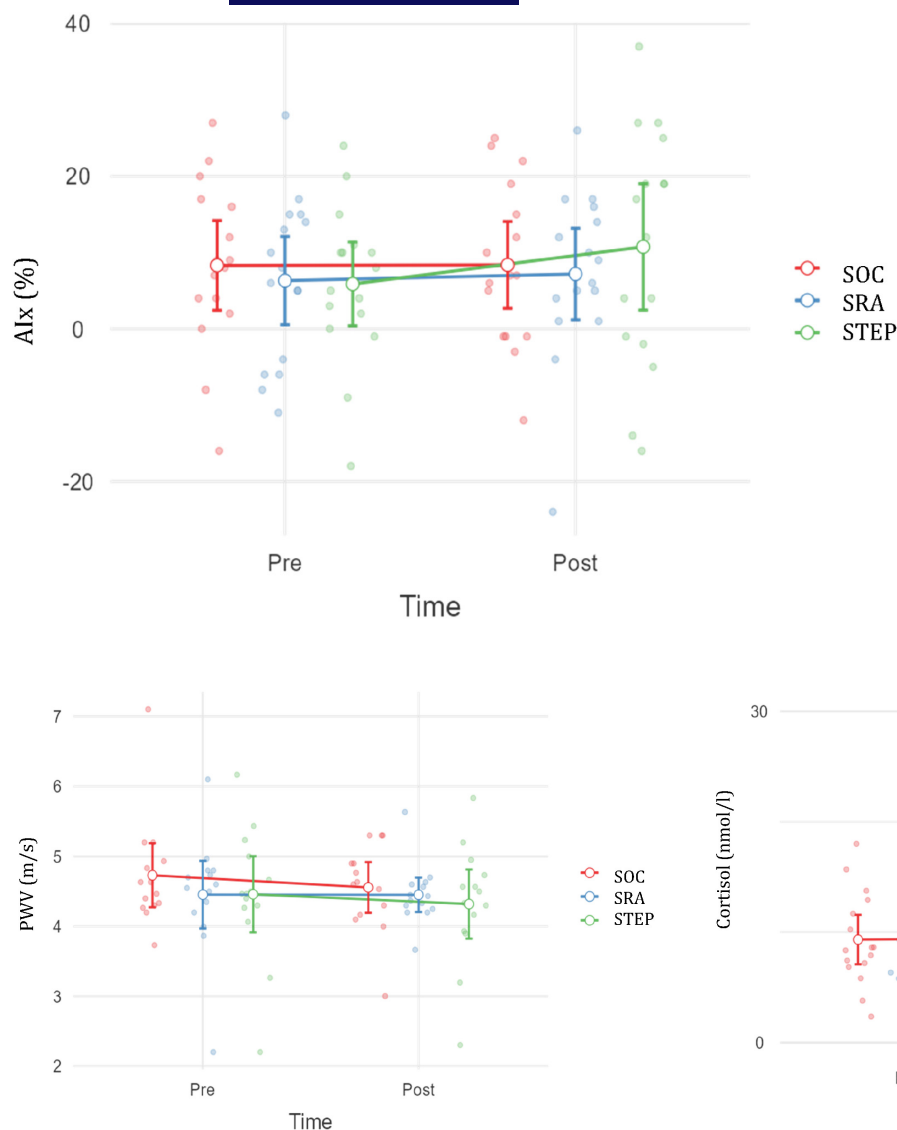
muscle sympathetic nerve activity can mediate constriction of vascular smooth muscles and thereby acutely increase arterial stiffness without changes in the blood pressure (19).

FIGURE 2 AIx (%) pre and post the three conditions, social break (SOC), simple resistance activity break (SRA) and step-up activity break (STEP), with no significant time×condition interactions ($F = 1.42$, df $= 2$, $p = 0.26$, partial eta $= 0.09$).

FIGURE 3 PWV (m/s) pre and post the three conditions, social break (SOC), simple resistance activity break (SRA) and step-up activity break (STEP), with no significant time×condition interactions ($F = 0.29$, df $= 2$, $p = 0.82$, partial eta $= 0.02$).
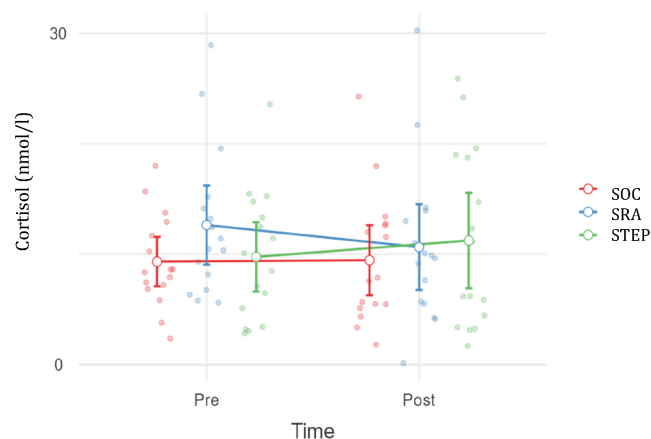

FIGURE 4 Salivary cortisol (nmol/l) pre and post the three conditions, social break (SOC), simple resistance activity break (SRA) and step-up activity break (STEP). No significant time×condition interactions were noted ($F = 2.30$, df $= 2$, $p = 0.12$, partial eta $= 0.13$).

respond (24), but the short duration (3 min) may not have been long enough for this response, compared to 12 or 15 min in similar studies (25). Alternative possibilities are that the saliva collection after the intervention may have been too late to detect a significant increase even if it existed (26), or that the intensity was below the lactate threshold, since increased cortisol and lactate after exercise have been shown to correlate (27). This may indicate that the STEP intervention represents a form of activity at a 'sweet spot' (28).

Physical activity during school lessons has been shown to elicit some beneficial effects on academic achievement and cognitive functions (29). Some studies indicate that higher-intensity physical activities, elicit a stronger effect on cognition and learning (30). However, increases in cortisol have been shown experimentally to reduce cognitive abilities acutely (31). Therefore, it is of interest that the step-up condition, a moderate-intensity activity of

short duration, did not increase the cortisol concentration in this investigation.

3.4 | Psychological factors: Mood, alertness and sleepiness

The hypothesis that short physical activity breaks during prolonged sitting would improve the participants' experienced mood was not supported by the findings of the current study. By contrast, a previous study, the Earlybird 65 study, showed that poor metabolic health was associated with low mood, and that intermittent activities in adolescents affected enjoyment and mood (10). In this study, none of the self-reported psychological parameters (mood, alertness and sleepiness) demonstrated a statistically significant change in any of

TABLE 3 Results on psychological factors. Positive and negative affects score (PANAS), Karolinska Sleepiness Questionnaire (KSS) and Alertness (VAS). Data are expressed as means and standard deviations (SD).

	Pre-SOC	Post-SOC	Pre-SRA	Post-SRA	Pre-STEP	Post-STEP
Positive affects (score)	21.6 ± 6.1	21.4 ± 7.2	24.2 ± 7.6	23.2 ± 8.3	24.3 ± 7.2	24.1 ± 6.9
Negative affects (score)	11.2 ± 1.8	11.1 ± 1.6	12.2 ± 4.2	11.9 ± 3.6	11.6 ± 1.8	11.2 ± 2.0
KSS score	5.6 ± 1.2	4.8 ± 1.4	5.1 ± 1.4	4.2 ± 1.4	4.8 ± 1.2	4.2 ± 1.8
Alertness (cm)	4.8 ± 1.4	5.3 ± 1.9	4.9 ± 2.1	6.4 ± 1.94	5.2 ± 1.64	5.8 ± 1.9

the conditions. A potential explanation could be that mood was relatively high already before the activity breaks and that the duration of the activity was too short. Nevertheless, increased physical activity in the transition from childhood to adulthood is important in the prevention of long-lasting negative effects on vascular health (32) and possibly also short activity breaks during the school day, compared with the usual prolonged sitting, may help to counteract the negative effects of sedentarism.

The finding, that the measure of vascular health AIx@75 was related to the STEP condition, potentially mediating the beneficial effects of regular physical activity, needs further investigation before being implemented in schools. It needs to be confirmed that the reduction in rest and recovery following frequent physical activity breaks during lessons do not lead to unfavourable consequences. In adults, limited time for rest and recovery during working hours has been proposed to be related to compromised vascular and metabolic health (33), while similar activities during leisure time show opposing relations. This is known as the 'physical activity paradox' (34). Although this paradox has been disputed, the fact is that it is unknown to which extent similar relations occur in adolescents, thus warranting further investigations.

In summary, the arterial stiffness measures of AIx, AIx@75 and PWV, remained unchanged during prolonged sitting with social breaks or breaks with simple resistance exercise. The more intense, endurance type of break, the step-up activity, however, induced significant increases in AIx@75 as compared to both social breaks and breaks with resistance activity, but no difference was observed on AIx and PWV. No changes were seen for cortisol and psychological factors. If physical activity breaks during school days are implemented, it is possible that short-term increases in augmentation over time may lead to long-term improvements in vascular health due to vascular remodelling. Further studies are needed to evaluate the long-term effects of regular physical activity breaks during school days.

4 | CONCLUSION

Frequent, short endurance activity breaks such as step-up activity may be a feasible way to counteract the negative effects on vascular structure and function from a sedentary lifestyle that has become more common in adolescents. The results from this study highlight the importance of further investigations on the long-term effects of frequent, short activity breaks, during school days, for vascular health in Swedish adolescents.

CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Upon reasonable request, data may be available by contacting the project investigator Örjan Ekblom.

CONSENT FOR PUBLICATION

Not applicable.

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SUPPORTING INFORMATION

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

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