UHASSELT
KNOWLEDGE IN ACTION

Maastricht University

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

The impact of green spaces on the general well-being and stress among college students

Lore Verheyen
Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :
Prof. dr. Michelle PLUSQUIN
MENTOR :
De heer Kenneth VANBRABANT
Mevrouw Rossella ALFANO

Transnational University Limburg is a unique collaboration of two universities in two countries: the University of Hasselt and Maastricht University.

UHASSELT
KNOWLEDGE IN ACTION
www.uhasselt.be
Universiteit Hasselt
Campus Hasselt:
Martelarenlaan 42 | 3500 Hasselt
Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

2020
2021

Faculty of Medicine and Life Sciences
School for Life Sciences
Master of Biomedical Sciences

Master's thesis

The impact of green spaces on the general well-being and stress among college students

Lore Verheyen

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences, specialization Environmental Health Sciences

SUPERVISOR :
Prof. dr. Michelle PLUSQUIN

MENTOR :
De heer Kenneth VANBRABANT
Mevrouw Rossella ALFANO

The impact of green spaces on the general well-being and stress among college students

Lore Verheyen[1,2], Kenneth Vanbrabant[2], Rossella Alfano[2] and Michelle Plusquin[2,*]

[1]*Student Environmental Health Sciences, Hasselt University, Diepenbeek, Belgium*
[2]*Centre for Environmental Sciences (CMK), Hasselt University, Diepenbeek, Belgium*

Running title: *Nature-based solutions for school-related stress*

Keywords: Stress, green space exposure, cognitive function, college students, general well-being

ABSTRACT

Background – Due to growing demands and high pressure, college students are exposed to high amounts of stress. Chronic stress can have deleterious effects on physical and mental health, and may contribute to health problems later in life. Current stress relief strategies do not seem to be sufficient. Therefore, it is crucial to find an effective method to prevent chronic stress among college students by easily accessible and low-cost solutions, such as spending time in nature. This study hypothesizes that green space exposure is linked to stress reduction and an improved cognitive function among college students.
Methods – In the framework of this interventional case-control study, stress levels were determined in 29 healthy volunteers before and after a four-week intervention period. General well-being and mental health were assessed by self-reported questionnaires. Eye-tracking software was used as an indicator of neurocognition and attentional bias. Optimization of a protocol to determine cortisol levels in hair samples was initiated.
Findings – Results of the 12-item General Health questionnaire showed a significant interaction between intervention and time for GHQ-12 scores ($\beta = -2.56$, 95% CI -4.74 to -0.38, $p = 0.021$). No significant association was found for the interaction between intervention and time for the eye-tracking based cognitive scores and attentional bias.
Conclusion – Based on the self-reported questionnaires, there is an indication that a regular thirty-minute walk in a natural environment increases general well-being and mental health among college students. Future research is crucial to explore the effects of green space exposure to cognition and attention bias.

INTRODUCTION

Stress is pervasive in today's society. Stress is defined in the stress theory as the nonspecific response of the body to any demand [1]. Whereas acute stress is crucial for induction of adaptive responses to environmental stressors, chronic stress might negatively impact physical health and mental well-being [2, 3]. Chronic stress contributes to deleterious health problems in later life, such as cardiovascular disease, hypertension, diabetes mellitus, and mental illnesses, including depression and anxiety [4, 5].

Students are particularly susceptible to stress, as they are often exposed to high pressure during their education [6, 7]. Stress can impede students' academic performance by diminishing achievement, reducing motivation, and increasing the likelihood of dropping out of school [8]. The number of students who experience school-related stress has been increasing annually. According to the National College Health Assessment II of the American College Health Association, up to 90% of the interviewed students experienced average to tremendous stress in 2015 due to school-related and personal reasons [9]. This percentage increased to 92% in 2019 [10]. Additionally, the American College Health Association reported that the prevalence of depression in the interviewed students increased by 5.2% in May 2020 relative to fall 2019 [11]. In Belgium, 20% of all university students have experienced burnout symptoms during their education [12].

*Corresponding author: Michelle Plusquin Tel: +32 11 26 82 89; Email: michelle.plusquin@uhasselt.be

Stress triggers activation of the hypothalamic-pituitary-adrenal (HPA) axis, which causes the secretion of glucocorticoids, such as cortisol [13]. During the chronic stress response, the hypothalamus secretes corticotropin releasing hormone (CRH), which causes secretion of adrenocorticothropic hormone (ACTH) by the adenohypophysis [3]. ACTH signals the adrenal cortex to produce corticosteroids. Cortisol is the main glucocorticoid, synthesized from cholesterol and characterized as a stress hormone. This hormone has a broad action on several organ systems [14]. Broadly, it maintains blood glucose, central nervous system function, and cardiovascular function. Cortisol can cross the blood-brain barrier, access the brain, and may bind to receptors in the hippocampus, amygdala, and frontal lobes [15]. These structures are known to be involved in learning and memory [2]. Previous research indicates that chronic elevated cortisol levels may cause cognitive impairment [16, 17]. However, the exact mechanism remains unknown. Scott *et al.* [18] suggested that chronic stress can cause a continuous change in the body's allostatic load [19-21], which can induce dysregulation of the HPA-axis and inflammation. These changes may impair cognitive performance and result in poorer cognitive function due to changes in neural structure [15, 17, 22]. Several theories suggested that cognitive processing towards decreased attention for positive emotions and increased attention for negative emotions is associated with increased risk for the development of emotional disorders, such as burnout [23, 24].

Cortisol is often analyzed from biological sources such as saliva, blood, and urine [25, 26]. However, cortisol determined in these matrices reflects acute and short-term concentrations of cortisol secretion, due to the circadian rhythm of cortisol release, and not the chronic HPA activation [25, 27]. To overcome this limitation, Raul *et al.* [28] were the first to develop a method of cortisol measurement in hair samples. A 1-cm hair segment reflects the retrospective cortisol concentration over the last month [26, 29]. Cortisol extraction from hair provides a reliable, non-invasive method to measure chronic stress by reflection of the long-term central HPA basal activity [3, 27, 30, 31]. Previous research already showed a positive correlation between stress and hair cortisol levels [26, 32-34].

In recent years, several studies have revealed the beneficial effects of green spaces on stress [35]. The term 'green space' comprises natural environments surrounded by green, such as woods and parks. The benefits of these natural environments include improved mental health and general well-being, as well as a reduced disease prevalence [36-40]. Ulrich and colleagues [41] state in the 'Stress Reduction Theory' that visual exposure to natural environments improves recovery from stress. Additionally, natural environments may restore concentration after mental fatigue according to the 'Attention Restoration Theory' [42]. With regard to the growing demands and pressure, increasing the allostatic loads of students, nature has been suggested to be an easily accessible and cost-effective resource for stress prevention [19, 43]. Thus, managing stress levels by spending time in nature could provide an opportunity to prevent stress-related diseases. Recently, there has been growing interest in the positive effects of spending time in nature on college students' mental functioning. However, these studies focused on subjective self-reported questionnaires alone [44-46] or in combination with salivary cortisol assays [47] as a physiological measure, which may not reliably reflect chronic stress due to the circadian rhythm of cortisol production.

In the framework of this interventional case-control study, we hypothesize that a regular thirty-minute walk for four weeks in a natural environment is linked to stress reduction and an improved cognitive function among college students. During this study, stress levels and cognitive function of college students were determined before and after the four-week intervention period using psychological measures. Furthermore, a protocol was optimized to determine cortisol levels in hair samples using high-performance liquid chromatography (HPLC) in order to provide a more objective, physiological measure for stress level analysis.

This study aims at providing evidence that intervention in natural environments ameliorates perceived stress and cognitive function among college students, based on validated, self-reported questionnaires and eye-tracking analysis. We hope that this study inspires policymakers to implement natural environments as an easily accessible and low-cost method to boost mental health in schools.

EXPERIMENTAL PROCEDURES

Study population

This interventional case-control study targeted college and PhD students of Hasselt University, University College PXL, and UC-Leuven-Limburg. Information regarding the study was disseminated using e-mails and using social media. Interested participants received an invitation to an online information session. The inclusion criteria were being able (i) to perform a regular thirty-minute walk during four weeks, and (ii) to fill in questionnaires in Dutch. A total of 59 volunteers were recruited. During follow-up, 2 participants dropped out due to personal reasons, resulting in 57 students taking part of the study. Participants were subdivided at random using a computer algorithm into a control group (n=28) and an intervention group (n=29). This study was performed according to the principles outlined in the Declaration of Helsinki for investigation of human subjects [48]. Furthermore, this study was approved by the commission for Medical Ethics of Hasselt University (CME2020/006) and all participants gave their informed consent prior to inclusion in the study.

Study design

The study consisted of an intervention period of four weeks, during which participants of the intervention group performed a regular thirty-minute walk in a natural environment (≥ 4 walks per week). Participants of the control group maintained their regular daily lifestyle. At baseline (day 0) and at the end of the four-week intervention period (day 28), an examination was performed, consisting of self-reported questionnaires, neurocognitive tests, and sample collection. An overview of the study design of this interventional case-control study is provided in Figure 1.

Questionnaires

The general research questionnaire and a questionnaire regarding the coronavirus (SARS-CoV-2) pandemic [11, 49], were used to gather information on the study population, including age, sex, weight, height, smoking status (coded as ‘does not smoke currently’ or ‘currently smokes’), previous medical diagnose of mental illness (coded as ‘no’ or ‘yes’), and previous infection with SARS-CoV-2, diagnosed by a PCR test (coded as ‘yes’, ‘no’, or ‘presumptive’). The body mass index (BMI) (kg/m^2) was calculated as weight divided by squared height and categorized as ‘underweight’ (BMI ≤18.5), ‘normal’ (BMI >18.5 and <25), ‘overweight’ (BMI ≥25 and <30), and ‘obese’ (BMI ≥30). The participants completed three questionnaires at baseline (day 0) and at the end of the four-week intervention period (day 28) to assess mental and general health (Supplementary info 1). Firstly, the 12-item General Health Questionnaire (GHQ-12) [50] estimated general well-being and psychological distress. Secondly, the Burnout Assessment Tool (BAT) [51] identified symptoms of burnout. Lastly, the World Health Organization–Five Well-Being Index (WHO-5) [52] assessed current mental well-being.

Neurocognitive tests

The commercially available eye tracking device Tobii Pro Nano (Tobii, Stockholm, Sweden) was used to assess whether green space exposure has beneficial effects on cognitive functioning and the ability to improve performance. Each participant

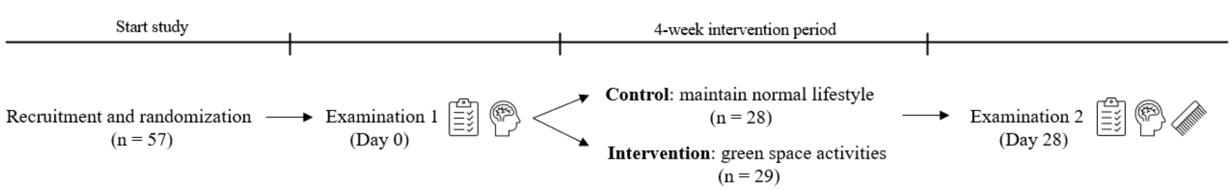


Figure 1: Overview of the study design. Participants were divided into an intervention group and a control group. The intervention group performed a four-week intervention period, the control group maintained their normal lifestyle. Self-reported questionnaires and neurocognitive tests were performed during examination 1 and 2. Additionally, examination 2 contained collection of a hair sample.

was seated in front of a monitor with the eye-tracking device connected at the bottom of the screen (Figure S1). Eye position was calibrated using the eye-tracking software (Tobii Pro Lab, version 1.152). The participant was asked to look at the monitor, where short task pictures were showed as comparable to the tasks described by Oyama *et al*. [53]. In addition, attention bias was determined by emotion recognition using emotional faces [54]. The participants completed the neurocognitive tasks at day 0 and day 28. Data was retrieved by setting an area of interest (AOI) on the correct answer, and the % fixation duration on the AOI was used as a measure of cognitive score. A total cognitive score was calculated as total percent fixation duration on the AOIs for all tasks. Attention bias was calculated by calculating the ratio of first fixation duration (msec) on the AOI of the emotion 'happy' to 'anxious' and to 'sadness'. More information regarding the cognitive tasks can be found in Supplementary info 2.

Sample collection

Hair samples were only collected at the end of the four-week intervention period (day 28). Hair was cut in the posterior vertex region with iron scissors as close as possible to the scalp and taped at the cut end [55]. Hair samples were stored at room temperature until further analysis.

Optimization of cortisol extraction from hair samples using test samples

Sample preparation and cortisol extraction was performed as previously described by Greff *et al*. [30] with few minor modifications. Briefly, the hair sample was segmented; the first centimeter from the scalp represented average stress levels during the previous month, while the next centimeter represented the average cortisol exposure at baseline. The hair sample was minced using scissors and washed two to three times using isopropanol for 3 min at room temperature followed by air drying. The segments of hair were placed into glass vials and weighed to allow determination of the concentration per a given mass of hair. Cortisol was extracted by adding alternating 1.5 ml methanol and acetone, followed by shaking at 40°C for 16 h and 5 min at room temperature, respectively [56]. After extraction, the supernatant was evaporated under nitrogen at both room temperature and 40 °C until completely dry. Lastly, dry residues were resuspended in 250 µl phosphate buffered saline (PBS) and 50 µl methanol, for enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC), respectively. Samples were vortexed until completely resolved and immediately analyzed.

Optimization of extraction methods of cortisol

Optimization of cortisol extraction from hair samples included extraction and drying experiments. Extraction experiments including a two-step and four-step extraction method were compared. The two-step extraction method consisted of alternating the addition of methanol and acetone. The four-step extraction method consisted of alternating the addition of methanol and acetone, repeated twice, Drying experiments include drying samples under nitrogen at room temperature or 40 °C until completely dry.

Determination of cortisol concentration in hair samples using ELISA

The total yield of cortisol in test samples was determined using the commercially available Cortisol Competitive ELISA kit (Thermo Fisher Scientific Inc., Frederick, MD, USA) according to manufacturer's instructions.

Identification of the detection limit of cortisol by high-performance liquid chromatography

The Chromaster HPLC system (Chromaster, VWR Hitachi, Tokyo, Japan) was used to determine cortisol levels in hair samples. The system was equipped with a diode array detector (DAD, VWR no. 5430), a column oven (VWR no. 5310), an auto sampler (VWR no. 5260), and a HPLC pump (VWR no. 5160). Analytes were separated on an ACE® Equivalence™ 5 C18 (250 x 4.6 mm) (ACE, Aberdeen, Scotland) column. The injected volume was 10 µl. The mobile phase flow rate was 0.4 mL min^{-1} and the column temperature was kept constant at 30 °C. The binary mobile phase consisted of water (Merck KGaA, Darmstadt, Germany) and acetonitrile (VWR, Fontenay-sous-Bois, France). The gradient was performed as described by Vanaelst *et al*. [57] with minor modifications: 10% acetonitrile was increased to 60% in 6 min, kept constant for 3 min, decreased to 10% in 2.5 min, and finally kept constant in the following 13.5 min. Under these conditions, the total run time was 25 min. Cortisol was detected at a wavelength of 247 nm as determined by Ray *et al*. [58]. The minimal detectable amount of cortisol was determined using known concentrations cortisol standard (C–106, Cerilliant ®, TX, USA), diluted in methanol (e.g. 1 mg/ml [standard]; 0.025 mg/ml; 0.0025 mg/ml; 0.00025 mg/ml).

Statistical analysis

Statistical analysis was performed using RStudio software, version 1.2.1335 (RStudio Inc., Boston, USA). Continuous data are presented as means and standard deviations (SD) and categorical data are presented as numbers and frequencies (%). Data normality was checked using the Shapiro-Wilk test. Differences between control and intervention group were analyzed by a Student's t-test or a Chi Squared test to indicate whether randomization was successful. To quantify the effect of the intervention on repeated measurements of perceived stress and cognition, linear mixed effect models were used that included random effects on participants and on an interaction term between intervention and time of examination (day 28, further referred to as 'time'). We adjusted for the following potential confounders and covariates which were selected a priori: sex, age, BMI, and smoking status. The model was additionally adjusted for previous diagnose of mental illness, and previous infection with SARS-CoV-2. For attention bias and cognitive score, the model was additionally adjusted for date and hour of examination. A Paired t-test was used to assess the differences of cortisol concentrations obtained by the two-step and four-step extraction methods, and by the drying experiments at room temperature and 40 °C. Correlation between extraction methods were compared using Pearson correlation. P-values less than 0.05 were considered significant.

RESULTS

Study population

Characteristics of the study population are reported in Table 1. Briefly, the mean ± SD age of the participants was 22.2 ± 2.06 years old. The majority of the participants were females (61.4%), had a normal BMI (75.4%), did not smoke currently (89.5%), were never diagnosed with mental illnesses (87.7%), and were never diagnosed with SARS-CoV-2 (80.7%). No differences in population characteristics were found between the intervention and control group (Table 1).

Table 1: Characteristics of participants (n = 57). Mean ± SD or numbers and frequencies (%) for all variables. P-value for difference between groups is given.

Characteristics	**Mean ± SD or numbers and frequencies (%)**		
	Control (n=28)	**Intervention (n=29)**	**p-value**
Age, years	22.4 (2.18)	22.1 (1.96)	0.692
Sex			0.867
Male	10 (35.7%)	12 (41.4%)	
Female	18 (64.3%)	17 (58.6%)	
BMI			0.757
Underweight	0 (0%)	0 (0%)	
Normal	21 (75.0%)	22 (75.9%)	
Overweight	7 (25.0%)	7 (24.1%)	
Obese	0 (0%)	0 (0%)	
Smoking status			0.633
Current smoker	24 (85.7%)	27 (93.1%)	
No current smoker	4 (14.3%)	2 (6.9%)	
Mental illness			1
No	25 (89.3%)	25 (86.2%)	
Yes	3 (10.7%)	4 (13.8%)	
SARS-CoV-2			0.654
No	23 (82.1%)	23 (79.3%)	
Yes	3 (10.7%)	2 (6.9%)	
Presumptive	2 (7.1%)	4 (13.8%)	

Abbreviations: BMI; Body Mass Index

General well-being based on the 12-item General Health Questionnaire

Scores of GHQ-12 are presented in Figure 2(a). A linear mixed effects model was conducted to assess the interaction between intervention and time on GHQ-12 scores using GHQ-12 as outcome variable (Table 2). For both the unadjusted and the adjusted model, a significant interaction between intervention and time for GHQ-12 scores was found ($p_{unadjusted,adjusted}$ = 0.021). After adjusting for sex, age, BMI, smoking status, previous diagnose of

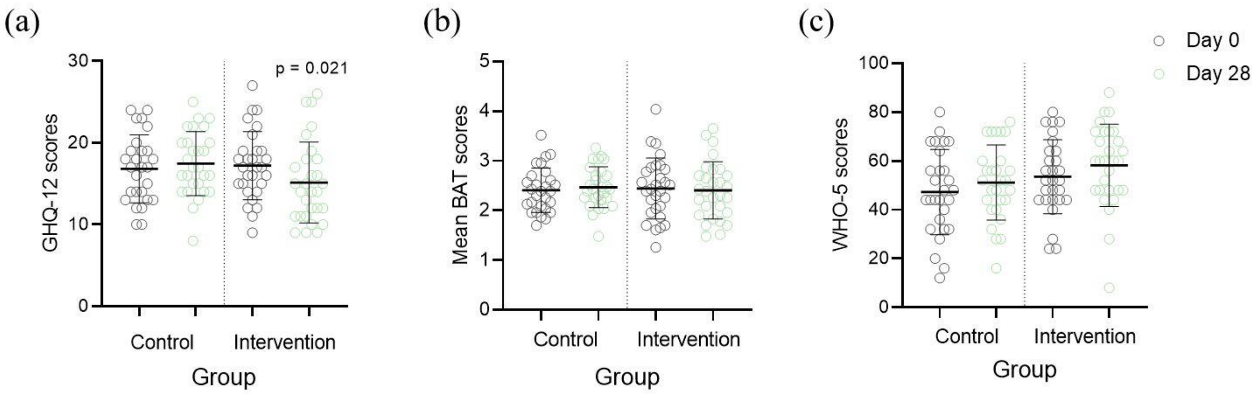


Figure 2: Scatter dot plots depict the scores of (a) GHQ-12, (b) mean BAT scores and (c) WHO-5 scores in control (n = 28) and intervention groups (n = 29). Circles indicate individual scores, bars indicate mean score ± SD. Grey indicates individual scores on day 0; green on day 28. P-value indicates significant effect of the interaction term intervention*time.

mental illness, and infection with SARS-CoV-2, the GHQ-12 score was significantly and negatively associated with the interaction between intervention and time (β = -2.56, 95% CI -4.74 to -0.38, p = 0.021).

Risk of burnout based on Burnout Assessment Tool

Results of the linear mixed model, using mean BAT scores as outcome variable, are shown in Table 2. Mean BAT scores are presented in Figure 2(b). No significant interaction between intervention and time was found for mean BAT scores ($p_{unadjusted,adjusted}$ = 0.179). A negative trend was shown for the association between mean BAT score and interaction between intervention and time in the adjusted model (β = -0.10, 95% CI -0.24 to 0.05, p = 0.179).

Current mental health based on World Health Organization - Five Well-Being Index

A linear mixed effects model was conducted to assess the interaction between intervention and time on WHO-5 scores. Figure 2(c) presents scores of the WHO-5 questionnaire. Results of the linear mixed model using WHO-5 scores as outcome variable are shown in Table 2. No significant interaction between intervention and time was found for WHO-5 scores ($p_{unadjusted}$ = 0.741, $p_{adjusted}$ = 0.734). After adjusting for sex, age, BMI, smoking status, previous diagnose of mental illness, and infection with SARS-CoV-2, a positive trend was shown for the association between the WHO-5 scores and interaction between intervention and time (β = 1.16, 95% CI -5.53 to 7.85, p = 0.734).

Table 2: Linear mixed model analysis outcomes for the self-reported questionnaires, attention bias, and cognitive scores, including the interaction term intervention*time.

Predictor	Intervention*time					
	Unadjusted			Adjusted¹		
	β	*CI*	*p*	*β*	*CI*	*p*
GHQ-12	-2.57	-4.75 – -0.39	0.021	-2.56	-4.74 – -0.38	0.021
Mean BAT scores	-0.10	-0.24 – 0.05	0.179	-0.10	-0.24 – 0.05	0.179
WHO-5 scores	1.13	-5.56 – 7.82	0.741	1.16	-5.53 – 7.85	0.734
	Unadjusted			Adjusted²		
Happy/anxious	-0.04	-0.16 – 0.08	0.518	-0.04	-0.17 – 0.08	0.523
Happy/sad	0.0006	-0.15 – 0.15	0.993	0.01	-0.14 – 0.16	0.906
Cognitive score	1.92	-3.84 – 7.68	0.513	1.85	-3.95 – 7.86	0.530

Adjusted¹: model adjusted for sex, age, BMI, smoking status, previous diagnose of mental illness, and infection with SARS-CoV-2.
Adjusted²: model adjusted for sex, age, BMI, smoking status, previous diagnose of mental illness, infection with SARS-CoV-2, date of examination, and hour of examination.

Attentional processing of emotional information

A linear mixed effects model was conducted to assess the interaction between intervention and time on the total fixation duration to a specific emotion compared to the emotion 'happy'. Outcomes of the linear mixed effect models using the ratio 'happy'/'anxious' and 'happy'/'sad' as outcome variables are presented in Table 2 . After adjusting for sex, age, BMI, smoking status, previous diagnose of mental illness, infection with SARS-CoV-2, date of examination, and hour of examination, analysis showed no association between the bias score for the ratio happy/anxious and the interaction between intervention and time (β = -0.04, 95% CI -0.17 to -0.08, p = 0.523). Using the same adjusted model, no association was found for the ratio happy/sad and the interaction between intervention and time (β = 0.01, 95% CI –0.14 to 0.16, p = 0.906). Results of the unadjusted model, excluding the interaction term intervention and time, show a significant main effect of time of examination on the ratio happy to anxious (β = 0.13, 95% CI -0.07 to 0.19, p < 0.001).

Cognitive score

Total percent fixation duration on all tasks as a cognitive score are presented in Figure 3. The results of the linear mixed model using cognitive scores as outcome variable are shown in Table 2. After adjusting for sex, age, BMI, smoking status, previous diagnose of mental illness, infection with SARS-CoV-2, date of examination, and hour of examination, a positive trend was found for the association for the cognitive score and the interaction between intervention and time (β = 1.86, 95% CI -3.95 to 7.68, p = 0.53).

Quantification of cortisol in test samples by ELISA

Extraction and drying experiments for cortisol quantification were performed using ELISA to optimize the extraction procedure. Figure 4 shows results of the extraction experiments. Cortisol concentrations using the four-step extraction method were non-significantly higher than those assessed with the two-step method (p = 0.58). Pearson correlation suggested that these methods are strongly inversely correlated (r = -0.98, p = 0.14). Sample drying under nitrogen at room temperature showed non-significant higher cortisol concentrations as compared to drying at 40 °C (p = 0.08). The mean of the total yield of cortisol was 2785.8 pg/mL and 13.1 pg/mg hair (Figure 4(b), blue).

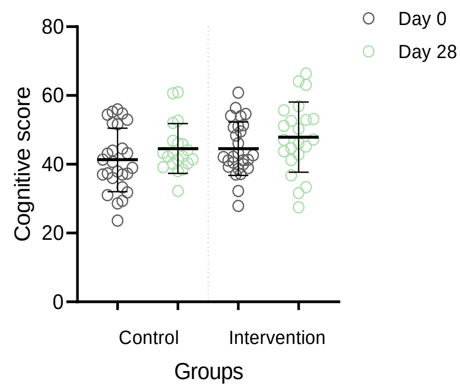


Figure 3: Scatter dots plot depicts the total cognitive scores in the control (n = 19) and intervention group (n = 19) on day 0 and 28. Circles indicate individual scores, bars indicate mean score ± SD. Grey indicates mean score on day 0; green on day 28.

Detection limit of cortisol using high performance liquid chromatography

HPLC performed on the ACE® Equivalence™ 5 C18 column demonstrated no optimal separation. As shown in Figure 5(a), chromatography of the blank sample (MeOH) showed no peak, while chromatography of the standard cortisol (0.025 mg/mL Figure 5(b); 0.0025 mg/mL Figure 5(c)) showed a peak around 15.28 min, which could be attributed to cortisol. The detection limit was 0.0025 mg/mL.

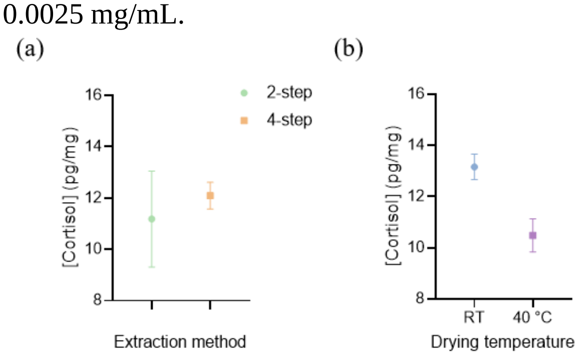


Figure 4: Dot plot visualizes mean ± SD cortisol concentrations (pg per mg weighed hair) for the (a) extraction and (b) drying experiments (n=3). Green and orange indicate cortisol concentrations using the two-step extraction and the four-step extraction method, respectively. Blue and purple indicate drying under nitrogen at room temperature and at 40 °C, respectively.

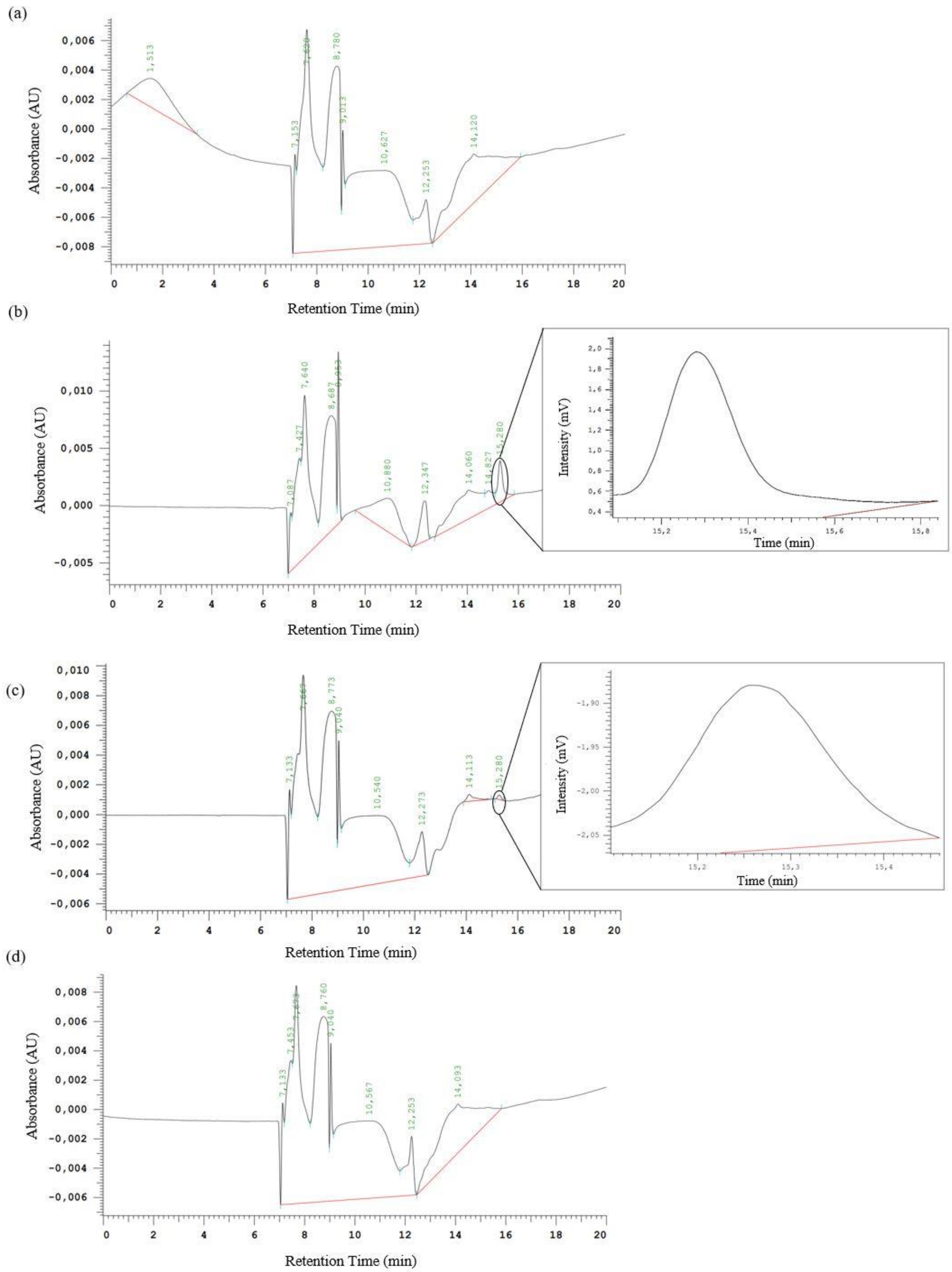


Figure 5: HPLC chromatography of (a) methanol (blank), (b) dilution series cortisol of 0.025 mg/mL [standard], (c) 0.0025 mg/mL, and (d) 0.00025 mg/mL. Outputs show the retention time (minutes) on the x-axis and absorbance (AU) on the y-axis. The peak that occurs after 15.28 minutes was assigned to cortisol. No peak of cortisol was detected for 0.00025 mg/mL. Figure (b) and (c) additionally show the intensity (mV) of the peak after a retention time of 15.28 minutes.

DISCUSSION

Due to the harmful consequences of stress, this study aimed at providing evidence for activities in natural environments as an effective method to prevent chronic stress among college students. We have shown a significant association between the GHQ-12 score and the interaction between intervention and time, which indicates that current mental health improved in the intervention group after the four-week intervention period. A significant ($p<0.05$) interaction between intervention and time indicates that the changes over time between the intervention and control group are different. Results from the WHO-5 index and BAT only suggest a decrease in perceived stress in the intervention group after the intervention period. Eye-tracking results showed no significant association between the interaction between intervention and time for both attention bias and cognitive score as outcome variables. Moreover, we initialized the design of a protocol to determine cortisol concentrations from hair samples.

During this study, we used three validated questionnaires as a tool to estimate perceived stress among students. Firstly, the GHQ-12, as designed by Goldberg *et al.* [50], was used to indicate the likelihood of psychological distress. This questionnaire includes six positive items and six negative items. Scoring was performed as determined by Sánches-Lòpez and Dresch [59]. The positive items were scored from 0 (always) to 3 (never), whereas the negative items were scored from 0 (never) to 3 (always). The final score ranged from 0 to 36, with higher scores indicating an increased likelihood of psychological distress. Secondly, the work-related version of the BAT, as designed by Schaufeli *et al.* [51], was used as a measure for burnout complaints. A total score was calculated by adding the scores on the four core dimensions, which are categorized from 1 (never) to 5 (always). A mean score was obtained by dividing the total score by the number of items – in this case 23. Higher scores indicate worse quality of life. Results of the BAT only suggest a beneficial effect of intervention and time on burnout complaints, whereas worse quality of life was reported in the control group after the four-week intervention period (Figure 3b). Lastly, the WHO-5 scales positive aspects of well-being [52]. This questionnaire includes five questions regarding positive emotions over the last two weeks. The items are scored from 0 (none of the time) to 5 (all of the time). Higher scores indicate better current mental health. Scores increased non-significantly in both the control and intervention group (Figure 3c). It is interesting to note that the findings in the control group are inconsistent, as results from the BAT indicate more burnout symptoms after the intervention period, whereas results from the WHO-5 suggest better current well-being. These inconsistent findings could be due to the fact that the BAT might trigger negative emotions, whereas the WHO-5 might trigger positive emotions. Perceived stress in college students using self-reported questionnaires has also been determined by other studies. Our findings are consistent with existing research on the beneficial effects of green space on perceived stress in children and adolescents using different validated questionnaires [60]. Holt and colleagues [45] reported a better quality of life, better mood and lower perceived stress in university students who actively use green space. Against our expectations, the observed effect of green space is not as prominent as expected. Future studies on the current topic are therefore recommended.

No significant associations for the interaction between intervention and time on the outcome variables attention bias and cognitive score were obtained based on the eye tracking results. For attention bias, it was expected that the first fixation duration for the emotion ‘happy’ would be longer as compared to the emotion ‘anxious’ and ‘sad’ in the intervention group after 28 days. Surprisingly, we have found a non-significant negative estimate ($p = 0.523$) for the association between the interaction term intervention and time for the ratio ‘happy’ to ‘anxious’ and a very small positive estimate for the ratio ‘happy’ to ‘sad’. When excluding the interaction term in the unadjusted model, a significant main effect of time of examination on the outcome variable was found, including a positive estimate. Therefore, the negative estimate could possibly be explained by limitations in the statistic model. However, our findings are in line with other studies [61-63], implying that altered attention is specific for patients diagnosed with depression or burnout. A positive trend was found for the association between the cognitive score and the interaction between intervention and time, indicating cognitive scores tended to increase in the intervention group after the intervention period. We acknowledge a limitation for the method which was used to determine the cognitive score. The total task duration was used as the denominator to calculate percent fixation duration on the AOI. For further research, we suggest to use the time for which valid gaze plots were detected as the denominator. This method takes data loss into account due to blinking or looking away from the screen. According to a recent review by Norwood *et al*. [64], activities in nature improve attention and cognitive performance in adolescents. Results of the review favor the use of natural environments as a low-cost method to promote well-being and mental health.

We initialized a method to determine hair cortisol levels using HPLC. Although immunoassays are the golden standard to determine hair cortisol levels, we opted to use HPLC to quantify hair cortisol as this method is known to have higher sensitivity and specificity. Moreover, multiple components can be identified using HPLC, while when using ELISA, only one component can be determined, e.g. cortisol. Liquid chromatography-mass spectrometry (LC-MS) could provide the opportunity to determine cortisol levels more accurately as compared to immunoassays, as the latter may overestimate cortisol concentrations due to cross-reactivity with exogenous glucocorticoids and endogenous cortisol metabolites [57, 65-68]. However, other studies have found no significant differences in the different methodologies. Surprisingly, Slominski *et al.* [26] reported a greater sensitivity of ELISA-based assays as compared to LC-MS-based assays. In order to optimize extraction methods for cortisol extraction from hair samples, we compared cortisol concentrations in a two-step and four-step extraction method. Results show a higher concentration obtained by the four-step extraction method. As the values of the two-step extraction method are highly variable, the four-step method is preferred. Additionally, we compared cortisol concentrations after drying under nitrogen at both room temperature and 40 °C. The concentrations obtained by drying the sample under nitrogen at 40 °C were non-significantly lower as compared to drying at room temperature. This could be due to degradation of cortisol caused by heat. Therefore, we opted to continue the extraction procedures by drying the samples under nitrogen at room temperature. Although a higher concentration was obtained using the four-step extraction method, we continued the experiments using the two-step extraction method, as the four-step extraction method is very time-consuming. We initialized the design of an optimized protocol for cortisol determination. The detection limit of cortisol was set at 0.0025 mg/mL, while we measured a mean total yield cortisol of 2785.8 pg/mL using ELISA. This indicates that the detector, provided by the device, is not sensitive enough to measure cortisol at low concentrations. To overcome this limitation in future research, we recommend to add mass spectrometry or fluorescent labelling of cortisol to detect higher amounts. Moreover, a calibration curve needs to be included to ensure reliable results. We observed a mean concentration cortisol of 13.1 pg/mg in hair using hair of a volunteer as test sample. Similar concentrations cortisol per weighed hair in healthy individuals have been observed by other studies. Li *et al*. [55] reported an average cortisol content ranging from 9.2 to 40.95 pg/mg. Similarly, Gao *et al*. [27] showed concentrations ranging from 3.28 to 24.83 pg/mg.

We acknowledge there are some weaknesses in our study. Firstly, the intervention period comprises an activity in a natural environment, surrounded by green. However, there is no clarity on the features of green space. People can interpret the term ‘green space’ differently, which could possibly add some form of bias. During our study, we defined green space as ‘natural environments surrounded by green, such as woods and parks’. Secondly, self-reported questionnaires are a subjective method to assess perceived stress. Although we use widely used, validated questionnaires, uncertainty about the results should be kept in mind when interpreting the results. Thirdly, this study was conducted during the global outbreak of SARS-CoV-2. Although we corrected for SARS-CoV-2 in the statistic model, results have to be interpreted with care, as a negative impact on college students’ well-being was reported due to the global development of the coronavirus outbreak [10, 11, 69]. Lastly, we were not able to detect cortisol levels in hair using the extraction methods as described above. Further research is necessary to complete optimization of the protocol, including determination of the most suitable column and optimization of the mobile phase.

Our study also has several strengths worth mentioning. Firstly, we included a control group in this interventional study. Not including a control group could lead to overestimation of the beneficial effects of green space. It should be noted that participants in the control group, for who activities in green environments are a part of their daily routine, should continue doing so, as the body is adjusted to these activities. Quitting could lead to false interpretation of the study results as perceived stress could increase. Secondly, to the best of our knowledge, this is the first study to assess perceived stress after an intervention in green spaces using a combination of these validated questionnaires and eye-tracking analysis. Thirdly, participants were asked to register their activities on paper or by using the free app ‘Strava’ (©2021 Strava, CA, USA). This motivated participants to effectively perform the activities. Next, we only included college students. As there are limited studies investigating stress reduction in college students, this study provides new insights on the beneficial effects of natural environments on perceived stress and cognitive performance in a population wherein high pressure is a part of their daily life. Lastly, we adjusted for a wide range of potential confounding variables, which provides more reliable results.

CONCLUSION

We observed a significant negative association between the interaction between intervention and time of examination on GHQ-12 scores. These results indicate that psychological distress decreased in the intervention group after a four-week intervention period, which included a regular activity in a natural environment. Moreover, results have shown a non-significant increase in cognitive score after the intervention period. Findings show beneficial effects of exposure to natural environments on general health and mental well-being. In conclusion, our results favor the use of nature as a method for stress reduction. Nonetheless, future research is needed to provide evidence for implementation of nature as an approach for stress reduction in educational settings.

REFERENCES

1. Tan S, Yip A: **Hans Selye (1907–1982): Founder of the stress theory**. *Singapore Medical Journal* 2018, **59**:170-171.
2. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A: **The impact of stress on body function: A review**. *EXCLI J* 2017, **16**:1057-1072.
3. Lee DY, Kim E, Choi MH: **Technical and clinical aspects of cortisol as a biochemical marker of chronic stress**. *BMB Rep* 2015, **48**(4):209-216.
4. Kivimäki M, Steptoe A: **Effects of stress on the development and progression of cardiovascular disease**. *Nature reviews Cardiology* 2018, **15**(4):215-229.
5. Madsen IEH, Nyberg ST, Magnusson Hanson LL, Ferrie JE, Ahola K, Alfredsson L, Batty GD, Bjorner JB, Borritz M, Burr H *et al*: **Job strain as a risk factor for clinical depression: systematic review and meta-analysis with additional individual participant data**. *Psychological medicine* 2017, **47**(8):1342-1356.
6. Kumaraswamy N: **Academic stress, anxiety and depression among college students: A brief review**. *International review of social sciences and humanities* 2013, **5**(1):135-143.
7. Ramón-Arbués E, Gea-Caballero V, Granada-López JM, Juárez-Vela R, Pellicer-García B, Antón-Solanas I: **The Prevalence of Depression, Anxiety and Stress and Their Associated Factors in College Students**. *International Journal of Environmental Research and Public Health* 2020, **17**(19):7001.
8. Pascoe MC, Hetrick SE, Parker AG: **The impact of stress on students in secondary school and higher education**. *International Journal of Adolescence and Youth* 2020, **25**(1):104-112.
9. American College Health Association: **American College Health Association-National College Health Assessment II: Reference Group Undergraduates Executive Summary Fall 2015**. *American College Health Association* 2016.
10. American College Health Association: **American College Health Association-National College Health Assessment II: Undergraduate Student Executive Summary Spring 2019**. 2019.
11. The Healthy Minds Network, American College Health Association: **The impact of COVID-19 on college student well-being** In.; 2020
12. Knipprath H, medewerking van Sam Desiere M, De Wandeler L: **Leren en Leven in Leuven**. *Een kwantitatieve analyse van de Nederlandstalige* 2017.
13. Jeanneteau F, Chao MV: **Are BDNF and glucocorticoid activities calibrated?** *Neuroscience* 2013, **239**:173-195.
14. **7-The Adrenal Gland** In: *Endocrine and Reproductive Physiology (Fourth Edition).* Edited by White BA, Porterfield SP. Philadelphia: Mosby; 2013: 147-175.
15. Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE: **The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition**. *Brain and Cognition* 2007, **65**(3):209-237.
16. Kline SA, Mega MS: **Stress-Induced Neurodegeneration: The Potential for Coping as Neuroprotective Therapy**. *American Journal of Alzheimer's Disease & Other Dementiasr* 2020, **35**:1533317520960873.
17. Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL, Meaney MJ: **Cortisol levels during human aging predict hippocampal atrophy and memory deficits**. *Nature neuroscience* 1998, **1**(1):69-73.
18. Scott SB, Graham-Engeland JE, Engeland CG, Smyth JM, Almeida DM, Katz MJ, Lipton RB, Mogle JA, Munoz E, Ram N *et al*: **The Effects of Stress on Cognitive Aging, Physiology and Emotion (ESCAPE) Project**. *BMC Psychiatry* 2015, **15**:146-146.
19. Goldstein DS, McEwen B: **Allostasis, homeostats, and the nature of stress**. *Stress (Amsterdam, Netherlands)* 2002, **5**(1):55-58.

20. McEwen BS: **Protective and damaging effects of stress mediators: central role of the brain**. *Dialogues in clinical neuroscience* 2006, **8**(4):367-381.
21. McEwen BS: **Stress, adaptation, and disease. Allostasis and allostatic load**. *Annals of the New York Academy of Sciences* 1998, **840**:33-44.
22. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, Launer L, Kuller L, Rubin S, Harris T: **Inflammatory markers and cognition in well-functioning African-American and white elders**. *Neurology* 2003, **61**(1):76.
23. Beck AT: **Cognitive therapy and the emotional disorders**: Penguin; 1979.
24. Mathews A, MacLeod C: **Cognitive vulnerability to emotional disorders**. *Annual review of clinical psychology* 2005, **1**:167-195.
25. Staufenbiel SM, Penninx BWJH, Spijker AT, Elzinga BM, van Rossum EFC: **Hair cortisol, stress exposure, and mental health in humans: A systematic review**. *Psychoneuroendocrino* 2013, **38**(8):1220-1235.
26. Slominski R, Rovnaghi CR, Anand KJ: **Methodological Considerations for Hair Cortisol Measurements in Children**. *Therapeutic drug monitoring* 2015, **37**(6):812-820.
27. Gao W, Xie Q, Jin J, Qiao T, Wang H, Chen L, Deng H, Lu Z: **HPLC-FLU detection of cortisol distribution in human hair**. *Clinical Biochemistry* 2010, **43**(7):677-682.
28. Raul JS, Cirimele V, Ludes B, Kintz P: **Detection of physiological concentrations of cortisol and cortisone in human hair**. *Clin Biochem* 2004, **37**(12):1105-1111.
29. Wennig R: **Potential problems with the interpretation of hair analysis results**. *Forensic Science International* 2000, **107**(1):5-12.
30. Greff MJE, Levine JM, Abuzgaia AM, Elzagallaai AA, Rieder MJ, van Uum SHM: **Hair cortisol analysis: An update on methodological considerations and clinical applications**. *Clin Biochem* 2019, **63**:1-9.
31. Xie Q, Gao W, Li J, Qiao T, Jin J, Deng H, Lu Z: **Correlation of cortisol in 1-cm hair segment with salivary cortisol in human: hair cortisol as an endogenous biomarker**. *Clinical chemistry and laboratory medicine* 2011, **49**(12):2013-2019.
32. Wosu AC, Valdimarsdóttir U, Shields AE, Williams DR, Williams MA: **Correlates of cortisol in human hair: implications for epidemiologic studies on health effects of chronic stress**. *Annals of epidemiology* 2013, **23**(12):797-811.e792.
33. O'Brien KM, Tronick EZ, Moore CL: **Relationship between hair cortisol and perceived chronic stress in a diverse sample**. *Stress and health : journal of the International Society for the Investigation of Stress* 2013, **29**(4):337-344.
34. Meyer JS, Novak MA: **Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity**. *Endocrinology* 2012, **153**(9):4120-4127.
35. Haluza D, Schonbauer R, Cervinka R: **Green perspectives for public health: a narrative review on the physiological effects of experiencing outdoor nature**. *Int J Environ Res Public Health* 2014, **11**(5):5445-5461.
36. Maas J, Verheij RA, Groenewegen PP, de Vries S, Spreeuwenberg P: **Green space, urbanity, and health: how strong is the relation?** *Journal of epidemiology and community health* 2006, **60**(7):587-592.
37. Takano T, Nakamura K, Watanabe M: **Urban residential environments and senior citizens' longevity in megacity areas: the importance of walkable green spaces**. *Journal of epidemiology and community health* 2002, **56**(12):913-918.
38. van den Berg AE, Maas J, Verheij RA, Groenewegen PP: **Green space as a buffer between stressful life events and health**. *Social science & medicine (1982)* 2010, **70**(8):1203-1210.
39. Mitchell R, Popham F: **Effect of exposure to natural environment on health inequalities: an observational population study**. *Lancet* 2008, **372**(9650):1655-1660.

40. Beil K, Hanes D: **The influence of urban natural and built environments on physiological and psychological measures of stress--a pilot study**. *Int J Environ Res Public Health* 2013, **10**(4):1250-1267.
41. Ulrich R, Simons R, Losito B, Fiorito E, Miles M, Zelson M: **Stress Recovery During Exposure to Natural and Urban Environments. Journal of Environmental Psychology. 11: 201-230**. *Journal of Environmental Psychology* 1991, **11**:201-230.
42. Kaplan S: **The restorative benefits of nature: Toward an integrative framework**. *Journal of Environmental Psychology* 1995, **15**(3):169-182.
43. Vercambre M-N, Brosselin P, Gilbert F, Nerrière E, Kovess-Masféty V: **Individual and contextual covariates of burnout: a cross-sectional nationwide study of French teachers**. *BMC Public Health* 2009, **9**:333-333.
44. Shrestha T, Di Blasi Z, Cassarino M: **Natural or Urban Campus Walks and Vitality in University Students: Exploratory Qualitative Findings from a Pilot Randomised Controlled Study**. *Int J Environ Res Public Health* 2021, **18**(4).
45. Holt EW, Lombard QK, Best N, Smiley-Smith S, Quinn JE: **Active and Passive Use of Green Space, Health, and Well-Being amongst University Students**. *International Journal of Environmental Research and Public Health* 2019, **16**(3):424.
46. van den Bogerd N, Dijkstra SC, Seidell JC, Maas J: **Greenery in the university environment: Students' preferences and perceived restoration likelihood**. *Plos One* 2018, **13**(2):e0192429.
47. Olafsdottir G, Cloke P, Schulz A, Dyck Z, Eysteinsson T, Thorleifsdottir B, Vögele C: **Health Benefits of Walking in Nature: A Randomized Controlled Study Under Conditions of Real-Life Stress**. *Environment and Behavior* 2018, **52**:001391651880079.
48. **[The Helsinki Declaration of the World Medical Association (WMA). Ethical principles of medical research involving human subjects]**. *Pol Merkur Lekarski* 2014, **36**(215):298-301.
49. Smith BW, Dalen J, Wiggins K, Tooley E, Christopher P, Bernard J: **The brief resilience scale: assessing the ability to bounce back**. *International journal of behavioral medicine* 2008, **15**(3):194-200.
50. Goldberg DP, Hillier VF: **A scaled version of the General Health Questionnaire**. *Psychological medicine* 1979, **9**(1):139-145.
51. Schaufeli WB, Desart S, De Witte H: **Burnout Assessment Tool (BAT)-Development, Validity, and Reliability**. *Int J Environ Res Public Health* 2020, **17**(24).
52. Topp CW, Ostergaard SD, Sondergaard S, Bech P: **The WHO-5 Well-Being Index: a systematic review of the literature**. *Psychother Psychosom* 2015, **84**(3):167-176.
53. Oyama A, Takeda S, Ito Y, Nakajima T, Takami Y, Takeya Y, Yamamoto K, Sugimoto K, Shimizu H, Shimamura M *et al*: **Novel Method for Rapid Assessment of Cognitive Impairment Using High-Performance Eye-Tracking Technology**. *Sci Rep-Uk* 2019, **9**(1):12932.
54. Goeleven E, De Raedt R, Leyman L, Verschuere B: **The Karolinska Directed Emotional Faces: A validation study**. *Cognition and Emotion* 2008, **22**(6):1094-1118.
55. Li J, Xie Q, Gao W, Xu Y, Wang S, Deng H, Lu Z: **Time course of cortisol loss in hair segments under immersion in hot water**. *Clinica Chimica Acta* 2012, **413**(3):434-440.
56. Gow R, Thomson S, Rieder M, Van Uum S, Koren G: **An assessment of cortisol analysis in hair and its clinical applications**. *Forensic Science International* 2010, **196**(1):32-37.
57. Vanaelst B, Rivet N, Huybrechts I, Ludes B, Henauw S, Raul J: **Measurement of cortisol and cortisone in children's hair using ultra performance liquid chromatography and tandem mass spectrometry**. *Analytical Methods* 2013, **5**:2074.

58. Ray P, Steckl AJ: **Label-Free Optical Detection of Multiple Biomarkers in Sweat, Plasma, Urine, and Saliva**. *ACS Sensors* 2019, **4**(5):1346-1357.
59. Sánchez-López M, Dresch V: **The 12-Item General Health Questionnaire (GHQ-12): Reliability, external validity and factor structure in the Spanish population**. *Psicothema* 2008, **20**:839-843.
60. Zhang Y, Mavoa S, Zhao J, Raphael D, Smith M: **The Association between Green Space and Adolescents' Mental Well-Being: A Systematic Review**. *International journal of environmental research and public health* 2020, **17**(18):6640.
61. Bianchi R, Laurent E: **Emotional information processing in depression and burnout: an eye-tracking study**. *Eur Arch Psychiatry Clin Neurosci* 2015, **265**(1):27-34.
62. Hakanen JJ, Schaufeli WB: **Do burnout and work engagement predict depressive symptoms and life satisfaction? A three-wave seven-year prospective study**. *J Affect Disord* 2012, **141**(2-3):415-424.
63. Watson D, Clark LA: **Negative affectivity: the disposition to experience aversive emotional states**. *Psychological bulletin* 1984, **96**(3):465-490.
64. Norwood MF, Lakhani A, Fullagar S, Maujean A, Downes M, Byrne J, Stewart A, Barber B, Kendall E: **A narrative and systematic review of the behavioural, cognitive and emotional effects of passive nature exposure on young people: Evidence for prescribing change**. *Landscape and Urban Planning* 2019, **189**:71-79.
65. Kirschbaum C, Tietze A, Skoluda N, Dettenborn L: **Hair as a retrospective calendar of cortisol production—Increased cortisol incorporation into hair in the third trimester of pregnancy**. *Psychoneuroendocrino* 2009, **34**(1):32-37.
66. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS: **Analysis of endogenous cortisol concentrations in the hair of rhesus macaques**. *Gen Comp Endocrinol* 2006, **147**(3):255-261.
67. Perogamvros I, Owen LJ, Newell-Price J, Ray DW, Trainer PJ, Keevil BG: **Simultaneous measurement of cortisol and cortisone in human saliva using liquid chromatography–tandem mass spectrometry: Application in basal and stimulated conditions**. *Journal of Chromatography B* 2009, **877**(29):3771-3775.
68. Miller R, Plessow F, Rauh M, Gröschl M, Kirschbaum C: **Comparison of salivary cortisol as measured by different immunoassays and tandem mass spectrometry**. *Psychoneuroendocrino* 2013, **38**(1):50-57.
69. Grubic N, Badovinac S, Johri AM: **Student mental health in the midst of the COVID-19 pandemic: A call for further research and immediate solutions**. *Int J Soc Psychiatry* 2020, **66**(5):517-518.

Acknowledgements – I would like to thank prof dr. Plusquin for the opportunity to work on this interesting topic. I would like to thank Mr. Vanbrabrant, Ms. Rasking and Ms. Alfano for feedback, useful tips and correcting my report. A special word of gratitude to Mr. Vanbrabant for the assistance in the lab and opportunity to train my lab skills. Additionally, a word of gratitude to Ms. Alfano for the assistance on the statistic part of my research.

Author contributions – Prof dr. Plusquin and Mr. Vanbrabant conceived and designed the research. Ms. Verheyen performed experiments and data analysis. Mr. Vanbrabant provided assistance with the experiments and data analysis. Verheyen wrote the paper. Mr. Vanbrabant, and Ms. Alfano corrected the report.

SUPPLEMENTARY INFORMATION

Supplementary info 1 – Questionnaires

General Health Questionnaire – 12 (GHQ-12)

Original Dutch version

"In de afgelopen weken, …"

	Helemaal niet	Niet meer dan gewoonlijk	Wat meer dan gewoonlijk	Veel meer dan gewoonlijk
Hebt u zich kunnen concentreren op uw bezigheden?	☐	☐	☐	☐
Bent u door zorgen veel slaap tekort gekomen?	☐	☐	☐	☐
Hebt u het gevoel gehad zinvol bezig te zijn?	☐	☐	☐	☐
Voelde u zich in staat om beslissingen (over dingen) te nemen?	☐	☐	☐	☐
Hebt u het gevoel gehad dat u voortdurend onder druk stond?	☐	☐	☐	☐
Hebt u het gevoel gehad dat u uw moeilijkheden niet de baas kon?	☐	☐	☐	☐
Hebt u plezier kunnen beleven aan uw gewone, dagelijkse bezigheden?	☐	☐	☐	☐
Bent u in staat geweest uw problemen onder ogen te zien?	☐	☐	☐	☐
Hebt u zich ongelukkig en neerslachtig gevoeld?	☐	☐	☐	☐
Bent u het vertrouwen in uzelf kwijtgeraakt?	☐	☐	☐	☐
Hebt u zich als een waardeloos iemand beschouwd?	☐	☐	☐	☐
Hebt u zich alles bij elkaar redelijk gelukkig gevoeld?	☐	☐	☐	☐

Burnout Assessment Tool (BAT)

Original Dutch version

Score

Dutch	Nooit	Zelden	Soms	Vaak	Altijd
#	1	2	3	4	5

Instructies

De volgende uitspraken hebben betrekking op hoe u uw werk beleeft en hoe u zich daarbij voelt. Wilt U aangeven hoe vaak iedere uitspraak op u van toepassing is?

The following statements are related to your work situation and how you experience this situation. Please state how often each statement applies to you.

Onderverdeling

Uitputting

		1	2	3	4	5
1.	Op het werk voel ik me geestelijk uitgeput	☐	☐	☐	☐	☐
2.	Alles wat ik doe op mijn werk, kost mij moeite	☐	☐	☐	☐	☐
3.	Ik raak maar niet uitgerust nadat ik gewerkt heb	☐	☐	☐	☐	☐
4.	Op het werk voel ik me lichamelijk uitgeput	☐	☐	☐	☐	☐
5.	Als ik 's morgens opsta, mis ik de energie om aan de werkdag te beginnen	☐	☐	☐	☐	☐
6.	Ik wil wel actief zijn op het werk, maar het lukt mij niet	☐	☐	☐	☐	☐
7.	Als ik me inspan op het werk, dan word ik snel moe	☐	☐	☐	☐	☐
8.	Op het einde van de werkdag voel ik me mentaal uitgeput en leeg	☐	☐	☐	☐	☐

Mentale distantie

		1	2	3	4	5
1.	Ik kan geen belangstelling en enthousiasme opbrengen voor mijn werk	☐	☐	☐	☐	☐
2.	Op mijn werk denk ik niet veel na en functioneer ik op automatische piloot	☐	☐	☐	☐	☐
3.	Ik voel een sterke weerzin tegen mijn werk	☐	☐	☐	☐	☐
4.	Mijn werk laat mij onverschillig	☐	☐	☐	☐	☐
5.	Ik ben cynisch over wat mijn werk voor anderen betekent	☐	☐	☐	☐	☐

Gebrek aan emotionele controle

		1	2	3	4	5
1.	Op mijn werk heb ik het gevoel geen controle te hebben over mijn emoties	☐	☐	☐	☐	☐
2.	Ik herken mezelf niet in de wijze waarop ik emotioneel reageer op mijn werk*	☐	☐	☐	☐	☐
3.	Tijdens mijn werk raak ik snel geïrriteerd als de dingen niet lopen zoals ik dat wil	☐	☐	☐	☐	☐
4.	Ik word kwaad of verdrietig op mijn werk zonder goed te weten waarom	☐	☐	☐	☐	☐
5.	Op mijn werk kan ik onbedoeld te sterk emotioneel reageren	☐	☐	☐	☐	☐

Gebrek aan cognitieve controle

		1	2	3	4	5
1.	Op het werk kan ik er mijn aandacht moeilijk bijhouden	☐	☐	☐	☐	☐
2.	Tijdens mijn werk heb ik moeite om helder na te denken	☐	☐	☐	☐	☐
3.	Ik ben vergeetachtig en verstrooid tijdens mijn werk	☐	☐	☐	☐	☐
4.	Als ik aan het werk ben, kan ik me moeilijk concentreren	☐	☐	☐	☐	☐
5.	Ik maak fouten in mijn werk omdat ik er met mijn hoofd ‘niet goed bij ben’	☐	☐	☐	☐	☐

Psychische spanningsklachten

		1	2	3	4	5
1.	Mijn gewicht schommelt zonder dat ik op dieet bent	☐	☐	☐	☐	☐
2.	Ik heb problemen met inslapen of doorslapen	☐	☐	☐	☐	☐
3.	Ik heb de neiging om te piekeren	☐	☐	☐	☐	☐
4.	Ik voel mij opgejaagd en gespannen	☐	☐	☐	☐	☐
5.	Ik voel me angstig en/of heb last van paniekaanvallen	☐	☐	☐	☐	☐
6.	Ik heb moeite met drukte en/of lawaai	☐	☐	☐	☐	☐

Psychosomatische spanningsklachten

		1	2	3	4	5
1.	Ik heb last van hartkloppingen of pijn in de borststreek	☐	☐	☐	☐	☐
2.	Ik heb last van maag- en/of darmklachten	☐	☐	☐	☐	☐
3.	Ik heb last van hoofdpijn	☐	☐	☐	☐	☐
4.	Ik heb last van pijnlijke spieren, bijvoorbeeld in de nek, schouder of rug	☐	☐	☐	☐	☐
5.	Ik word snel ziek	☐	☐	☐	☐	☐

World Health Organization - Five Well-Being Index (WHO-5)

Original Dutch version

U wordt verzocht voor ieder van de vijf uitdrukkingen aan te geven welke het best weergeeft hoe U zich heeft gevoeld tijdens de laatste twee weken. Hogere scores betekenen zich beter voelen.

Voorbeeld:
Als u zich gedurende de laatste twee weken en voor meer dan de helft van de tijd in een “vrolijk en in opperbeste stemming” heeft gevoeld, dan klikt u in de regel van beschrijving A op het rondje onder 3.

Uw stemming in de laatste 2 weken:	Constant	Meestal	Meer dan de helft van de tijd	Minder dan de helft van de tijd	Soms	Helemaal niet
	5	4	3	2	1	0
A. Ik voelde me vrolijk en in een opperbeste stemming	☐	☐	☐	☐	☐	☐
B. Ik voelde me rustig en ontspannen	☐	☐	☐	☐	☐	☐
C. Ik voelde me actief en doelbewust	☐	☐	☐	☐	☐	☐
D. Ik voelde me fris en uitgerust bij het opstaan	☐	☐	☐	☐	☐	☐
E. Mijn dagelijks leven was gevuld met dingen die me interesseren	☐	☐	☐	☐	☐	☐

Supplementary info 2 – Cognitive tasks

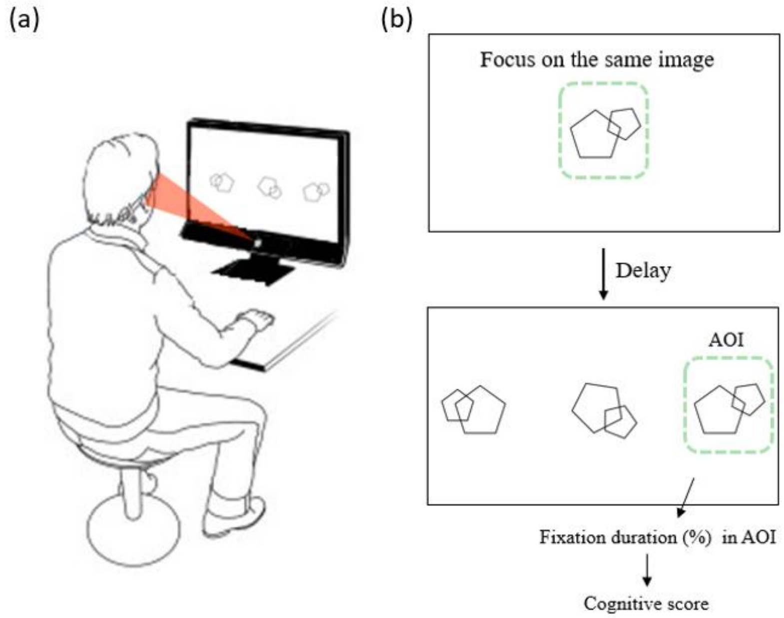
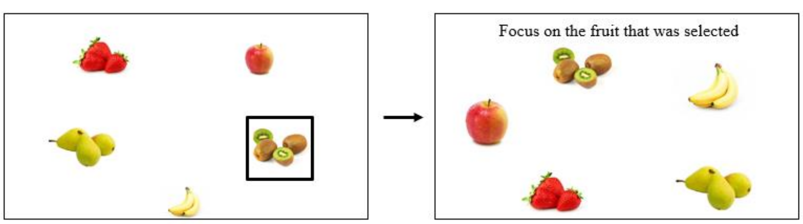
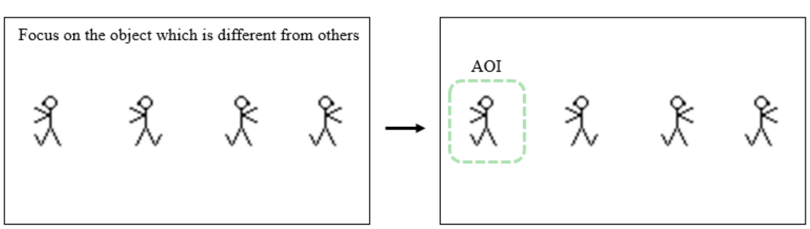


Figure S1: Cognitive assessment using eye-tracking software (Tobii Pro Lab, version 1.152). (a) The gaze point of the participant was recorded using an infrared light source, provided by the Tobii Pro Nano device. The device is located below the monitor. (b) Example of a visual working memory task in the cognitive assessment. An object was shown, followed by three distinct objects. The participant is asked to focus to the same image as the previous image, being the pentagon. Percent fixation duration on the AOI that was set on the target – image bottom right on the screen – was used as a measure of the cognitive score. Figure (a) partly derived from © 2021 Tobii AB (adjusted).

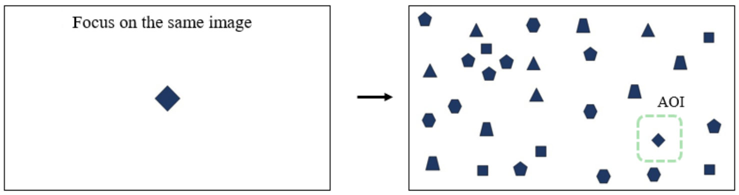
Task 1: Memory task (10s). During this task, a set of different types of fruits were shown. The participant was asked to remember which kind of fruit was selected. At the end of the test, the different types of fruits were displayed again. The participant was asked to focus on the kind of fruit that was selected at the beginning of the test.



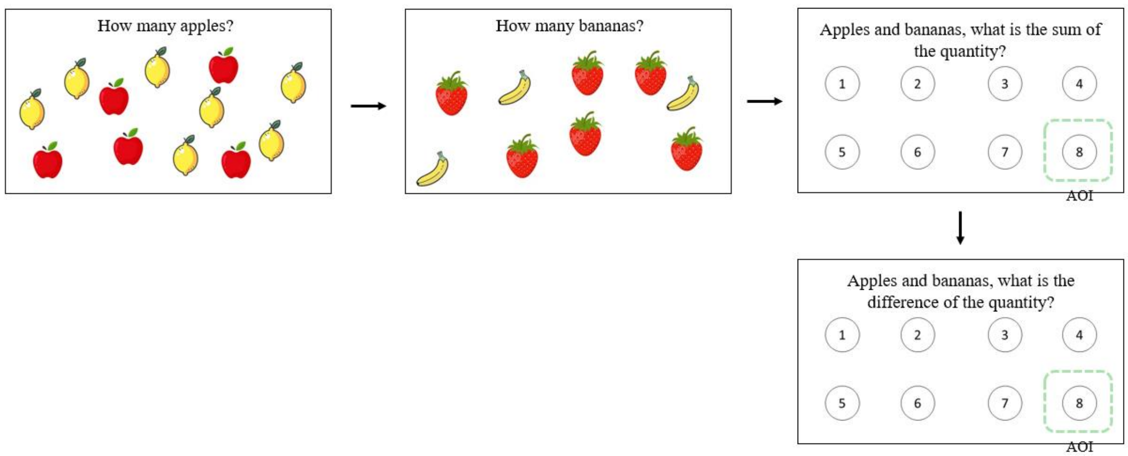
Task 2: Deductive reasoning (odd one out) (75s). Four different objects were shown. The participant was asked to focus on the object that was different from the others.



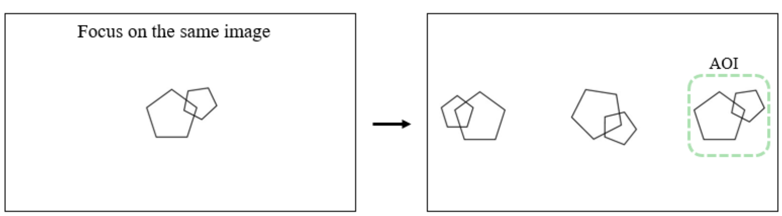
Task 3: Visual working memory (10s). During this task, an object was shown. The participant was asked to focus on the same image in the next part.



Task 4: Attention and calculation (14s). In the first part of this task was asked to calculate the amount of apples and bananas. In the next part, the participant was instructed to focus on the sum of the quantity. After this, the participant was instructed to focus on the difference of the quantity.



Task 5: Visuospatial function task (visual working memory). (10s) An object was shown, followed by three distinct objects. The participant was asked to focus to the same image as the previous image, being the pentagon.



Task 6: Attention bias. During this task, a set of face pairs was shown. Face pairs were retrieved from the Karolinska Directed Emotional Faces [54] database. These faces expressed a neutral emotion together with the emotion ‘happy’, ‘sad’, ‘angry’, or ‘anxious’. The background of the images were framed with a black background to remove non-informative aspects. Diameters of the circles indicate duration of fixation, numbers in the circles represent fixation sequence.