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RESEARCH ARTICLE



Intensity-dependent associations of physical activity and cardiorespiratory fitness with hair cortisol concentrations in the context of lifetime air pollution exposure

Veronika Cibulcova^{a,b}, Steriani Elavsky^a, Lukas Cipryan^a, Marta Jackowska^c, Tomas Dostal^a, Vitezslav Jirik^{b,d}, Daniel Jandacka^a and Vera Kr Jandackova^{a,b}

^aDepartment of Human Movement Studies, Faculty of Education, University of Ostrava, Ostrava, Czech Republic; ^bDepartment of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic; ^cDepartment of Clinical and Health Psychology, Faculty of Psychology, SWPS University, Sopot, Poland; ^dEpidemiology Research Centre, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

ABSTRACT

Hair cortisol concentration (HCC) is used as a biomarker of chronic stress. While evidence linking HCC to physical activity is mixed, associations with cardiorespiratory fitness remain unexplored, and the contribution of long-term ambient air pollution is unclear. This study examined associations of HCC with objectively and subjectively assessed moderate and vigorous physical activity, cardiorespiratory fitness, and estimated lifetime ambient air pollution exposure. Data were obtained from a subsample of adults ($N = 133$; 87% women) participating in the Healthy Aging in an Industrial Environment-Programme 4 study (4HAIE). HCC was quantified from 3-cm hair segments using liquid chromatography-tandem mass spectrometry, reflecting cortisol exposure over approximately 3 months. Physical activity was assessed using the Leisure-Time Exercise Questionnaire (LTEQ) and 14-day Fitbit monitoring, and cardiorespiratory fitness by peak oxygen uptake (VO_{2peak}). Lifetime exposure to particulate matter ($PM_{2.5}$ and PM_{10}) and nitrogen dioxide (NO_2) was estimated from residential histories and historical air pollution models. Associations were examined using multiple linear regression and hierarchical models. Vigorous physical activity was positively associated with HCC in both self-reported (LTEQ: $B = 0.010$, $p = 0.042$) and objectively measured data (Fitbit: $B = 0.006$, $p = 0.044$). VO_{2peak} was initially associated with higher HCC ($B = 0.019$, $p = 0.041$), but this association was attenuated after inclusion of vigorous physical activity. Neither moderate physical activity nor lifetime air pollution exposure was significantly associated with HCC. These findings suggest that vigorous physical activity may contribute to higher HCC, highlighting the importance of considering physical activity intensity when interpreting HCC as a biomarker of chronic stress.

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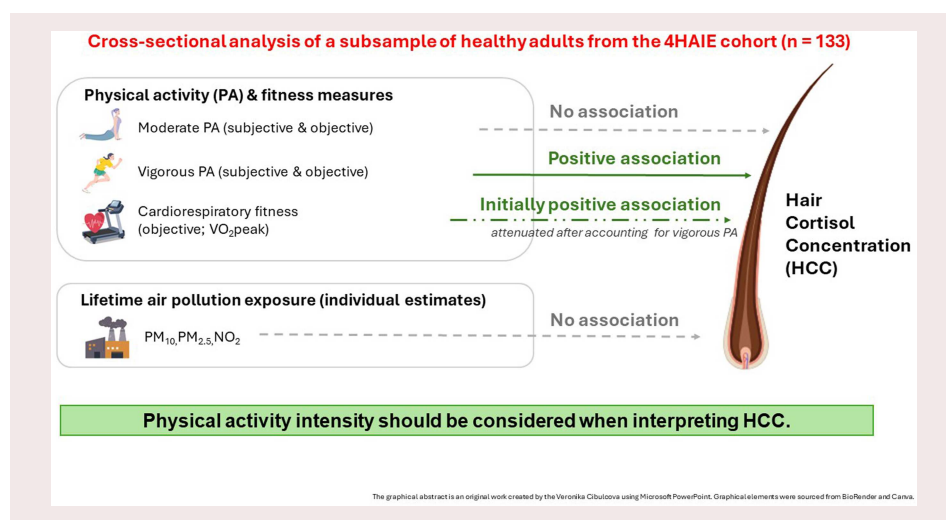
Hair cortisol; physical activity; cardiorespiratory fitness; air pollution; HPA axis; chronic stress

CONTACT Veronika Cibulcova  veronika.cibulcova@osu.cz  Department of Human Movement Studies, Faculty of Education, University of Ostrava, Syllabova 19, 703 00, Ostrava, Czech Republic

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Introduction

The hypothalamic-pituitary-adrenal (HPA) axis regulates many body and brain functions. Its main product is cortisol. Cortisol affects almost all body cells and is important for metabolism, immunity, emotions, and cognition (Kirschbaum, 2024). It is often called the “stress hormone,” but it is also essential for everyday functioning (Hackney & Walz, 2018). In human research, cortisol is most commonly measured in blood, saliva, or urine (Kirschbaum, 2024). While such measures provide valuable information on acute cortisol dynamics and are widely used to assess short-term stress responses, diurnal patterns, and stress reactivity, they are inherently limited for assessing chronic cortisol exposure, as they reflect secretion over relatively short time frames, typically minutes to hours (Garcia-Leon et al., 2018; Stalder et al., 2012). To address this limitation, hair cortisol concentration (HCC), first introduced as a biomarker around 2000, has been increasingly used to retrospectively assess long-term cortisol exposure (Cirimele et al., 2000). Hair cortisol concentration reflects average secretion over several months, is non-invasive to collect, and is not strongly influenced by short-term fluctuations such as circadian rhythm or acute stress (Iglesias et al., 2015). Validation studies in humans and animals support HCC as a robust marker of cumulative cortisol output (Stalder et al., 2012), and its use as a marker of chronic stress has grown substantially in recent years (Iob & Steptoe, 2019; Wilczyńska et al., 2024). Nevertheless, HCC quantifies overall cortisol load and does not distinguish between physical and psychological stressors. Both types of stressors, such as emotional strain or physical exertion, can activate the HPA axis and stimulate cortisol release to support adaptive physiological responses (Moyers & Hagger, 2023).

A large meta-analysis based on data from 124 (sub)samples and 10289 participants reported higher HCC in association with physical stressors, while no significant associations were observed with perceived stress or depressive symptoms. In that meta-analysis, studies involving physical activity were classified as physical stressors (Stalder et al., 2017). A recent study of female football players confirmed this distinction: HCC was positively associated with accumulated training load but showed no significant link with psychological health indicators (Sato et al., 2024). Skoluda et al. (2012) reported that amateur endurance athletes ($N = 304$) had ~46% higher HCC levels than body mass index (BMI)- and age-matched non-athletic controls ($N = 70$), with a dose-response effect by training volume. Gerber et al. (2013), using accelerometry in 46 students, reported a positive association with vigorous, but not moderate, activity. Other studies have yielded mixed findings: García-León et al. (2018) observed slightly higher HCC in exercisers who self-reported regular physical exercise, but with a small effect (Cohen’s $d = 0.11$). Stalder et al. (2013) found no associations when using self-reported activity. While findings across studies point to a possible link between HCC and physical activity, the overall evidence remains inconsistent and is limited by methodological weaknesses. These include reliance only on self-reports or overly broad activity measures, as well as homogeneous samples (e.g. only university students), all of which reduce comparability and weaken the strength of conclusions. Understanding how physical activity may influence HCC is further impeded by the lack of consideration of

cardiorespiratory fitness in the literature, a gap explicitly highlighted by several studies on physical activity and HCC (Gerber et al., 2012, 2013; Skoluda et al., 2012). Specifically, a recent review indicated that higher cardiorespiratory fitness is often associated with lower cortisol reactivity to acute exercise as assessed using short-term cortisol measures, although findings remain mixed (Molina-Hidalgo et al., 2023). Consistent with this, higher fitness has been linked to reduced markers of HPA-axis dysregulation, assessed via salivary cortisol in aging men (Lucertini et al., 2015), and to attenuated age-related cortisol responses assessed using blood cortisol measures in women (Traustadóttir et al., 2005). Together, these findings suggest that higher cardiorespiratory fitness may be associated with more adaptive HPA-axis functioning and cortisol regulation in response to stress. However, to date, no study in adults has examined whether cardiorespiratory fitness is associated with long-term cortisol output as captured by HCC.

While exploring the contribution of physical activity and fitness to HCC, it is important to consider that many forms of physical activity, particularly outdoor activities such as running, may involve exposure to air pollution, a recognised global health risk (Dolcini et al., 2024; WHO, 2021). Consequently, the interplay between air pollution and physical activity, as well as their combined effects on health outcomes, constitutes an emerging area of investigation with important public health implications (Hahad et al., 2021). Physical activity is associated with increased minute ventilation and pulmonary gas exchange, potentially leading to greater inhalation of ambient pollutants (Wu et al., 2024). This may be particularly relevant in regions with persistently elevated pollution levels, such as the Moravian-Silesian Region of the Czech Republic, which ranks among the most polluted regions in the European Union (Machaczka et al., 2023; Michalik et al., 2022). Although air pollution levels have declined since the 1990s, they still exceed recommended limits (Jiřík et al., 2017). Air pollution may act as an environmental stressor capable of activating the HPA axis and increasing glucocorticoid secretion as part of a systemic stress response (Thomson, 2019). In line with this hypothesis, elevated cortisol levels have been observed following air pollution exposure in both animals (Sirivelu et al., 2006; Thomson, 2013) and humans (Hajat et al., 2019; Niu et al., 2018). However, these findings are based primarily on salivary and serum cortisol as short-term markers of HPA-axis activity and may therefore not fully reflect potential alterations in HPA-axis regulation associated with chronic environmental stress exposure (Thomson, 2019). To the best of our knowledge, evidence linking ambient air pollution to HCC is limited to two studies from Flanders, reporting higher HCC in adolescents (Verheyen et al., 2021a) and in pregnant women (Verheyen et al., 2021b), with no data in the general adult population and no adjustment for physical activity. Overall, the available literature suggests that multiple behavioural and environmental factors, as well as potentially physiological processes adaptations related to fitness, may influence HPA-axis activity through distinct mechanisms and contribute to variation in HCC.

Aim and hypotheses

The aim of this study was to examine associations between HCC, moderate and vigorous physical activity assessed using both self-reported leisure-time physical activity and objectively measured physical activity indicators, and cardiorespiratory fitness. Additionally, we tested whether HCC is associated with lifetime exposure to air pollution. We used data from a subsample of the 4HAIE study, including runners and non-runners from both high- and low-pollution areas, which was specifically designed to assess multiple objective and subjective indicators of physical activity and fitness, as well as long-term exposure to air pollutants in relation to health outcomes and injury. We hypothesised that higher cardiorespiratory fitness would be associated with lower HCC, and that vigorous physical activity and lifetime air pollution exposure would be associated with higher HCC. To our knowledge, this is the first study to combine measures of physical activity, cardiorespiratory fitness, and long-term air pollution exposure in relation to HCC in an adult sample.

Methods

Participants

Participants ($N = 150$) were a randomly selected subsample of the Healthy Aging in an Industrial Environment - Programme 4 study (4HAIE, 2019-2021), a prospective study on air pollution, health, and aging in adults

aged 18-65 years. A detailed description of the recruitment process, cohort formation, and eligibility criteria has been published previously (Plesek et al., 2025). The parent study was designed to recruit both runners and physically inactive controls (non-runners) according to predefined eligibility criteria (Cipryan et al., 2020; Elavsky et al., 2021; Jandacka et al., 2020). Briefly, eligible runners met the World Health Organisation (WHO) aerobic physical activity recommendations, reported running at least 10 km per week during the previous six weeks, and intended to continue running. Inactive controls (non-runners) did not meet these criteria but were otherwise capable of engaging in regular physical activity, including running. Participants were recruited from regions with historically higher (Moravian-Silesian Region) and lower (South Bohemian Region) levels of air pollution to capture a broad gradient of environmental exposure. Eligible participants were required to have lived in one of these regions for at least five years and have no plans to move away during the following year. Hair cortisol concentration was analysed in a randomly selected subset of samples available at the time of laboratory analysis, while data collection in the parent study was still ongoing. Because of funding constraints, HCC could be measured only in this subset. Therefore, samples were selected using simple random sampling without replacement from all eligible samples available at that time. General 4HAIE exclusion criteria comprised smoking, acute health conditions limiting activity, and Magnetic Resonance Imaging (MRI)/Dual-Energy X-ray Absorptiometry (DEXA) contraindications. For the present study, additional exclusion criteria included hair length < 3 cm or otherwise insufficient hair sample (e.g. due to baldness or a shaved scalp), systemic glucocorticoid use, and self-reported HPA-axis disorders. Ethical approval was obtained from the university ethics committee, and all participants gave written informed consent in accordance with the Declaration of Helsinki.

Measures

Hair cortisol concentration

Hair samples were collected from all participants meeting the hair-length eligibility criteria at the end of the first day of the two-day 4HAIE baseline protocol, following cardiorespiratory fitness assessment. A 3 cm strand from the posterior vertex was cut close to the scalp by trained staff, reflecting cortisol exposure over the past three months (≈ 12 weeks) (Kirschbaum et al., 2009). Samples (≈ 3 mm diameter) were wrapped in foil, sealed, labelled, and stored at room temperature in a dark, dry environment. Hair cortisol concentration was analysed at the Dresden University of Technology using liquid chromatography-mass spectrometry (LC-MS/MS) with standard washing and extraction (Gao et al., 2013). This gold-standard method is recognised for its high specificity (Martins-Silva et al., 2025). All samples were run in a single batch, expressed in pg/mg, with intra-assay variation <12%.

Demographics, health and leisure-time physical activity

Participants completed online questionnaires to provide demographic and health information (e.g. age, sex, education, medication use, chronic medical conditions, residential history). In women, menopausal status was determined during telephone screening based on menstrual cycle history and hormonal status according to the Stages of Reproductive Aging Workshop criteria (Harlow et al., 2012). When classification based on menstrual history was not possible, menopausal status was determined using self-reported menopausal status. Further details regarding the assessment procedure are available elsewhere (Elavsky et al., 2024). Subjective physical activity was assessed with a modified Godin Leisure-Time Exercise Questionnaire (LTEQ; Godin & Shephard, 1985), which captures self-reported frequency of leisure-time physical activity at different intensity levels (light, moderate, and vigorous). Weighted scores (vigorous $\times 9$, moderate $\times 5$, light $\times 3$) were calculated to derive component and total LTEQ indices (Godin, 2011), with higher scores indicating greater self-reported leisure-time physical activity. Perceived stress was assessed using the 10-item Perceived Stress Scale (PSS-10; Cohen & Williamson, 1988), which evaluates the extent to which life situations are perceived as unpredictable, uncontrollable, or overloading. Items were rated on a 5-point Likert scale ranging from 1 (never) to 5 (very often), with higher scores indicating greater perceived stress. Internal consistency in the present sample was Cronbach's $\alpha = 0.872$. Comprehensive details on all variables are available at <https://haie-lerco.cz/data>.

Objectively measured physical activity using a Fitbit device

Objectively measured physical activity was recorded for 14 consecutive days following the baseline assessment using Fitbit Charge 3 devices (Fitbit, San Francisco, CA). Minutes spent in moderate and vigorous activity were tracked across 10 weekdays and two weekends. The data were retrieved via the Fitbit cloud through a publicly available application programming interface using the HealthReact platform (<https://www.healthreact.eu/>). For descriptive purposes (Table 1), participants were categorised according to adherence to WHO guidelines on physical activity (WHO, 2020), which recommend 150–300 minutes of moderate-intensity, 75–150 minutes of vigorous-intensity aerobic activity, or an equivalent combination per week.

Cardiorespiratory fitness

Cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$) was assessed using a graded exercise test (GXT) on a motorised treadmill (Rodby RL 2500E). After a 3-min warm-up at 5.0 km/h, speed increased by 1.0 km/h per minute with 1% fixed incline until exhaustion. Expired gases were continuously analysed (Blue Cherry, Geratherm Medical AG), heart rate was monitored (Polar Electro H9), and perceived exertion was rated using the Borg 20-point scale. $\text{VO}_{2\text{peak}}$ was defined as the highest 30-s average of O_2 consumption before test termination. Tests were performed under controlled conditions (21 °C, 40% RH), at least 3 h postprandial, with 24-h abstinence from vigorous activity. Participants with elevated blood pressure (>150/90 mm Hg) or those who failed the Physical Activity Readiness Questionnaire could perform the test only with medical approval. Otherwise, they continued with the study protocol without GXT. A more detailed description of the GXT is provided in the protocol article of the 4HAIE study (Cipryan et al., 2020).

Lifetime exposure to air pollution

The methodology for calculating personal lifetime exposure was developed by a team of authors based on an analysis of historical concentrations of air pollutants at the district level in two differently environmentally polluted regions of the Czech Republic (see Machaczka et al., 2023; Michalik et al., 2022). This study used time series of concentrations of PM_{10} , $\text{PM}_{2.5}$, and NO_2 originating from national databases for model calculations. Average annual concentrations were derived by integrating measured and mathematically modelled air pollution data provided by the Czech Hydrometeorological Institute (CHMI) for the years 1997–2017. A spatial and temporal correlation and regression analysis of these data, emission balance data

Table 1. Descriptive characteristics of the study participants.

Sample size ($N = 133$)	
Age (years), mean (SD)	38.72 (9.66)
Sex (%)	
Male	17 (12.78)
Female	116 (87.22)
Menopausal status (women), N (%)	
Premenopausal	103 (88.79)
Perimenopausal	7 (6.03)
Postmenopausal	6 (5.17)
University degree, N (%)	61 (45.86)
Region	
Moravian-Silesian Region (high air pollution), N (%)	76 (57.14)
South Bohemian Region (low air pollution), N (%)	57 (42.86)
Baseline physical activity status (runner/non-runner)	
Runner, N (%)	98 (73.68)
Non-runner (inactive control), N (%)	35 (26.32)
No self-reported economic strain (food or clothing), N (%)	82/132 (62.12)
Weekly alcohol intake (sum of reported alcoholic beverages), median (IQR)	2 (1–5)
Daily wear time based on heart rate recording (minutes; Fitbit), mean (SD)	919.63 (104.11)
Presence or diagnosis of chronic medical conditions (self-reported), N (%)	28 (21.05)
Self-reported prescribed medication use, N (%)	26 (19.55)
Including hormonal contraception or other hormone-related medication among women	5 (4.31)
Body mass index, mean (SD)	23.44 (3.37)
Hair cortisol concentration (pg/mg), median (IQR)	6.07 (3.63–11.50)
VO_2 peak ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), mean (SD)	40.76 (8.53)
Meeting WHO recommendations, Fitbit (N , %)	127 (95.49)
$\text{PM}_{2.5}$ ($\mu\text{g}/\text{m}^3$), median (IQR; range)	22.22 (15.68–30.70; 7.21–41.47)
PM_{10} ($\mu\text{g}/\text{m}^3$), median (IQR; range)	29.70 (20.67–39.90; 9.45–53.86)
NO_2 ($\mu\text{g}/\text{m}^3$), median (IQR; range)	13.45 (9.30–19.38; 5.88–25.97)

and dust fallout data for the years 1960–1997, and correlation analyses of documented data on industrial production, i.e. a key factor in pollutant emissions, were performed. For districts where specific industrial production data were missing, constant trend extrapolation was used to extend the time series before 1960. Lifetime exposure to air pollutants was quantified as the lifetime average exposure concentration (LC_{px}) of each individual air pollutant (x) and proband (p). This calculation used the annual average concentration derived from historical time series of air pollutant concentrations at all districts (d), as described previously. These district-level estimates were linked to individual participants using their residential histories across the life course. Lifetime exposure was then calculated using the following formula:

$$LC_{px} = \frac{\sum_{i=y}^{i=y+a} (C_{xdi})}{a}$$

where (C_{xdi}) is the average annual concentration of pollutant (x) in the district (d) where the participant resided in year (i). The annual concentrations from the participant's birth year (y) to their current age (a) are summed and then divided by their age (a) to obtain a lifetime average.

Covariates

Several covariates were considered due to their potential associations with HCC, physical activity, cardio-respiratory fitness, and air pollution exposure. These included age (continuous), sex (categorical), and education level (categorical; categorised as basic, vocational, secondary, higher, or university). For models involving Fitbit-derived activity variables, daily wear time in minutes based on heart rate recording was included to account for variability in device compliance (continuous). In models examining the association between air pollution exposure and HCC, objectively measured vigorous physical activity (Fitbit, minutes/day, continuous) was included as a covariate to account for its potential confounding effect. In additional exploratory analyses, interaction terms between lifetime air pollution exposure and vigorous physical activity were tested.

Additional covariates included BMI (continuous), self-reported perceived household economic strain (categorical), self-reported presence or diagnosis of chronic medical conditions (categorical), use of prescribed medication (categorical) and weekly alcohol intake (continuous). Body mass index was calculated from objectively measured height and weight; for further details, see Cipryan et al. (2020). Self-reported perceived household economic strain was assessed using a single-item measure of financial difficulties in meeting basic needs (food and clothing), rated on a six-point scale ranging from 1 (“always”) to 6 (“never”), with higher scores indicating lower perceived economic strain. The prevalence of chronic medical conditions was assessed by self-reported presence or diagnosis of any of the following: coronary heart disease, stroke, diabetes mellitus, cancer, asthma, bronchitis, dementia, depression and hypertension. Medication use (including hormonal contraception or other hormone-related medication) was assessed by self-report of prescribed medication use within the last 14 days. Weekly alcohol intake (sum of reported alcoholic beverages) was computed as the sum of self-reported weekly servings of wine (1 dL per glass), beer (0.5 L per glass), and distilled spirits (0.5 dL per glass), based on responses to individual questionnaire items.

Statistical analyses

All analyses were conducted in Stata/IC 16.1 (StataCorp LLC, College Station, TX, U.S.A.) with a significance level of $\alpha = 0.05$. Intentionally, p -values were not corrected for multiple comparisons (Rothman, 2014, 1990; Saville, 1990). Hair cortisol concentration showed a positively skewed distribution (Shapiro-Wilk $p < 0.001$) and was therefore log-transformed (ln-HCC), which improved normality ($p = 0.88$). Table 1 presents untransformed HCC values, while continuous variables are summarised as means with standard deviations or medians with interquartile ranges, according to their distribution. For selected variables, the observed range (minimum-maximum) is additionally reported. Categorical variables are presented as N (%). Air pollution variables (PM_{10} , $PM_{2.5}$, NO_2) were also positively skewed. Several transformations (log, square-root, cubic, square, inverse) were tested but did not normalise the data, hence, untransformed values were used in all analyses. Associations between ln-HCC (pg/mg) and physical activity (subjective and objective),

cardiorespiratory fitness, and lifetime air pollution exposure were examined using multiple linear regression. Because several physical activity and fitness indicators (VO₂peak, LTEQ vigorous physical activity, and vigorous physical activity by Fitbit) were moderately intercorrelated (Spearman's rho $\approx$ 0.48–0.56), they were initially examined in separate multiple regression models. Diagnostic analyses also indicated substantial collinearity among the air pollutants (mean VIF = 4.77). Therefore, only single-pollutant models were tested. Assumptions of linear regression were evaluated separately for each model by assessing linearity through visual inspection of residual-versus-fitted plots, normality of residuals (Shapiro-Wilk test), homoscedasticity (Breusch-Pagan test), and multicollinearity (variance inflation factors). No violations of assumptions were detected. Results are reported as unstandardised coefficients with 95% CIs, SEs, and R² values. Additionally, associations of the covariates age and sex with ln-HCC, physical activity measures, and cardiorespiratory fitness were examined using separate regression models corresponding to the primary analyses. In additional explanatory analyses, we used hierarchical linear regression models to evaluate the contribution of moderate and vigorous physical activity to the VO₂peak-HCC association. Model 1 included VO₂peak together with age, sex, and education. Model 2 additionally contained self-reported moderate LTEQ activity. Model 3 further contained self-reported vigorous LTEQ activity. Separately, Model 4 added Fitbit-derived moderate activity to Model 1, and Model 5 added Fitbit-derived vigorous activity to Model 4. Variance inflation factors indicated no evidence of problematic multicollinearity among predictors (all VIF < 2.5; mean VIF = 1.52).

Adjusted associations of physical activity (subjective and objective) and cardiorespiratory fitness with ln-HCC were visualised using partial regression plots, whereas associations with lifetime air pollution exposure are reported in tables only.

Additional and sensitivity analyses

Additional analyses were conducted to support the interpretation and robustness of the primary findings. First, we examined PSS both as an independent predictor of ln-HCC and as an additional covariate in sensitivity analyses of the fully adjusted regression models. However, the PSS was not included in the primary regression models because it was not central to the research question. Second, in order to evaluate the representativeness of the analytical HCC subsample was evaluated we compared its baseline characteristics with those of the broader pool of eligible participants from whom hair samples had been collected. Depending on the distribution of the data, continuous variables were compared using independent-samples t-tests or Mann-Whitney U tests, whereas categorical variables were compared using chi-square tests. Third, the association between self-reported runner status (runner vs. inactive control) and ln-HCC was examined using multiple linear regression adjusted for the same covariates as the primary analyses. Finally, to explore whether objectively measured vigorous physical activity (Fitbit, minutes/day) moderated the association between lifetime air pollution exposure and ln-HCC, we included interaction terms (physical activity x air pollution exposure) in the models assessing lifetime air pollution exposure as a predictor.

Results

Participants

Of the 150 randomly selected participants, one withdrew, one had HCC values below the detection limit, and two were excluded due to prednisone use. In addition, lifetime air pollution exposure data were missing for eight participants and VO₂peak data for five. The final analytical sample therefore comprised 133 participants (mean age = 38.7 years, SD = 9.66; age range: 18–63 years). Sample sizes for analyses that included self-reported leisure-time physical activity ranged from 115 to 121 due to missing responses, with exact numbers reported in the tables and figures. Fitbit devices were worn for a mean of 919.63 minutes per day during the 14-day monitoring period. The median untransformed HCC was 6.07 pg/mg (IQR: 3.63–11.50; range: 0.60–37.83 pg/mg). Detailed sample characteristics are presented in [Table 1](#). The majority of participants were women (87%), most of whom were premenopausal (88.8%). The resulting imbalance in sex distribution reflects the inclusion criteria for HCC analysis, as several male participants did not have sufficient hair in the posterior vertex region. Overall, 46% of participants held a university degree.

All participants were of White ethnic origin. A self-reported chronic medical condition was reported by 21.1% of participants, and prescribed medication use by 19.6%. Overall, the sample was characterised by a normal mean BMI, relatively high cardiorespiratory fitness, and high levels of physical activity, with the majority meeting WHO recommendations based on Fitbit-derived data. Compared with the broader pool of eligible participants from whom hair samples had been collected ($N = 546$), the analytical HCC subsample did not differ significantly in age or education. However, it included a higher proportion of women and participants with higher cardiorespiratory fitness and higher levels of vigorous physical activity, as assessed by both self-report and Fitbit-derived measures (Supplementary Table S1).

The associations of hair cortisol concentration with physical activity measures and cardiorespiratory fitness (separate regression models)

Among the self-reported LTEQ measures, vigorous leisure-time physical activity was significantly positively associated with ln-HCC ($R^2 = 0.086$, $B = 0.010$, $SE = 0.004$, 95% CI = 0.0004–0.019, $p = 0.042$), whereas moderate leisure-time activity showed a non-significant negative trend ($R^2 = 0.043$, $B = -0.010$, $SE = 0.008$, 95% CI = -0.027–0.006, $p = 0.211$); see Table 2 and Figure 1. The association with LTEQ total score and LTEQ light activity was non-significant ($p > 0.113$), data not shown.

Similar findings were observed in the analyses using Fitbit-derived measures, daily minutes of vigorous physical activity were significantly positively associated with ln-HCC ($R^2 = 0.071$; $B = 0.006$, $SE = 0.003$, 95% CI = 0.0001–0.012, $p = 0.044$; see Table 2 and Figure 2, right), while moderate physical activity based on Fitbit showed no significant association ($R^2 = 0.044$; $B = -0.005$, $SE = 0.005$, 95% CI = -0.012–0.006, $p = 0.455$; see Table 2 and Figure 2, left). Additionally, average daily moderate and vigorous physical activity (MVPA) derived from Fitbit was not significantly associated with ln-HCC ($R^2 = 0.050$, $B = 0.003$, $p = 0.248$), data not shown.

Finally, VO_{2peak} was significantly positively associated with ln-HCC ($R^2 = 0.072$; $B = 0.019$, $SE = 0.009$, 95% CI = 0.001–0.038, $p = 0.041$), suggesting a positive association between cardiorespiratory fitness and HCC (see Table 2, Figure 3).

Associations of age and sex with ln-HCC, physical activity measures, and cardiorespiratory fitness are presented in Supplementary Table S2. Age and sex were associated with selected objectively measured physical activity and fitness measures but not with ln-HCC or self-reported physical activity (LTEQ). Specifically, age was significantly associated only with VO_{2peak} , whereas men exhibited higher objectively measured physical activity and higher VO_{2peak} than women.

To further explore the observed associations, we conducted additional explanatory analyses to test whether moderate and/or vigorous physical activity accounts for the relationship between VO_{2peak} and HCC. In the base model adjusted for age, sex, and education, VO_{2peak} was positively associated with ln-HCC ($B = 0.021$, $p = 0.045$). After adding self-reported moderate physical activity (LTEQ; Model 2), the association remained significant ($B = 0.023$, $p = 0.033$). When vigorous LTEQ activity was subsequently included (Model 3), the VO_{2peak} coefficient decreased and became non-significant ($B = 0.015$, $p = 0.237$), suggesting shared variance between VO_{2peak} and leisure-time vigorous physical activity. A similar pattern was observed in models using Fitbit-derived physical activity. Adding Fitbit moderate physical activity in Model 4 did not change the VO_{2peak} -HCC association ($B = 0.021$, $p = 0.026$). However, including Fitbit vigorous physical activity (Model 5) reduced the VO_{2peak} coefficient to non-significance ($B = 0.013$, $p = 0.241$). These results

Table 2. Results of separate multiple linear regression models examining the associations between measures of physical activity and cardiorespiratory fitness and ln-HCC, adjusted for age, sex, and education.

Physical activity and fitness measures	R^2	B	Std. Err	95% CI	p-value
Self-reported leisure-time physical activity (LTEQ)					
LTEQ moderate ($N = 115$)	0.043	-0.010	0.008	-0.027 to 0.006	0.211
LTEQ vigorous ($N = 121$)	0.086	0.010	0.004	0.0004 to 0.019	0.042*
Objectively-measured physical activity (based on Fitbit, minutes/day)					
Minutes in moderate PA per day ($N = 133$)	0.044	-0.005	0.005	-0.012 to 0.006	0.455
Minutes in vigorous PA per day ($N = 133$)	0.071	0.006	0.003	0.0001 to 0.012	0.044*
Cardiorespiratory fitness					
VO_{2peak} ($N = 133$)	0.072	0.019	0.009	0.001 to 0.038	0.041*

Note: Each row represents a separate regression model.

* $p < 0.05$.

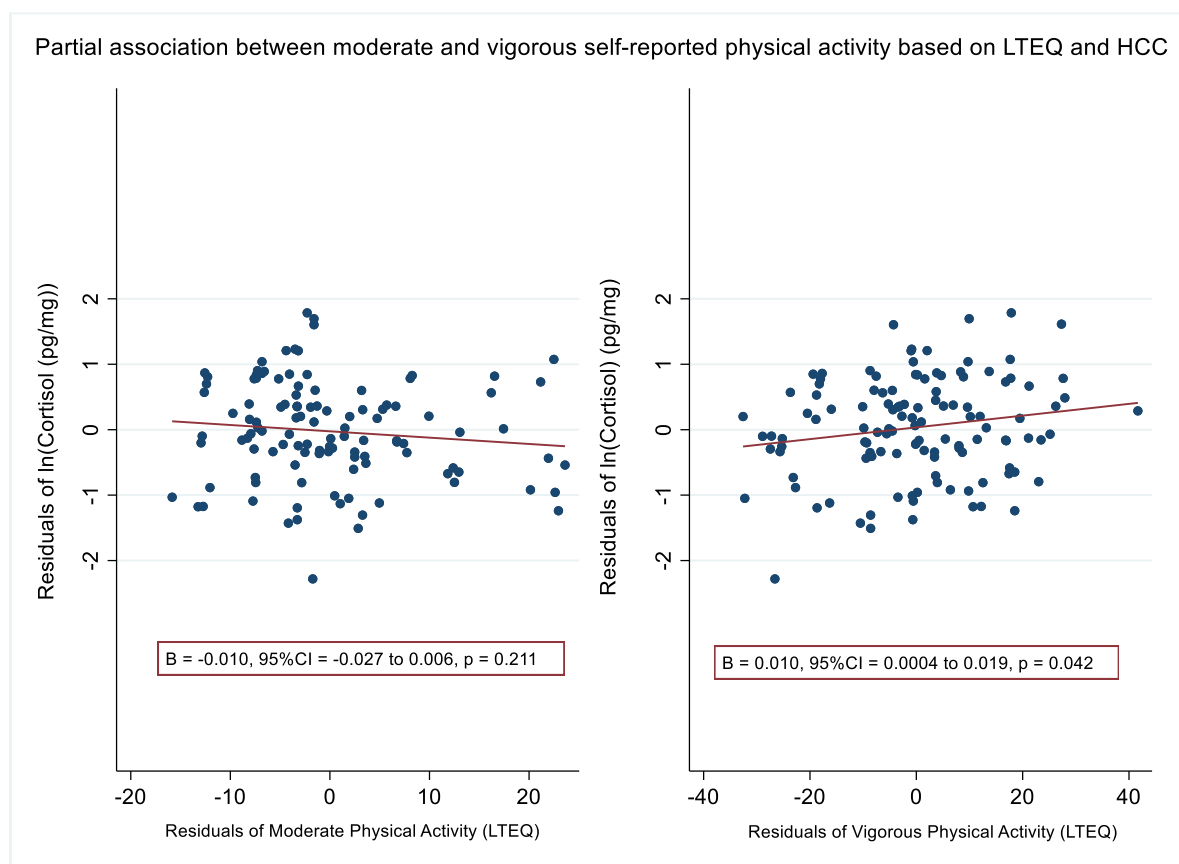


Figure 1. Partial regression plot showing the association between self-reported moderate (left) ($N = 115$) and vigorous ($N = 121$) leisure-time physical activity (LTEQ) and ln-HCC, adjusted for age, sex, and education.

may reflect differences in the contribution of vigorous versus moderate physical activity to the association between VO_{2peak} and HCC. Consistent with this pattern, only models including vigorous physical activity showed a noticeable increase in explained variance (R^2 rising from 0.059 to 0.082 in the LTEQ model and from 0.059 to 0.085 in the Fitbit model), whereas adding moderate activity resulted in a more modest improvement in model fit (R^2 rising from 0.059 to 0.072 in the LTEQ model and from 0.059 to 0.068 in the Fitbit model). A summary of these hierarchical regression models is presented in Table 3.

Sensitivity and robustness analyses

To examine the robustness of the findings, additional models including further covariates (BMI, self-reported chronic medical condition, use of prescribed medication (including hormonal contraception or other hormone-related medication), weekly alcohol intake, and perceived household economic strain) were estimated. The results were comparable to those of the primary analyses, with previously observed statistically significant associations between physical activity and fitness measures and HCC remaining evident (see Supplementary Table S3). The inclusion of PSS as an additional covariate in the fully adjusted model yielded similar estimates (see Supplementary Table S4). In a separate regression model, PSS was not statistically significantly associated with ln-HCC ($p = 0.386$; see Supplementary Table S5). In addition, self-reported runner status showed a significant association with ln-HCC, with inactive controls exhibiting lower ln-HCC than runners, consistent with the primary analyses (Supplementary Table S6).

The association between lifetime air pollution exposure and hair cortisol concentration (separate regression models)

As shown in Supplementary Table S7, none of the examined lifetime exposures to air pollutants was significantly associated with ln-HCC. $PM_{2.5}$ showed a non-significant positive association with ln-HCC

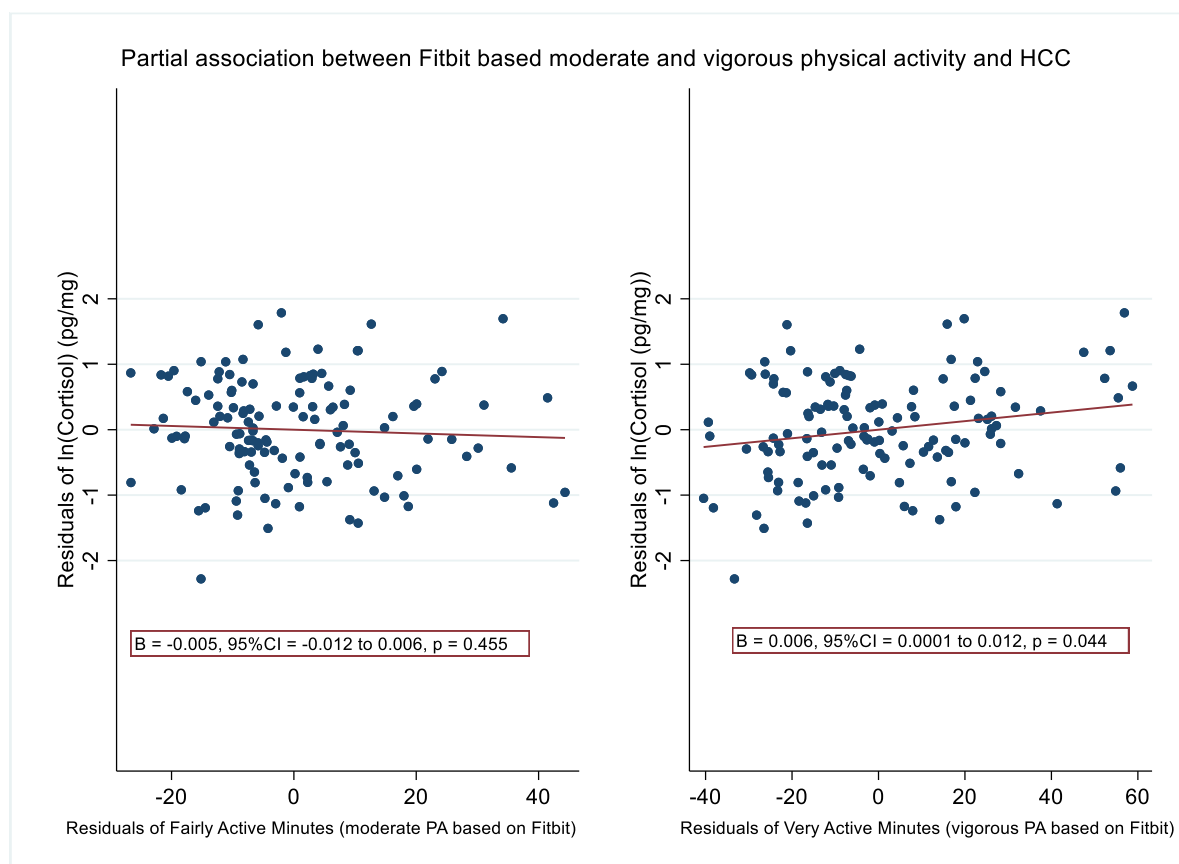


Figure 2. Partial regression plots showing the associations between Fitbit-derived minutes in moderate (left) and vigorous (right) physical activity per day and ln-HCC, adjusted for age, sex, education and Fitbit minutes of wear time based on HR values per day ($N = 133$).

($R^2 = 0.071$; $B = 0.002$, $SE = 0.008$, 95% CI: -0.012 to 0.017 , $p = 0.749$). A similarly non-significant relationship was observed for PM_{10} ($R^2 = 0.071$; $B = 0.002$, $SE = 0.006$, 95% CI: -0.009 to 0.013 , $p = 0.740$). For NO_2 , the regression coefficient was close to zero, with no indication of an association ($R^2 = 0.070$; $B = -0.001$, $SE = 0.012$, 95% CI: -0.025 to 0.022 , $p = 0.920$).

Exploratory models including interaction terms between air pollution exposure ($PM_{2.5}$, PM_{10} , and NO_2) and objectively measured vigorous physical activity (Fitbit, minutes/day) in relation to HCC did not yield statistically significant results (all p -values > 0.84). No significant interaction effects were observed, and adding interaction terms did not meaningfully improve model fit ($R^2 = 0.070$ - 0.071). Full results are presented in Supplementary Material (see Supplementary Table S8).

Discussion

Using cross-sectional data from a subsample of the Czech 4HAIE study, we found consistent positive associations of vigorous-intensity physical activity, measured both subjectively and objectively, as well as cardiorespiratory fitness, with HCC. In contrast, no such associations were observed for moderate-intensity physical activity. Further analyses suggested that vigorous physical activity may be more closely associated with HCC than moderate physical activity or cardiorespiratory fitness. Lifetime exposure to ambient air pollution was not significantly associated with HCC.

Several mechanisms may explain our finding that vigorous, but not moderate, physical activity was associated with higher HCC. First, this pattern is consistent with the intensity-dependent activation of the HPA axis. Exercise performed at moderate-to-high intensities (approximately 60-80% VO_{2max}) has been shown to elicit acute increases in circulating cortisol, whereas lower-intensity activity ($\sim 40\%$ VO_{2max})

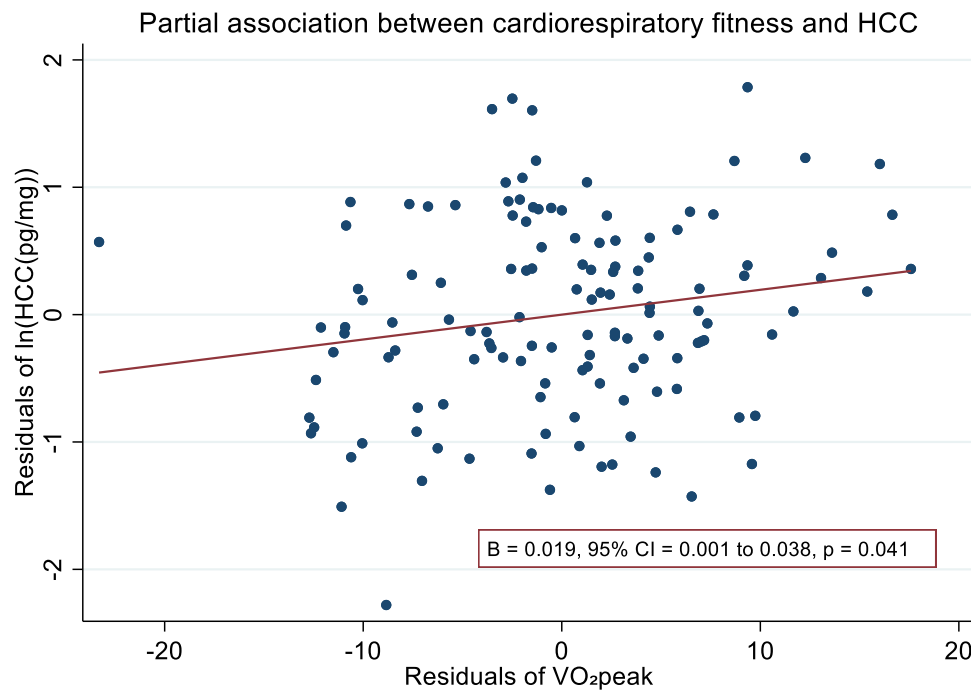


Figure 3. Partial regression plots showing the associations between cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$) and $\ln\text{-HCC}$, adjusted for age, sex, and education ($N = 133$).

Table 3. Hierarchical regression models examining the association between cardiorespiratory fitness ($\text{VO}_{2\text{peak}}$) and $\ln\text{-HCC}$, with additional subjective (LTEQ) and objective (Fitbit) measures of moderate and vigorous physical activity.

Models	Model 1 (age, sex, education, $\text{VO}_{2\text{peak}}$)	Model 2 (Model 1 + LTEQ moderate)	Model 3 (Model 2 + LTEQ vigorous)	Model 4 (Model 1 + Fitbit moderate)	Model 5 (Model 4 + Fitbit vigorous)
Sample size	133	106	106	133	133
Age	-0.005 (0.010), $p = .631$	-0.005 (0.010), $p = .586$	-0.006 (0.010), $p = .509$	-0.0037 (0.0079), $p = .645$	-0.0057 (0.0080), $p = .476$
Sex	-0.365 (0.266), $p = .173$	-0.374 (0.265), $p = .162$	-0.321 (0.271), $p = .238$	-0.358 (0.226), $p = .115$	-0.371 (0.225), $p = .101$
Education	-0.082 (0.072), $p = .254$	-0.089 (0.072), $p = .216$	-0.087 (0.072), $p = .231$	-0.094 (0.063), $p = .137$	-0.088 (0.063), $p = .161$
$\text{VO}_{2\text{peak}}$	0.021 (0.011), $p = .045^*$	0.023 (0.011), $p = .033^*$	0.015 (0.013), $p = .237$	0.021 (0.009), $p = .026^*$	0.013 (0.011), $p = .241$
LTEQ moderate	—	-0.010 (0.009), $p = .238$	-0.011 (0.009), $p = .188$	—	—
LTEQ vigorous	—	—	0.006 (0.006), $p = .317$	—	—
Fitbit moderate	—	—	—	-0.0047 (0.0045), $p = .296$	-0.0067 (0.0046), $p = .151$
Fitbit vigorous	—	—	—	—	0.0053 (0.0034), $p = .121$
R^2	0.059	0.072	0.082	0.068	0.085
Adjusted R^2	0.022	0.026	0.026	0.031	0.042

produces minimal or no cortisol responses (Anderson et al., 2019; Caplin et al., 2021; Hill et al., 2008). Moreover, cortisol elevations following vigorous exercise may extend into the post-exercise recovery period (Montero et al., 2021). Repeated exposure to such physiological activation could therefore be associated with higher cumulative cortisol output over time, as reflected in hair samples. A second explanation involves sweat-related cortisol incorporation into hair. While experimental evidence indicates that cortisol is present in sweat and can be absorbed by hair under controlled conditions (Russell et al., 2014), well-controlled physiological studies have not consistently demonstrated acute effects of sweating on HCC (Grass et al., 2015). Observational findings linking self-reported scalp sweating to higher HCC further support this possibility; however, these results are limited by the subjective assessment of sweating (Abdulateef, 2021). Thus, it remains unclear whether elevated HCC associated with vigorous activity

primarily may reflect intensity-dependent HPA axis activation, sweat-related processes, or other mechanisms that have not yet been identified.

Physical activity and cardiorespiratory fitness

As hypothesised, vigorous, but not moderate, physical activity was positively associated with HCC in our sample. Our findings are broadly consistent with observational studies using objective activity assessment (Gerber et al., 2013) and in interventional research, where exposure to high-intensity exercise led to increases in HCC, and moderate-intensity activity was associated with decreasing HCC levels (Wilczyńska et al., 2024). Studies have also found elevated HCC in endurance athletes (as compared to non-athletic controls; Skoluda et al., 2012), individuals classified as regularly exercising (Garcia-Leon et al., 2018), and in relation to higher self-reported training load (Sato et al., 2024). In contrast, activity assessed using self-reported frequency ratings was not associated with HCC (Stalder et al., 2013).

Contrary to our initial hypothesis, higher cardiorespiratory fitness was associated with higher HCC. This finding contrasts with previous evidence, such as the study by Gerber et al. (2017), who found no significant association between HCC and cardiorespiratory fitness assessed using the 20-m shuttle run test in a sample of 318 children. The authors suggested that this null finding may reflect the generally low HCC levels and limited variability in their young, healthy sample, potential floor effects, and the possibility that associations between fitness and HPA-axis activity may become more pronounced at older ages. Our findings also contrast with those of Lucertini et al. (2015), who observed an association between higher cardiorespiratory fitness and lower daily cortisol output in healthy older men ($N = 22$); however, these findings were based on a small sample and salivary cortisol, which reflects short-term HPA-axis activity rather than long-term cortisol exposure indexed by HCC. Together, these studies illustrate the heterogeneity in how physical activity has been assessed in research on HCC, with most relying on self-reported data and lacking sufficient differentiation by intensity, objective measurements, or assessments of cardiorespiratory fitness in adult populations. To the best of our knowledge, only one study has examined the association between cardiorespiratory fitness and HCC, as discussed above, and this study was conducted in a children population.

Our results suggest that the observed association between cardiorespiratory fitness and HCC may be more closely related to vigorous physical activity than to fitness itself, although this interpretation should be made with caution. In our hierarchical models, an initial link between higher VO_{2peak} and higher HCC was observed. However, this association was attenuated once vigorous physical activity was added to the hierarchical model, while the explained variance remained largely unchanged. Nonetheless, cardiorespiratory fitness reflects not only habitual physical activity but also genetic, behavioural, and socioeconomic factors, which may independently influence HPA-axis functioning (Oggionni et al., 2025).

Although HCC has become an increasingly popular biomarker and is often interpreted as an indicator of poor health (Lawes & Eid, 2025), such interpretation warrants caution. Elevated HCC may, in some contexts, reflect adaptive physiological activation, particularly in physically active individuals. To improve the interpretability of HCC, future studies should incorporate robust and intensity-specific measures of physical activity, with particular emphasis on vigorous-intensity activity, as insufficient consideration of activity intensity may contribute to confounding and variability across findings.

Lifetime air pollution exposure

Although lifetime exposure to ambient air pollution was expected to be associated with higher HCC, this association was not observed in the present sample. This null association was consistently observed across unadjusted models, models adjusted for objectively measured vigorous physical activity, as well as exploratory interaction analyses, suggesting that vigorous physical activity was unlikely to have materially influenced these findings. Current evidence linking ambient air pollution to HCC is limited, with only two prior studies reporting positive associations. The first, conducted in pregnant women ($n = 133$, plus 81 postpartum samples), found that higher residential exposure to NO_2 , $PM_{2.5}$ and black carbon was associated with higher second- and third-trimester HCC (Verheyen et al., 2021b). In the second, separate study of Flemish adolescents ($n = 395$), the authors reported that higher residential concentrations of NO_2 and PM_{10}

were linked to elevated HCC and inflammatory markers, but only in boys (Verheyen et al., 2021a). Several methodological and population characteristics may help explain these differences. Both studies focused on populations with distinct HPA-axis profiles, pregnant women and adolescents, rather than adults. Second, exposure was estimated using short-term, residence-based models (3-month or 1-year averages), which differ conceptually from the estimated lifetime exposure applied in the present study. Moreover, discrepancies with previous findings may also reflect broader patterns in scientific publishing, including rising journal rejection rates and a bias against negative or non-significant results (Fanelli, 2012). Regarding studies using different cortisol measures, the large MESA cohort, which examined salivary cortisol in relation to PM_{2.5}, NO₂ and NO_x, reported mostly null associations, with the exception of higher waking cortisol at higher NO₂ levels (Hajat et al., 2019). More recent evidence from a cross-sectional study of 203 overweight and obese Latino youth found the opposite pattern for some pollutants: higher O₃ exposure was associated with higher fasting morning serum cortisol, whereas long-term PM_{2.5} and NO₂ exposures were linked to lower cortisol levels, particularly in pre-pubertal teens (Toledo-Corral et al., 2021). Experimental studies have shown that short-term elevations in particulate matter can acutely activate neuroendocrine stress responses, as reflected by increases in circulating cortisol and catecholamines (Li et al., 2017). However, most previous studies have focused on short-term exposure and short-term cortisol markers, whereas the present study examined estimated lifetime exposure to air pollution. Although HCC reflects cumulative cortisol exposure over approximately the previous three months, lifetime environmental exposure may still influence long-term HPA-axis functioning and thereby contribute to cortisol secretion patterns reflected in HCC. In line with the allostatic load framework (McEwen, 1998), prolonged or repeated activation of stress-response systems may initially increase cortisol secretion, but over time may contribute to adaptive downregulation, impaired feedback regulation, or altered cortisol secretion patterns rather than persistently elevated cortisol levels (Fries et al., 2005; Miller et al., 2007; Nunez et al., 2025). Thus, although long-term air pollution exposure may still influence HPA-axis functioning, these alterations may not necessarily be reflected by elevated HCC concentrations. Consistent with this interpretation, longitudinal analyses have reported subtle alterations in diurnal cortisol patterns, such as cortisol slope flattening, rather than overall increases in cortisol levels in relation to long-term NO₂ exposure (Hajat et al., 2019).

Overall, the limited evidence linking air pollution with HCC remains inconsistent and appears to depend strongly on population characteristics, exposure assessment, and cortisol measurement. The present study adds to this limited body of evidence by examining estimated lifetime exposures in healthy adults using HCC as a long-term biomarker, while acknowledging the possibility of publication bias favoring positive findings. Larger longitudinal studies with harmonised exposure assessments and detailed behavioural covariates are needed to clarify the potential effects of long-term air pollution exposure on HPA-axis functioning and the extent to which these processes may be reflected in HCC.

Strengths and limitations

One strength of this study is the standardised collection of all hair samples by a small team of trained technicians, together with the use of a highly sensitive LC-MS/MS method for steroid quantification, which minimises methodological variability and enhances measurement reliability. We also employed objective measures of both physical activity and cardiorespiratory fitness, providing more reliable estimates than self-reported indicators. In addition, detailed socioeconomic, health, and behavioural data enabled adjustment for key covariates and informative sensitivity analyses. However, these strengths must be considered alongside several limitations. The cross-sectional design limits conclusions regarding causality of the observed associations. The sample size was limited by the resource-intensive nature of LC-MS/MS cortisol quantification rather than a formal a priori power calculation. Although hair samples were collected from all participants meeting the hair-length eligibility criteria, HCC was assessed only in a randomly selected subsample of participants, which may have reduced both the representativeness and statistical power of the analytical sample. Nevertheless, the achieved sample size is comparable to previous studies examining HCC in relation to objectively measured physical activity (Gerber et al., 2013) and air pollution exposures (Verheyen et al., 2021b). However, smaller associations may still have remained undetected and the absence of certain associations should be interpreted with caution. The parent study was designed to recruit runners and inactive controls (non-runners), with a target ratio of approximately 60% and 40%,

respectively. Consequently, the study sample represents a specific population with a higher proportion of runners than would be expected in the general population. Furthermore, compared with the broader pool of eligible participants from whom hair samples had been collected, the analytical HCC subsample was characterised by higher cardiorespiratory fitness and higher levels of vigorous physical activity. Therefore, caution is warranted when generalising these findings to populations with lower levels of physical activity and cardiorespiratory fitness. Moreover, the relatively narrow range of physical activity observed in the present sample may also have attenuated associations and contributed to the modest explained variance observed in the models. Future studies including individuals with a broader range of physical activity and fitness levels are needed to confirm and extend these findings. In addition, the predominance (87%) of women in the final analytical sample, a common limitation in HCC research, may have introduced sex-related bias and may further restrict the generalisability of the findings, particularly to men. This may be relevant, as previous literature has discussed potential sex differences in HCC related to hormonal regulation and cortisol-binding mechanisms (Lanfear et al., 2020), although findings regarding sex differences in HCC have remained inconsistent across studies (Binz et al., 2018; Fischer et al., 2017; Wosu et al., 2013). Nevertheless, most women were premenopausal (88.8%), hormonal contraception or other hormone-related medication use was reported only by a very small number of women ($N = 5$), and sex was included as a covariate in all analyses. Across all models, the explained variance in HCC was modest ($R^2 = 0.043$ – 0.086). Comparable levels of explained variance have also been reported in previous studies examining associations between physical activity and HCC (e.g. Gerber et al., 2013; Skoluda et al., 2012), suggesting that relatively low R^2 values are common in this area of research. Nevertheless, the modest explained variance limits the predictive strength of the models. In addition, no formal correction for multiple testing was applied, which may increase the risk of Type I error. Nevertheless, formal correction procedures were not applied intentionally, as their use remains debated in the statistical literature (e.g. Rothman, 2014; Saville, 1990). Another limitation is the differing temporal windows of the variables relative to HCC, which reflects cumulative cortisol exposure over approximately the previous three months. Specifically, the LTEQ assessed a typical week of leisure-time physical activity in the past month (Godin & Shephard, 1985), VO_{2peak} was measured on the day of hair sampling, and device-based physical activity was monitored for 14 days thereafter. However, previous work suggests that aggregating multiple days of accelerometer data provides reliable estimates of habitual physical activity (Hilden et al., 2023), and the physical activity and fitness measures in the present study were moderately to strongly correlated. Nevertheless, these temporal differences may have influenced the observed associations. In addition, the use of self-reported questionnaire data may be subject to reporting and recall bias. Furthermore, several factors that may influence HCC, such as hair treatment and hair washing frequency, were not considered in the present analyses. Nevertheless, previous studies examining the associations between these hair-related characteristics and HCC have reported heterogeneous and inconsistent findings (Stalder et al., 2017; Wosu et al., 2013). In addition, natural hair colour was not considered in the present analyses. Although hair pigmentation has been suggested as a potential factor influencing HCC concentrations (Abell et al., 2016; Neumann et al., 2017; Staufenbiel et al., 2015), previous findings remain inconsistent (Dettenborn et al., 2012; Faresjö et al., 2024; Stalder et al., 2017; Wosu et al., 2013). Therefore, although the potential influence of these hair-related factors cannot be entirely excluded, their overall impact on HCC concentrations remains unclear. Another limitation is that, although the lifetime exposure modelling approach is innovative, district-level estimates may not fully capture individual exposure variability. Nevertheless, area-level exposure assessment is widely used in air pollution research, as precise individual long-term exposure measurement remains challenging. This limitation may reduce the ability to detect associations and could partly contribute to the absence of observed associations in the present study.

Conclusions

In summary, this study is the first to examine HCC in relation to both subjective and objective indicators of physical activity, fitness, and lifetime air pollution exposure. We observed no significant associations between HCC and long-term air pollution exposure, nor any interaction between air pollution and objectively measured vigorous physical activity. These findings suggest that HCC may not fully capture potential alterations in HPA-axis functioning associated with long-term air pollution exposure. In contrast,

higher HCC was associated with greater vigorous, but not moderate, physical activity and with higher cardiorespiratory fitness, suggesting that HCC may partly reflect physiological adaptations related to physical activity and fitness. As HCC is increasingly used as an indicator of chronic psychological stress, these findings highlight the importance of considering vigorous physical activity when interpreting HCC levels, particularly in physically active populations where exercise-related physiological adaptations may contribute substantially to HCC variability. Further studies in larger and more diverse populations are needed to better understand factors contributing to HCC and the potential effects of air pollution on HPA-axis functioning and HCC.

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Author contributions

CRedit: **Veronika Cibulcova:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Steriani Elavsky:** Formal analysis, Supervision, Writing – review & editing; **Lukas Cipryan:** Supervision, Writing – review & editing; **Marta Jackowska:** Writing – review & editing; **Tomas Dostal:** Data curation, Writing – review & editing; **Vitezslav Jirik:** Methodology, Writing – review & editing; **Daniel Jandacka:** Conceptualization, Funding acquisition, Writing – review & editing; **Vera Kr Jandackova:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing.

Disclosure statement

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

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Data availability statement

Data are available upon reasonable request. Data from the 4HAIE cohort will be shared in accordance with ethical guidelines and participant consent. Researchers may request access to pseudoanonymised data for approved studies. Please contact the corresponding author for further information. A detailed description of the data set is available at www.4HAIE.cz, and data access guidelines are published at <https://haie-lerco.cz/data>.

Consent statement

The University of Ostrava ethics committee approved the Healthy Aging in Industrial Environment Study and all participants gave informed consent.

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