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Corresponding author: Christopher Gidlow, Centre for Sport, Health and Exercise Research, Staffordshire University, Leek Road, Stoke-on-Trent, Staffordshire, ST4 2DF, 01782 294330, c.gidlow@staffs.ac.uk

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Highlights

- Hair cortisol concentration (HCC) is a novel, non-invasive biomarker of chronic stress
- We explored natural environment in neighbourhoods and HCC
- HCC-measured chronic stress was higher in areas with less natural environment
- When considered simultaneously neither natural environment nor income deprivation were significant HCC predictors
- HCC has potential as a chronic stress biomarker in larger studies of natural environment and health.

ABSTRACT

Neighbourhood green space is positively associated with health. Stress reducing effects of nature might underpin this relationship, but researchers have often used objective stress measures to characterise *acute* responses to natural environments, or used self-reported measures in observational research. Hair cortisol concentration (HCC) is a novel non-invasive biomarker, with potential to improve our understanding of natural environments and *chronic* stress. We collected data from 132 healthy employed adults from the West Midlands region of the UK (June –Sept 2014), including socio-demographics, health, lifestyle and perceived stress and stress appraisal. Postcode was used to determine overall deprivation (Index of Multiple Deprivation, IMD), material deprivation (% income deprived) and the amount of natural environment in participants' home neighbourhoods. Hair samples (3cm) were taken from the scalp and HCC was determined to reflect past three months of cortisol secretion. Separate linear regression models, adjusting for potential confounders, indicated that HCC-measured chronic stress was higher in participants who lived in areas that were more deprived overall (β=-.235, p=.008), more income deprived (β=-219, p=.017), and comprised less natural environment (β=-212, p=.019). When income deprivation (i.e., material well-being) and natural environment were entered in the same model, associations for both were attenuated beyond significance (β=.168, p=.077 and β=-.160, p=.086, respectively). Overall, chronic stress measured by HCC was higher in areas with less natural environment. The relative contribution of neighbourhood natural environment, deprivation and other neighbourhood characteristics to chronic stress using HCC warrants further study in larger, more diverse samples.

1. Introduction

1.1 Natural environments, health and stress

There is convergent evidence that living in *greener neighbourhoods* is associated with better health outcomes (Maas et al., 2009; Mitchell & Popham, 2007; Mitchell, Astell-Burt, & Richardson, 2011; Tamosiunas et al., 2014; Triguero-Mas et al., 2015; Vaghri et al., 2013). Residential proximity to water (or *blue space*) has also been linked with better mental health (White, Alcock, Wheeler, & Depledge, 2013a), but perhaps less consistently (Gascon et al., 2015). Possible explanatory mechanisms include the opportunities for physical activity and social interaction afforded by access to natural environments, stress-reducing and restorative effects, and protection from pollutants, such as noise and air pollution (Bowler, Buyung-Ali, Knight, & Pullin, 2010; Hartig, Mitchell, de Vries, & Frumkin, 2014; Nieuwenhuijsen et al., 2014).

This paper focuses on possible stress-reducing effects of living in neighbourhoods with more natural environment. This is in line with Ulrich's Psycho-evolutionary Theory (PET) or Stress Reduction Theory (SRT), which proposes that natural environments improve affective states, influencing the brain and neuroendocrine system to promote stress recovery (Ulrich et al., 1991; Ulrich, 1983). To date, related studies have tended to be experimental, measuring *acute* stress-reducing effects of natural environments. Improvements in self-reported affect (or mood) in response to natural environments are consistently reported (Bowler et al., 2010), but there is little evidence of concurrent beneficial effects on the neuroendocrine system. Some have objectively measured stress using cortisol, a hormone end-product of Hypothalamus-Pituitary-Adrenal (HPA) axis activity, which is increased in response to physical or psychological stress (Hellhammer, Wüst, & Kudielka, 2009). To date, findings from studies of acute salivary cortisol responses to natural environment exposure have been inconsistent (Beil & Hanes, 2013; Bowler et al., 2010; Lee et al., 2011; Park, Tsunetsugu, Kasetani, Kagawa, & Miyazaki, 2010; Van den Berg & Custers, 2010) and often subject to the methodological challenge of controlling the various influences on cortisol secretion that might mask environmental effects, whilst maintaining an ecologically valid experience of nature.

Objectively measured *chronic* stress and natural environments are far less studied. This has been piloted (Ward Thompson et al., 2012) and studied proper (Roe et al., 2013) in Scotland. Roe et al. (2013) measured average salivary cortisol and diurnal pattern using multiple samples (3, 6 and 9 hours post-waking), on two consecutive days, in 88 middle-aged residents of deprived urban areas who were not in work. Chronic stress was determined through analysis of the diurnal slope, on the basis that a steeper slope is indicative of a more efficient and healthier daily cortisol response (Ward Thompson et al., 2012). A steeper diurnal cortisol decline was associated with higher levels of green space at the level of the Census Area Statistics (CAS) ward, geographical units used in the UK census, with an average of 4942 residents per ward. There were also some gender effects (such that cortisol response was less healthy in women in less green areas).

Chronic stress is also implicated in health inequalities (Chrousos, 2009; Kristenson, Eriksen, Sluiter, Starke, & Ursin, 2004). The increase in HPA axis activity in response to external stressors promotes generally beneficial metabolic and physiological changes that allow rapid physical exertion (fight-or-flight). However, *chronic* stimulation of this response can over-tax this system, resulting in *allostatic load*; non-adaptive reactions that alter baseline physiology and increase the likelihood of disease (Flier, Underhill, & McEwen, 1998). In lower socio-economic groups, higher stress exposure compounded by lower *resilience*, are thought to play a part in the greater susceptibility to disease observed in socio-economic heath inequalities (Kristenson et al., 2004). There is some evidence of socio-economic differences in absolute levels, or diurnal patterns of, cortisol (Li, Power, Kelly, Kirschbaum, & Hertzman, 2007). But, socio-economic patterning of cortisol as a marker of allostatic load has, so far, been inconsistent (Dowd, Simanek, & Aiello, 2009).

1.2 Hair cortisol for chronic stress measurement

The present study aimed to further our understanding of how the natural environment and deprivation characteristics of residential neighbourhoods might be associated with chronic stress, using hair cortisol concentration (HCC), a novel, non-invasive biomarker. Traditional collection of cortisol through saliva, blood or urine is limited by sensitivity to diurnal cortisol changes, acute stress and consumption. Hair cortisol concentration offers a feasible method for *chronic* stress measurement. Cortisol levels extracted from a 3cm sample of scalp hair can reflect the past 3 months of cortisol secretion, offering a stable and feasible measure of long term stress exposure, where higher HCC reflects higher chronic stress levels (Gow, Thomson, Rieder, Van Uum, & Koren, 2010). Evidence for the utility of HCC is growing (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013; Wester & van Rossum, 2015; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Hair cortisol concentration has been linked with conditions characterised by abnormal HPA axis activity (e.g., Cushing's Syndrome; Manenschijn et al., 2012), cardiovascular disease risk (Manenschijn et al., 2013), stressors that disrupt the circadian rhythm (Manenschijn, van Kruysbergen, de Jong, Koper, & van Rossum, 2011), anxiety (Steudte et al., 2011) and depression (Dettenborn et al., 2012).

Despite the recognised potential of HCC for use in larger epidemiological studies (Wosu et al., 2013), there has been little specific exploration of environmental correlates or socio-economic patterning. For example, elevated HCC has been observed in unemployed adults (Dettenborn, Tietze, Bruckner, & Kirschbaum, 2010) and children whose parents are less well educated (Vaghri et al., 2013), but we are not aware of any studies of area-level deprivation. Nor are we aware of investigations in to HCC-natural environment associations. This paper addresses these issues and further contributes to the area by building on the work of Roe et al. (2013) in a larger and more socio-economically diverse sample, using smaller geographical units and more inclusive indicators of natural environment. Data were collected from a sample of generally healthy, employed adults, representing a socio-economic range, from both urban and rural areas.

1.3 Study aims

The present analysis had three aims, to explore:

i. HCC patterning in relation to the amount of natural environment in the home neighbourhood, including green and blue space;
ii. HCC patterning in relation to material and overall deprivation;
iii. The relative contribution of material deprivation and amount of natural environment in the home neighbourhood.

2. Methods

2.1 Participants and procedures

Participants were recruited from two large public sector employers in the West Midlands, UK. Data were collected June to September 2014 as part of a wider study, with further details reported elsewhere (Gidlow, Randall, Gillman, Silk, & Jones, 2015). Exclusion criteria included: hair typically shorter than 2 cm; conditions known to affect cortisol levels (pregnancy, Cushing syndrome, Addison disease). Employees were invited to take part via an email that included a link to a brief online eligibility screening questionnaire. All eligible individuals who completed the screening survey were contacted to arrange appointments for baseline data collection at the worksite, with follow-up appointments 12 weeks later. Participants were offered £15 in retail vouchers on completion of the project as an incentive. This study was approved by the University ethics committee.

2.2 Measures

2.2.1 Baseline

A series of data were gathered at baseline to profile participants in terms of potential individual-level confounders of HCC and to characterise their residential neighbourhood.

- Demographic: Age, gender and ethnicity.
- Health: Self-reported height and weight were used to determine Body Mass Index (BMI, kg/m^2); SF1 general health question (1=Poor to 5=Excellent).
- Lifestyle: Physical activity was measured using the short version of the International Physical Activity Questionnaire (IPAQ-short; www.ipaq.ki.se); alcohol consumption (Audit-C); smoking status (yes/no).
- Stress: Perceived Stress Scale, PSS-10 (Cohen, Kamarck, & Mermelstein, 1983) provided a measure of general stress. The PSS asks participants to report the frequency of different feelings and thoughts over the last month (5-point scale, from 0=Never to 4=Very often) and scores are summed (with some reverse scored items taken in to account).
- Hair-related: Hair washing frequency (from 1 to >7 times/week) and use of hair dye / treatment (yes/no).
- Neighbourhood factors: Participants' home postcodes were used to derive three measures based on the corresponding Lower Super Output Area (LSOA), geographical units designed to have approximately homogenous population composition and size (mean population approximately 1600):
    i. Natural environment: Percentage of area within 400m of the population-weighted centroid of the LSOA classified as natural (including private gardens and water). This estimate of total area of natural land use per LSOA was generated from the Generalised Land Use Database (GLUD) 2005 (Communities and Local Government, 2005), which includes green space,

woodland, farming and agricultural land, water bodies (blue space) and residential gardens. Using a buffer around the population-weighted centroid accounted for populations being exposed to environments outside of the boundaries of their given LSOA. Within-sample tertiles were generated based on sample rank for natural environment (tertile 1=26.6-61.8%, mean=51.5±7.5%; tertile 2=62.0-67.4%, mean=64.6±1.7%; tertile 3=67.9-98.6%, mean=77.0±9.0%). These were used for basic difference tests. The continuous variable was used for regression.

ii. Overall deprivation: Index of Multiple Deprivation (IMD) 2010 (Communities and Local Government, 2010), which comprises seven domains of Income, Employment, Health and Disability, Education Skills and Training, Barriers to Housing and Other Services, Crime and Living Environment. The IMD rank, where lower rank indicates greater deprivation, was used given the non-linear scale of overall IMD score. Within-sample tertiles (where 1=most deprived) were generated for basic difference tests, with the continuous variable used for regression.

iii. Income deprivation: Percentage of residents classified as income deprived (from IMD) as marker of material deprivation, where higher values indicate higher deprivation. Within-sample tertiles (where 1=least income deprived) were generated for basic difference tests, with the continuous variable used for regression.

iv. Urbanicity: For participant profiling, a dichotomous urban-rural classification was used to classify LSOAs as urban (settlements with >10,000 residents) or rural (town and fringe; villages, hamlets and isolated dwellings; Bibby & Brindley, 2013).

2.2.2 Three-month follow-up

- Hair sample: a sample of at least 3 cm of hair was taken from the posterior vertex position of the scalp. Based on an average hair growth rate of 1 cm per month (Wennig, 2000), this is thought to represent hair grown in the 12 weeks between baseline and follow-up, reflecting cumulative cortisol secretion in that period (i.e., higher HCC reflects higher chronic stress).

- Stress appraisal: The Appraisal of Life Events Scale (ALES) (Ferguson et al., 1999) involved individuals describing the most stressful event that they had experienced in the last three months and rating it using 16 items (6-point scale, from 0 = not at all to 5 = very much so). Summary scores were calculated for dimensions of threat, challenge and loss. Building on a related analysis, the ALES-Loss score was used here as it has shown an association with HCC in this sample (Gidlow et al. 2015).

2.3 Hair sampling and analysis

Samples were obtained for 136 participants and analysed by Salimetrics Ltd, Cambridge, UK. All were analysed in duplicate and 15 using double extraction (i.e., two samples analysed from the same hair sample). Cortisol results from the ELISA (μg/dL) were corrected for hair sample weight, amount of methanol used and reconstitution volume. The correlation between HCC duplicates was r=.95, which is comparable to the r=.97 reported by Kirschbaum et al. (2009).

2.4 Statistical analysis

Hair cortisol data were significantly positively skewed and so were log transformed for analyses, but means and standard deviations are provided in original units (pg/mg). Associations between HCC and potential correlates were explored using Spearman's rank correlations (weak ρ <.3; moderate ρ =.3 to .49; strong ρ >.5; Cohen, 1988). ANCOVA were used to explore differences in HCC between tertiles for natural and deprivation environment, controlling for the strongest covariate (hair treatment), with follow-up contrast using Bonferroni adjustment. Linear regression models regressed overall deprivation, income deprivation and natural environment (all continuous variables) against HCC, adjusting for possible confounders.

3. Results

3.1 Sample characteristics

Following the exclusion of outliers (>4 SD), HCC data were available for 132 employed adults (Table 1). There were more females than males. There was a relatively even distribution across the quintiles of overall deprivation based on national rankings (Most deprived Q1=16.5%; Q2=17.3%, Q3=26.3%, Q4=15.8%, Least deprived Q5=21.1%), with mean level of income deprivation in neighbourhoods of 12.9±9.2 (range 2-40%). There was sample variation in terms of participants' residential neighbourhoods accounted for by natural environment (mean 65.1±13.0%, range 26.6-98.6%). This range and the presence of high values is keeping with 14.7% of participants living in rural areas.

3.2 Hair cortisol concentration

Mean HCC was 10.8±9.4 pg/mg. This was consistent with a generally healthy sample (92.4% reported *Good* to *Excellent* health; mean BMI 25.1±4.2 kg/m^2) who were not chronically stressed (mean PSS 16.6±7.2). Potential HCC confounders were explored and most were not associated with HCC (Table 2). There was a weak positive association for alcohol consumption, lower HCC in females versus males, and those who did versus did not report use of hair dye/treatment. However, gender and use of hair treatment were correlated (ρ=.607, p<.001) as only females reported use of hair treatment (n=79). As the strongest apparent confounder of HCC, use of hair treatment was included as a covariate in subsequent different tests. In terms of perceived stress, as reported in a related analysis (Gidlow et al. 2015), mean PSS was not associated with HCC, however, the ALES-loss score showed significant association with HCC and was, therefore, included in subsequent analysis (Table 2).

3.3 Hair cortisol concentration, deprivation and natural environments

Differences in HCC by socio-economic group (with hair treatment use as a covariate) revealed significant differences by *overall deprivation* (F(2,122)=4.368, p=.015, η_p^2=.067). Follow-up contrasts (with Bonferroni adjustment) indicated a significant difference between those in most versus least deprived tertiles (p=.020), with a non-significant difference between the most deprived and middle tertile (p=.062; Figure 1a). However, when this was repeated for *income deprivation*, there was no effect (F(2,122)=.772, p=.464, η_p^2=.035; Figure 1b). ANCOVA comparing mean HCC in tertiles of natural environment revealed a significant effect (F(2,122)=4.127, p=.018, η_p^2=.063), with follow-up contrasts showing a significant difference between those in most versus least natural tertiles (tertile 1 vs. 3, p=.015; Figure 1c). Figure 1d illustrates the consistency of this pattern across tertiles of income deprivation.

Adjusted linear regression analyses confirmed that overall deprivation, income deprivation and the amount of natural environment in participants' residential neighborhood were independent predictors of HCC, when entered in separate regression models (Models 1-3); i.e., higher HCC in residents of neighbourhoods with higher levels of overall and income deprivation, and less natural environment (Table 3). When the relative importance of income deprivation (i.e., material well-being) and natural environment were explored in the same model (Model 4), the associations for both were attenuated beyond significance (p=.077 and p=.086, respectively).

4. Discussion

Data from our sample of generally healthy adults suggested that living in neighbourhoods with more natural environment is associated with lower chronic stress measured by HCC, independent of other potential individual-level covariates of HCC. Although this association was attenuated beyond significance when income deprivation was considered, it warrants attention given the modest sample size and independent associations for income deprivation and natural environment observed in separate Models (2 and 3, respectively). As far as we know, this is the first exploration of HCC patterning by residential neighbourhood deprivation and natural environment, and adds to the case for further exploration of HCC as a chronic stress biomarker in epidemiological studies, particularly concerning the relationship between natural environments, stress and health.

Our findings add to the sparse evidence linking natural environments and objectively measured *chronic* stress. To date, experimental studies have explored *acute* objective stress response to natural environments (Beil & Hanes, 2013; Bowler et al., 2010; Lee et al., 2011; Park et al., 2010; Van den Berg & Custers, 2010) or self-reported mental health indicators have been used in observational studies (Astell-Burt, Feng, & Kolt, 2013; White, Alcock, Wheeler, & Depledge, 2013b). Our data are consistent with the only similar investigation of residential green space and cortisol in a community sample (Roe et al., 2013) and build on this important work in a number of ways. First, we observed effects within a larger sample, who lived in areas that represented a range in terms of deprivation, and urban/rural character. Second, we used a natural environment measure that included, not only green space, but private gardens and blue space; and did so with smaller geographical units (LSOA with approximate average population of 1600 versus CAS ward with approximate average population 5000), using 400m buffers around the population-weighted area centroid. Characterising the environment within an area defined around the population-weighted area centroid aimed to negate two problems: edge effects, where the need to define geographical units can lead to exposures from neighbouring areas boundaries being ignored; uneven distribution of populations within specific part(s) of an LSOA, which can be particularly problematic in rural areas, where populations might occupy small, discrete parts of a large LSOA. Finally, and perhaps most importantly, we also found a natural environment-cortisol association (based on ANCOVA and Model 3) using the low burden, non-invasive method of HCC measurement. This has the advantage of providing a stable measure of *chronic* stress through reflecting cortisol secretion over a prolonged period (not requiring several samples to determine the diurnal slope), in addition to the practical benefits of collection and storage of hair, rather than biological fluids.

In the context of the natural environment-stress literature, our data provide some support for the SRT by inferring that residence in more natural areas might have stress-reducing effects. Specifically, comparisons of HCC across different tertiles of natural environment showed that those living in areas with mean natural environment coverage of 51.5% (tertile 1) had higher HCC than those in areas with mean natural environment coverage of 77.0% (tertile 3). This highlights that HCC differences by natural environment were even observed in a sample where natural environment coverage was generally high (and included some rural dwellers). Future studies with larger samples would benefit from also including areas with less natural environment, which might show more pronounced effects and allow meaningful analysis of components of the natural environment (e.g., % green space; % gardens; % blue space). Moreover, this pattern was consistent across the tertiles of deprivation (Figure 1d) so the stress-reducing benefits of frequent exposure to natural environments in the living environment appeared consistent across the socio-economic range. One potential explanation is that living in natural, compared with urban, environments may be associated with different neural processing of stress. Indeed, while performing an arithmetic task under time pressure during functional magnetic resonance imaging (fMRI), individuals who currently lived in a city showed higher amygdala activity, a region of the brain which signals negative affect and threat, than those living in natural environments (Lederbogen et al., 2011). It is noteworthy that Lederbogen et al. (2011) did not find an association between acute salivary cortisol changes and amygdala activity, but suggested this may be reflective of the greater sensitivity of neural measures to downstream peripheral markers (such as cortisol).

As detailed earlier, we have focused on the stress-reducing mechanism, as one of several proposed to underpin the frequently observed beneficial natural environment-health relationship. There was no evidence of an association between natural environment and total physical activity (ρ=.056, p=.500), another posited mechanism, but for which evidence remains inconclusive (Lachowycz & Jones, 2011; Mytton, Townsend, Rutter, & Foster, 2012). It was not possible to explore other possible mechanisms of social interaction or attention restoration, which could be the subject of further, larger studies taking a similar approach. This notwithstanding, our data could help to explain the positive associations between green space and mental health that are observed with relative consistency in larger samples, both cross-sectionally (Astell-Burt et al., 2013; Triguero-Mas et al., 2015; White et al., 2013b) and longitudinally (Alcock, White, Wheeler, Fleming, & Depledge, 2014), as well as other green space-disease associations where elevated stress can contribute to pathology (e.g., Richardson & Mitchell, 2010; Tamosiunas et al., 2014). We also advocate the application of HCC to further explore the, so far, inconsistent evidence from observational studies linking mental health benefits with blue space (Gascon et al., 2015; White et al., 2013a).

Within our sample, it appeared that the amount of natural environment and the level of income deprivation in one’s neighbourhood were important for chronic stress when considered separately, but not in the same model. There is consistent evidence linking deprivation with poor health, and chronic stress is implicated as a possible causal mechanism (Kristenson et al., 2004). We observed an association between HCC and the composite deprivation indicator that included aspects of the physical environment (e.g., *living environment*, *barriers to housing* *and other services*). To separate the effects of *material* deprivation from environmental deprivation, we repeated analysis using income deprivation as the outcome variable and observed a slightly attenuated, but similar association (Model 2). When income deprivation and natural environment were entered in to the same model (Model 4), associations for both were attenuated beyond significance. This might be a result of the association between natural environment and income deprivation (r=-.374), such that both are related markers of general environmental quality, and a limitation of statistical power in our sample. Nevertheless, we can conclude that environmental characteristics of the neighbourhood are related to chronic stress (and, therefore, health), independent of measured individual participant characteristics. This should be further explored using HCC in larger, more powerful datasets, especially given the inconsistent socio-economic patterning of stress using cortisol to date (Dowd et al., 2009).

Our data, therefore, add to the growing literature supporting the utility of HCC for objective stress characterisation, but with particular application in the study of environment and health. Future research may wish to explore complementary approaches to measuring the stress-natural environment relationship, such as differences in diurnal slope (e.g., Roe et al. 2013) and how living in areas of high natural environment may predispose individuals to better deal with acute stress (cf. Lederbogen et al., 2011). It is also important to remain cognisant of potential hypocortisolemia. Extreme stress or 'burn out' might be reflected in lower cortisol secretion, similar to the patterns of lower HCC in individuals with post-traumatic stress disorder (Steudte et al. 2013). We are confident that this was not a problem in our sample based on the self-reported health and perceived stress data.

Limitations of this study are recognised. First, the sample size. Our sample compared well with many other studies of HCC and of salivary cortisol and green space, but our final model accounted for only 13.2% of HCC variance. We explored possible covariates or HCC confounders and adjusted for all those statistically or plausibly relevant, but a larger sample might have allowed for identification of other factors. Second, individual-level socio-economic indicators of salary grade band and education were collected, but not used as the need to collate data across two different workplaces prevented meaningful analysis. Third, a greater socio-economic range, including unemployed adults, might have allowed detection of larger effects, although we did have a reasonable distribution based on national deprivation rankings. Fourth, the natural environment indicator was calculated at area-level, not individual-level. In the absence of participants' residential addresses, amount of natural environment was derived for the 400m area around the population-weighted centre of their home LSOA. Fifth, we were not able to take the type of work place environment in to account. Finally, these data are cross-sectional so we cannot infer causality.

5. Conclusion

We found that living in neighbourhoods with higher levels of natural environment was associated lower levels of chronic stress, measured using hair cortisol. But when area-level income deprivation and natural environment were considered simultaneously, associations for both were attenuated beyond significance. These data contribute to the emerging literature on the health benefits of natural environments and the possible role of stress, and adds to the growing case for HCC as a feasible, non-invasive stress biomarker in epidemiological and health research. We advocate further longitudinal study in larger, more diverse samples to unpick the specific contribution of deprivation, natural environments, and individual and area-level factors.

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Table 1. Participant characteristics

			n	Mean / %	SD
Individual characteristics	Gender	Male (%)	25	18.9	
		Female (%)	107	81.1	
	Age	(years)	131	41.4	11.4
	General health	Very good or Excellent (%)	80	60.6	
		Good (%)	42	42.0	
		Fair or poor (%)	10	7.6	
	Body Mass Index	(kg/m^2)	128	25.1	4.8
	Smoking status	Smoker (%)	12	9.2	
		Non-smoker (%)	118	90.8	
	Alcohol	Audit-C score	128	3.9	2.7
	Physical activity	MET-minutes/week	132	2356.1	2151.7
	Perceived stress	PSS score	120	16.6	7.2
Hair-related characteristics	HCC	(pg/mg)	132	10.81	9.37
	Hair dye/treatment	No	51	39.2	
		Yes	79	60.8	
	Washing frequency	Washes per week	130	4.0	3.0-7.0
Neighbourhood characteristics	Overall IMD	Rank of LSOA (out of 34,753 in England)	128	16556.3	8883.9
	Income Deprivation	% of population income deprived	128	12.9	9.2
	Natural environment	% land natural cover within 400m of LSOA centroid	128	65.1	13.0

Table 2. Results of tests to explore possible confounders of HCC

Correlations[a]		Test statistic rho	p
	Age	.017	.850
	Body Mass Index	.101	.256
	Physical activity (total)	.022	.805
	Smoking status	-.011	.901
	Alcohol consumption (Audit-C score)	.175	.049
	Frequency of hair washing	-.161	.068
	Perceived Stress Scale (PSS)	.148	.107
	ALES-Threat score	.093	.292
	ALES-Challenge score	.164	.063
	ALES-Loss score	.168	.057
Difference tests[b]		t	p
	Gender (male > female)	2.006	.047
	Use of hair dye/treatment (no > yes)	2.270	.026

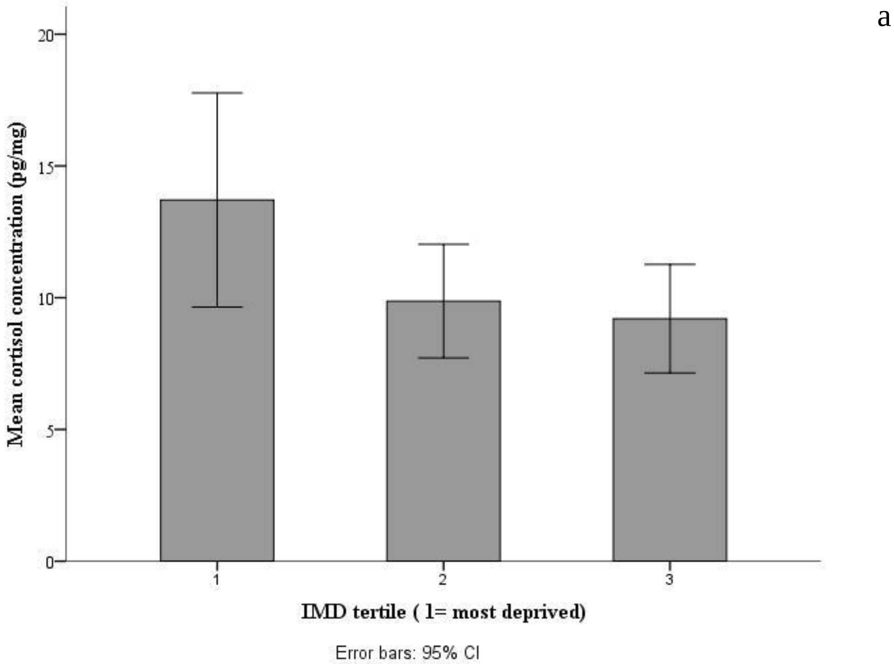
[a] Non-parametric correlations (test statistics rho, ρ)

[b] Independent samples t-test (test statistic t)

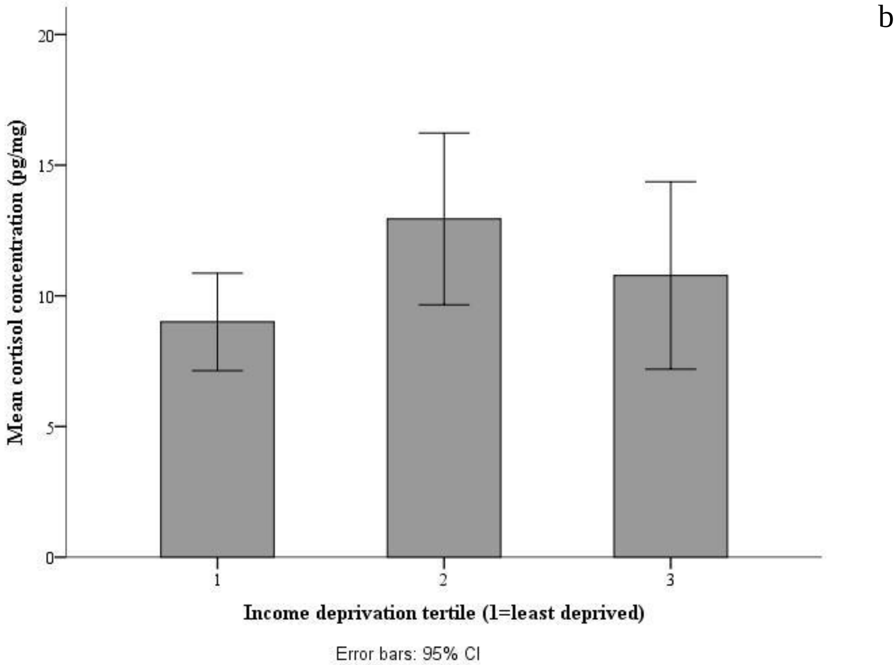
Table 3. Linear regression coefficients (unadjusted and adjusted) for overall deprivation, income deprivation and natural environment as predictors of Hair Cortisol Concentration (HCC)

Model		Unadjusted			Adjusted[a]		
		β	P	Adj R^2	β	p	Adj R^2
1	Overall deprivation (IMD)	-.183	.043	.026	-.235	.008	.126
2	Income deprivation (%)	.136	.137	.010	.219	.017	.117
3	Natural environment (%)	-.188	.039	.027	-.212	.019	.115
4	Income deprivation (%)	.081	.401	.025	.168	.077	.132
	Natural environment (%)	-.160	.097		-1.60	.086	

Model 1: IMD was the only neighbourhood level indicator entered
Model 2: % Income deprivation was the only neighbourhood level indicator entered
Model 3: % Natural was the only neighbourhood level indicator entered
Model 4: Income deprivation and Natural environment variables entered simultaneously.
[a] Adjusted for age, gender, use of hair dye, alcohol consumption; ALES Loss score
β, Standardised regression coefficient;
Adj R^2, proportion of variance explained by model (adjusted for the number of predictor variables in model)

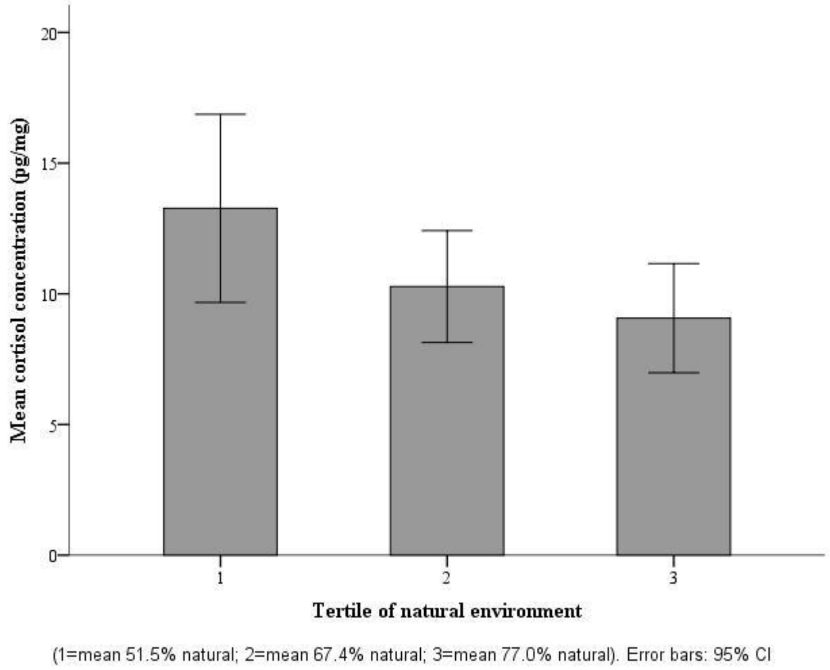


a
Mean cortisol concentration (pg/mg)
0
5
10
15
20
1
2
3
IMD tertile (1= most deprived)
Error bars: 95% CI



b
Mean cortisol concentration (pg/mg)
0
5
10
15
20
1
2
3
Income deprivation tertile (1=least deprived)
Error bars: 95% CI

c



d

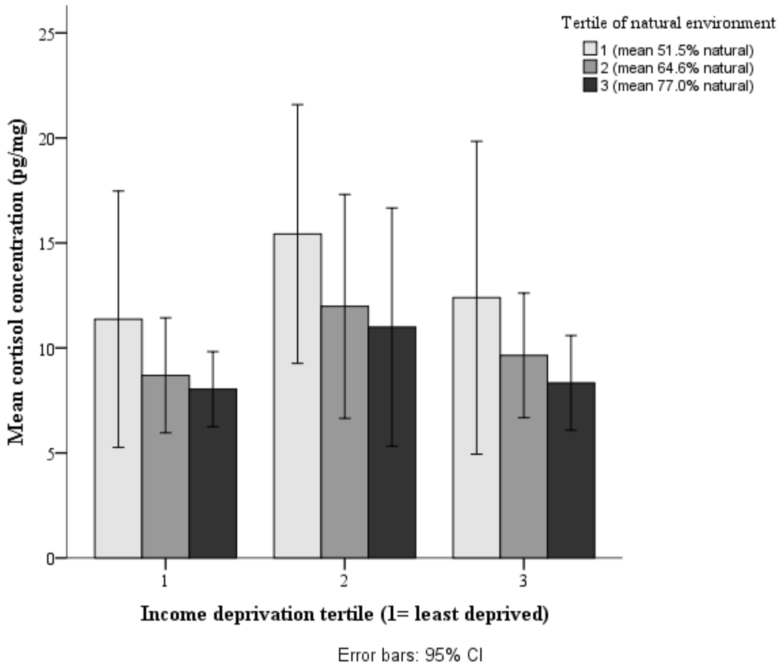


Figure 1. Mean Hair Cortisol Concentration (HCC) by overall deprivation (a), income deprivation (b), natural environment (c), and both income deprivation and natural environment (d). (*Note: HCC is raw concentration, not adjusted for use of hair dye/treatment*).