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ARTICLE

Cultural and socio-economic conditions as factors contributing to chronic stress in sub-Saharan African communities

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Abstract: Stress is known to contribute to overall health status. Many individuals in sub-Saharan Africa are believed to be stressed about their employment, income, and health. This study aimed to investigate hair cortisol as a biomarker of chronic stress in settlement communities in Kenya. Hair samples were collected from 108 volunteers from settlement communities in Kenya. An enzyme-linked immunosorbent assay technique was used to measure hair cortisol concentrations. In parallel, a health survey was completed. The mean ± SD for the cortisol concentration in the hair of volunteers from the settlement communities in Naivasha was 639 ± 300 ng/g, which was higher than found for a Caucasian reference group (299 ± 110 ng/g; one-way ANOVA, $P = 0.0003$). There were no differences in hair cortisol concentrations between members of slum settlements adjacent to large floriculture farms in Naivasha (Karagita, Kamere/Kwa Muhia/DCK, and Kasarani) compared with those well-removed from all floriculture in Mogotio (Mogotio and Westlands/Katorongot). However, hair cortisol concentrations were significantly higher in females, divorced volunteers, those who made below minimum wage, and those who reported feeling unsafe collecting water or using sanitation facilities within these 2 settlement groups. We found no evidence for increased chronic stress (measured by hair cortisol content) between members of slum settlements adjacent to versus distant to large floriculture farms. Cultural and socio-economic conditions that prevail in much of sub-Saharan Africa were found to be factors contributing to chronic stress.

Key words: hair, cortisol, socioeconomic status, Kenya, chronic stress, floriculture.

Résumé : On sait que le stress contribue à l'état de santé global. On soupçonne que plusieurs individus habitant l'Afrique sub-saharienne sont stressés à cause de leur emploi, de leur revenu et de leur santé. Cette étude visait à examiner le cortisol de cheveux comme bio-marqueur du stress chronique de collectivités de peuplement au Kenya. Les échantillons de cheveux ont été récoltés de 108 volontaires habitant des collectivités de peuplement au Kenya. Les concentrations de cortisol des cheveux ont été mesurées à l'aide d'un dosage par ELISA. En parallèle, une enquête sur la santé a été complétée. La concentration moyenne ± É.-T. de cortisol des cheveux des collectivités de peuplement de Naivasha était de 639 ± 300 ng/g, ce qui était supérieur à la concentration du groupe de référence Caucasien (299 ± 110 ng/g; ANOVA; $P = 0{,}0003$). Il n'y avait pas de différence entre les concentrations de cortisol des collectivités de peuplement des bidonvilles adjacents aux fermes floricoles de Naivasha (Karagita, Kamere/Kwa Muhia/DCK et Kasarani), et celles bien retirées de toute floriculture de Mogotio (Mogotio et Westlands/Katorongot). Cependant, les concentrations de cortisol des cheveux étaient significativement plus élevées chez les femmes, les volontaires divorcés, les personnes vivant sous le salaire minimum, et celles qui rapportaient ne pas se sentir en sécurité en allant chercher de l'eau ou en utilisant les lieux d'hygiène et ce, au sein de ces deux collectivités de peuplement. Les auteurs n'ont pas établi la preuve d'un stress chronique accru (mesuré par le contenu en cortisol des cheveux) entre les membres des collectivités de peuplement des bidonvilles adjacentes ou distantes des grandes fermes floricoles. Les conditions culturelles et socioéconomiques qui prévalent dans une grande partie de l'Afrique sub-saharienne se sont avérées constituer des facteurs contribuant au stress chronique. [Traduit par la Redaction]

Mots-clés : cheveux, cortisol, statut socioéconomique, Kenya, stress chronique, floriculture.

Background

Chronic stress is known to negatively impact overall health status (McEwen 2012). Many individuals in sub-Saharan Africa are believed to be stressed about their job and employment status, their income, their health, and the health of their children. The World Health Organization (WHO) projects that by 2030 the majority of the population in sub-Saharan Africa will live in urban areas. This transition will involve the expansion of settlement communities (i.e., slums) where risks such as food insecurity as well as inadequate water, sanitation, and health facilities and ser-

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vices exist, and are potential sources of stress for those who live there (Unger and Riley 2007; Kyobutungi et al. 2008; WHO 2010).

Traditionally, cortisol has been measured as a biomarker of stress in serum, saliva, or urine. However, these matrices have major disadvantages for stress measurements over long periods of time, as they represent only a single point in time. Cortisol levels in these matrices are also subject to significant daily physiological fluctuations, and thus, to characterize long-term stress, multiple samples must be taken, which is labour intensive for both participants and researchers. These setbacks make it difficult to measure chronic stress in large populations using the traditional matrices (Henley et al. 2013).

Circulating lipophilic compounds such as cortisol are incorporated into the hair shaft (Villain et al. 2004). A direct correspondence between hair cortisol levels and Cushing's syndrome, a physiological condition that exhibits well-defined changes in the classical hypothalamic–pituitary–adrenal (HPA) axis activity, demonstrates that systemic cortisol is deposited in the hair shaft, and reflects hair cortisol content (Thomson et al. 2010). Thus, cortisol levels in hair are increasingly being utilized as a biomarker of chronic stress (Kalra et al. 2007; Yamada et al. 2007; Van Uum et al. 2008; Kirschbaum et al. 2009; Stalder et al. 2010; Thomson et al. 2010; Karlen et al. 2011; Manenschijn et al. 2011; Steudte et al. 2011; Russell et al. 2012; Skoluda et al. 2012; Manenschijn et al. 2013). Hair grows an average of 1 cm per month, and thus 1 cm of hair represents 1 month of cortisol exposure, which mitigates the issue of inter- and intra-daily cortisol fluctuations. Hair samples are also easy to collect and store, making this an attractive matrix for field studies.

Increased systemic cortisol is implicated in the development of numerous health conditions; thus, this biomarker may allow insight into pathogenesis and disease progression. A Canadian study reported that the mean hair cortisol concentrations were higher in patients with major chronic pain compared with their non-obese controls (Van Uum et al. 2008). Another study by this group found that the median hair cortisol content in patients who had a confirmed acute myocardial infarction was significantly higher than in patients whose chest pains were attributed to other causes (Pereg et al. 2011). These studies lend strong support to the use of hair cortisol as a biomarker of chronic stress, particularly in relation to health effects. Examination of hair cortisol content may also shed light on factors that contribute to chronic stress in community-based studies such as ours. Personal and cultural factors may shape stress through differences in socio-economic status and in the perception of stressors.

Little research has explored levels of stress in any African population. To the best of our knowledge, this is the first study that employs hair cortisol as a biomarker of chronic stress in Kenya, and the second in any African community. The earlier work of Steudte et al. (2011) showed that participants with post-traumatic stress disorder (PTSD) had higher hair cortisol than those of traumatized controls from a civil war area in Uganda. By using hair cortisol as a biomarker, our study investigated the hypothesis that volunteers from settlement communities near large floriculture operations at Lake Naivasha in Kenya exhibit stress levels above those in members of settlement communities distant from Naivasha. We also set out to identify some of the sources of stress in these communities from completed health questionnaires.

Methods

Ethics statement

This project was approved by the Research Ethics Board of Western University, London, Ontario, Canada, and the Kenya Medical Research Institute (KEMRI), Kenya. Prior to administering the survey, clinicians completed an informed verbal consent process with each survey participant in Kiswahili. All hair samples were obtained following written receipt of informed consent, both in English and in Kiswahili, and were coded immediately upon collection.

Community-based participatory research

The Western University Ecosystem Health Research team has been continuously involved for 6 years in community-based participatory research (CBPR) at Naivasha, Kenya, in partnership with Egerton University, Njoro, Nakuru, Kenya. The CBPR model aims to enhance the significance of research by involving community members and local partners through collaborative, active partnerships (Isreal et al. 2005). Members of the clinical team, which included both Canadian and Kenyan investigators and community members, were actively involved in the research selection and decision-making processes, as well as in co-learning and capacity building.

Health and stress survey instrument

Health and socio-economic status (SES) were assessed using a survey instrument that asked questions surrounding indicators of SES, demographics, migration, water use, gender, general health status, and also involved a physical examination that incorporated measurement of height, weight, and blood pressure. Height was measured using a measuring tape and recorded in centimetres, weight was measured using a bathroom scale and recorded in kilograms, and blood pressure was measured using a sphygmomanometer and recorded as systolic-over-diastolic blood pressure in millimetres of mercury (Hg; 1 mm Hg = 133.322 Pa). The survey tool was reviewed by 9 multilingual medical professionals, a focus group discussion with 10 members from the communities in Naivasha, and by pilot testing. The survey tool was pilot-tested on 2 and 3 June 2011 amongst a sample of 20 participants in Kaptembo, Nakuru, Kenya, which is a settlement community similar to those in Naivasha and Mogotio. No problems with the survey tool were identified. The survey tool was finalized on June 10, 2011 at a workshop in Naivasha, Kenya, that comprised members from both Western and Egerton Universities as well as members from the Naivasha communities.

Survey implementation

Two days of clinician training consisted of developing survey understanding, sampling, and delivery, as well as pilot survey data collection to ensure quality delivery. One of 7 trained Kenyan clinicians administered each survey verbally in Kiswahili, recording responses on paper. Participants were selected and approached by a local community representative. Implementation included the administration of the survey to 800 volunteers in the communities in Naivasha, Kenya, between 14 June and 8 July 2011. The survey was distributed representatively amongst each of the settlement communities in Naivasha (Karagita, Kamere/Kwa Muhia/DCK, and Kasarani). Two-hundred surveys were also administered to the settlement communities in Mogotio (Mogotio and Westlands/Katorongot). Each participant was provided with a unique code for confidentiality purposes.

Hair collection

We randomly selected a subsample of approximately 200 of the 1000 participants from the settlements in Naivasha and Mogotio who participated in the health status survey for collection of hair samples. These samples were collected over 4 days (11, 12, 13, and 15 August 2011) from individuals at the Karagita Dispensary, and on 20 August 2011 at the Mogotio Chief's Camp. Participants were provided with compensation for transportation costs incurred getting to and from the clinic/camp. Each participant from the health survey was provided with a new code and with a random number generator (Random.org) we selected the new codes to contact by phone. Hair samples were analyzed from a group of European descent ($N = 15$) as a reference population. We also collected hair samples from the Kenyan members of our clinical

team (N = 9; 5 females, 4 males) who are certified clinicians that were living and working in Naivasha, Kenya, at the time of hair collection. Although relatively small, this is a well-educated Kenyan population removed from poverty and consequently less stressed than the settlement members.

Hair was normally collected for cortisol analysis from the posterior vertex region, as close to the scalp as possible, using scissors that were cleaned and disinfected with isopropyl alcohol and dried thoroughly between each use. Gloves were worn during hair collection to prevent contamination of the samples. The posterior vertex region was chosen because most hairs in this area have the same growth rate and because the proportion of hair in the telogen growth phase is low (Villain et al. 2004). Little is known about hair growth in Africans; however, one study demonstrated that African hair grows slower and has more hair in the telogen phase when compared with the hair of Caucasians (Loussouarn 2001). This same study showed no difference in hair growth parameters between sexes, and that there was wide variation between subjects in rate of hair growth (Loussouarn 2001). Owing to the prevalence of fake hairpieces worn by women in Kenya, many of the hair samples were taken from the hairline at the back of the neck to obtain real hair. For these reasons, we cannot approximate that 1 cm of hair growth is equal to one month of cortisol production. After collection, each sample was carefully inserted into an envelope, ensuring that the hair was not bent, and the envelope was sealed with tape. Hair was transported to London (Ontario, Canada) in August 2011. The hair was stored at room temperature in the dark until analysis. In total, hair samples were collected from 108 volunteers from the settlements in Naivasha (73) and Mogotio (35). Hair samples were also concurrently analyzed from a population of European descent (N = 15) as a reference group. Hair samples were excluded in the subsequent grouped analyses if there was < 8 mg of hair collected, if variation between duplicates was > 20%, or if the hair cortisol content was > 1500 ng/g (N = 14 excluded). The 14 individuals with hair cortisol levels above 1500 ng/g were excluded from our analysis for 2 reasons. First, the ELISA assay used was no longer linear at these very high cortisol concentrations. Secondly, based on several studies with hair cortisol, such concentrations are normally associated with contamination of the hair from an external cortisol source. Before excluding these individuals we checked to determine whether these high hair-cortisol values (>1500 ng/g) were more commonly associated with certain employment conditions, with socio-economic conditions, or with self-reported conditions leading to stress. In this analysis there were no differences in those with high hair cortisol vs. those with low hair cortisol based on sex; income level (both < and > 5000 Kenyan Shillings (KSh) per month); marital status (single, married or divorced; widowed); or feeling safe (yes or no).

Hair cortisol analysis

A 10–15 mg portion of hair was weighed using an analytical balance and transferred to a glass scintillation vial. Each sample was washed 3 times with HPLC grade isopropanol and left to dry for at least 5 h under a fume hood. Afterwards, 1 mL of HPLC-grade methanol was added and hair was finely minced using surgical scissors. The capped vials were then sealed with a strip of parafilm and incubated in a rotating incubator at 50 °C at 100 rpm for 16 h. After incubation, the vials were cooled to room temperature and the entire methanol extract was transferred to a glass tube. The methanol was evaporated from each sample by heating the tube to 50 °C and placing it under a gentle stream of nitrogen gas. The remaining residue was reconstituted by the addition of 250 μL of phosphate-buffered saline solution (PBS) at pH 8.0 to the sample with vortexing until well mixed. Subsequently, 50 μL of the buffer solution were added to the well of a 96-well plate, in duplicate, as per the enzyme-linked immunosorbent assay (ELISA) kit instructions. A sample of the PBS buffer alone was used as a negative control to assess non-specific binding and colour development. This value was subtracted from total values prior to interpretation. The positive controls were the cortisol standard samples included in the ELISA kit.

Cortisol measurement was performed using the ELISA cortisol kit originally developed for salivary cortisol content (Alpco Diagnostics, New Hampshire, USA) as per the manufacturer's directions, with the exception that the assay was shaken at 100 rpm instead of 200 rpm. The ELISA assay was conducted on a flat-bottomed, antibody coated 96-well plate and absorption was read on a V_{max} plate reader (Molecular Devices, Sunnyvale, California, USA) with absorption at 450 nm. The ELISA kit has cross-reactivities with other steroids as follows: corticosterone (31%), progesterone (<2%), deoxycortisol (<2%), dexamethasone (<2%), estriol (<0.001%), estrone (<0.001%), and testosterone (<0.001%). The absolute cortisol extraction recoveries were 88% and 87% in 100 ng/mL and 2 ng/mL standards, respectively. The cortisol concentrations derived in nanograms per millilitre were then corrected to the mass of hair used to produce a hair cortisol concentration in nanograms per gram of hair. The limit of detection for the ELISA is 1.14 ng/mL (Alpco Diagnostics).

Table 1. Mean (SD) for hair cortisol concentrations (ng/g) by population.

	N	Cortisol (ng/g)
Karagita	37	580 (198)
Kamere	13	575 (308)
Kasarani	16	684 (267)
Mogotio	17	737 (413)
Westlands/ Katorongot	11	698 (401)
		P = 0.332
Settlements combined	94	639 (300)
Reference	15	299 (110)
		P = 0.0003

Note: Statistical analysis using one-way ANOVA.

Health survey and stress scale analysis

We used GraphPad Prism 6.0 for statistical analyses. Normality of the data was tested with the D'Agostino and Pearson omnibus normality test. Hair cortisol data were not normally distributed, so log-transformed values were used for analysis. In the first step, relationships between hair cortisol content and health status indicators were investigated using simple difference of means tests. Second, multiple linear regression models in which all variables were entered simultaneously were conducted to investigate the independence of hair cortisol relationships with individual predictors. The multiple linear regression analyses were performed with SPSS statistical software (version 21; IBM SPSS Statistics). The level of significance was set at $P < 0.05$. Survey responses not adding up to total participant count indicate incomplete survey completion.

Results

The mean ± SD values for the hair cortisol levels among the participants from the different settlements (Karagita, Kamere, Kasarani, situated adjacent to major floriculture operations, and Mogotio and Westlands/Katorongot, well removed from large-scale floriculture) were not different from one another (Table 1; one-way ANOVA; P = 0.332). For subsequent analysis, we combined the hair cortisol values from the different settlements in Naivasha and Mogotio, as they are similar sociodemographic settings, to investigate potential sources of stress. The average values for hair cortisol content from all settlement communities (639 ± 300 ng/g) were significantly higher than the those for a Canadian Caucasian group (299 ± 110 ng/g; one-way ANOVA, P = 0.0003; Table 1) we had previously evaluated (Henley et al. 2013). The results of the simple difference between means tests and the standardized multiple linear regression coefficients (with mutual adjustment for all vari-

Table 2. Mean (SD) for hair cortisol concentrations (ng/g) by health status.

	N	Cortisol (ng/g)	SML
Sex			
Male	28	546 (224)	
Female	66	679 (320)	
		$P = 0.0486^a$	0.098; $P = 0.605^a$
Age (years)			
<20	4	650 (340)	
20–24	21	572 (217)	
25–29	16	642 (297)	
30–34	18	672 (359)	
35–39	8	603 (230)	
40–44	8	523 (225)	
45–49	9	682 (374)	
50–59	8	690 (302)	
60+	2	966; 1478*	
		$P = 0.0534^b$	–0.024; $P = 0.914^b$
Marital status			
Single	23	631 (287)	
Married	57	612 (263)	
Divorced	7	944 (367)	
Widowed	6	646 (451)	
		$P = 0.0485^b$	–0.178; $P = 0.427^b$
Education			
No formal	3	835 (478)	
Lower Primary	5	752 (225)	
Upper Primary	41	641 (316)	
Secondary	42	618 (286)	
Tertiary	3	525 (248)	
		$P = 0.599^b$	–0.013; $P = 0.955^b$
Income (KSh per month)			
<5000	14	752 (270)	
>5000	54	581 (235)	
		$P = 0.0216^a$	–0.462; $P = 0.027^a$
Do you smoke?			
Yes	13	660 (391)	
No	81	636 (286)	
		$P = 0.506^a$	–0.059; $P = 0.743^a$
Do you feel safe collecting water and going to the bathroom?			
Yes	70	607 (282)	
No	24	734 (335)	
		$P = 0.0370^a$	–0.090; $P = 0.667^a$
Have you ever been assaulted when collecting water or going to the bathroom?			
Yes	14	681 (298)	
No	86	628 (297)	
		$P = 0.541^a$	0.095; $P = 0.601^b$
Body mass index			
Underweight	4	604 (399)	
Normal	22	642 (261)	
Overweight	5	749 (149)	
Obese	6	604 (264)	
		$P = 0.330^b$	–0.116; $P = 0.530^b$

Note: SML, standardized multiple linear regression (with mutual adjustment for all variables input); KSh, Kenyan shillings. *, $N = 2$.

[a]Statistical analysis using Student's *t* test.

[b]Statistical analysis using one-way ANOVA.

ables) are presented in Table 2. Women from the settlements had a hair cortisol concentration of 679 ± 320 ng/g, which was significantly higher than among the men (546 ± 224 ng/g; analysis by Student's *t* test; $P = 0.0486$; Table 2).

The hair cortisol content for the Kenyan clinical team members was 360 ± 134 ng/g, which is not significantly different from our Canadian reference group of European descent (Student's *t* test, $P = 0.220$). There was also no difference between the hair cortisol content in the male ($N = 4$) vs. the female ($N = 5$) members of the Kenyan clinical team (348 ± 118 and 369 ± 160 ng/g, respectively). Using data from Kenyan clinical team members, we conducted an age- and sex-matched comparison with the participants from the settlement communities. The values for hair cortisol content of males aged 20–24 is higher in participants from the settlements (530 ± 118 ng/g vs. 348 ± 118 ng/g for the Kenyan clinical team; $P = 0.0234$). The values for hair cortisol content of females aged 20–24 is also higher in the participants from the settlements (618 ± 292 ng/g vs. 316 ± 121 ng/g for the Kenyan clinical team; $P = 0.0140$).

Higher concentrations and significant differences were also found between those participants from the settlements in Naivasha and Mogotio who made <5000 KSh per month (approximate minimum wage in Kenya for unskilled employees), compared with those who made >5000 KSh per month, or among settlement

volunteers who reported feeling unsafe collecting water or using sanitation facilities compared with those who reported feeling safe, or in participants who were divorced compared with those who were single, married, or widowed (Table 2). In the multiple regression analysis, income remained the significant determinant of hair cortisol content after mutual adjustment for each other factor. The fraction of variance in hair cortisol explained by this multiple linear regression model was $R^2 = 0.273$.

Discussion

We found no difference in hair cortisol concentrations between volunteers from the slum settlements in Naivasha living adjacent to large floriculture farms, and volunteers from Mogotio, who lived in slum settlements well-removed from large floriculture operations. Of some interest, hair cortisol concentrations in individuals living in these settlements was higher than in Canadians of European descent. We found no difference in the hair cortisol content of the 9 members of the Kenyan clinical team and the Canadians sampled. Thus, we believe the significantly higher hair cortisol content in individuals from the slum settlement communities than in Kenyan physicians on the sampling team is reflective, at least in part, of increased chronic stress. Of particular importance, in the simple difference of means tests conducted on combined participants from the settlement communities in Naivasha (Karagita, Kamere/Kwa Muhia/DCK, and Kasarani; intensive floriculture) and from the settlement communities in Mogotio (Mogotio and Westlands/Katorongot) we found that participants who are female, divorced, feeling unsafe using sanitation facilities, and (or) collecting water, and making less than the minimum wage in Kenya, have significantly higher hair cortisol content than their comparative controls. After conducting a multiple linear regression with mutual adjustment for all factors, income remained the significant determinant of hair cortisol content. The fact that those individuals from these settlement communities who self identify with marital break-up, low income, and (or) fear, have elevated hair cortisol content indicates that hair cortisol is also a biomarker for stress in African citizens. The role of cultural consensus or sociocultural congruity in the stress response appears to be contributing to the differences in hair cortisol we observed (Brown 1982; Dressler 2012). Incongruity with social or behavioral norms in a culture has been found to be positively associated with hypothalamic–pituitary–adrenal (HPA) activity, and thus, increased cortisol levels (Wilce Jr. 2003). Faresjo et al. (2013) compared hair cortisol levels and perceived stress in young Greek adults who were very concerned about the financial crisis to young Swedish adults under much less economic pressure. The Greeks had higher perceived stress, greater depression, and more anxiety than the Swedes, but had significantly lower hair cortisol. This suggests chronic stress in the Greek subjects led to decreased cortisol levels and a depressed hypothalamic–pituitary–adrenal (HPA) axis. It is known that chronically elevated levels of cortisol can down-regulate the HPA system, resulting in decreased cortisol. This association occurs in some pathological conditions, including PTSD (Heim et al. 2000). This is in marked contrast to our current findings, which showed the members of Naivasha and Mogotio settlements who reported more stressful conditions had higher hair cortisol levels. Differences in hair cortisol content will be discussed below, in the context of responses from the health status survey.

Hair habits

How the external environment impacts hair cortisol content is not well studied, and there are inconsistent reports on this subject in the literature. Repeated hair-washings in hot water and shampoo have been reported to decrease hair cortisol content by some, and this could perhaps be due to damage of the hair structure (Stout et al. 2007; Hamel et al. 2011; Li et al. 2012). Conversely, Kirschbaum et al. (2009), Manenschijn et al. (2011), and Stalder et al. (2012) found no significant differences in hair cortisol content in hair related to frequency of hair-washing or treatment. These results are consistent with those of a very recent study that found that sweating might increase hair cortisol levels, but that this increase cannot be effectively decreased by hair-washing procedures (Russell et al. 2014). In the same study, Russell and his colleagues also showed that cortisol is present in sweat and the cortisol content of the sweat correlated with salivary cortisol levels, indicating that cortisol levels in sweat could reflect acute HPA activity. All of these studies were performed using hair from Caucasians, with no data available on the effects of hair-washing on African hair. Consequently, hair-washing frequency and the effect of sweat could be contributing to the differences in hair cortisol content we found between sexes and between volunteers from the settlements compared to Kenyan members of the clinical sampling team. Females from the settlements had higher hair cortisol than males, whereas female members from the clinical team had similar hair cortisol levels to male members of the clinical team. This implies that the potential effects of hair-washing do not preclude the use of hair cortisol as a biomarker of chronic stress in sub-Saharan Africa. Many African women braid their hair and (or) wear wigs, fake hairpieces, or scarves on their head (Craig 2002). In general, women typically only wash their hair when they go to the salon, which is about once a month to once every 3 months, whereas men typically wash their hair every time they shower and also keep their hair very short (T. Wambua, personal communication). Females from the settlements had higher hair cortisol than the males, which could reflect hair-washing habits. The effects of hair washing frequency and of sweating need to be studied in African hair, to strengthen the use of hair cortisol as a biomarker of stress in this region of the world.

Sex and gender differences

Unlike many previous studies that examine sex differences in cortisol secretion, we found increased hair cortisol levels in females when compared with males from the settlement communities. Kirschbaum et al. (1999) showed that men have larger adrenocorticotrophic hormone (ACTH) increases than women, and thus would typically have higher levels of cortisol. A large (N = 360) study found that European men have higher hair cortisol content than European women (Dettenborn et al. 2012). Other studies found no differences in cortisol secretion in hair between sexes (Karlen et al. 2011; Stalder et al. 2012).

In Kenya, women are often responsible both for productive tasks such as running a household, caring for children, collecting water, gathering firewood, and food production, as well as their reproductive roles. Consequently, Kenyan women typically perform the largest share of labour, which could be reflective of their increased hair cortisol levels (Sobania 2003). In addition to assuming productive and reproductive roles, many women also experience stress from formal work outside the home.

There is increased HPA axis activity during pregnancy; concomitantly, this can also be a stressful period in a woman's life (Mulder et al. 2002; Kalra et al. 2007; Kirschbaum et al. 2009). We did not note whether the women we surveyed were pregnant or whether they recently had a child, as these factors could contribute to their hair cortisol levels. Based on 2007 estimates, women in Kenya on average give birth to 4.9 children (Yin and Kent 2007). Thus, the likelihood that some of the women we surveyed were pregnant or recently had a child is relatively high considering that 57% of our female participants were of prime reproductive age (between 18 and 35 years).

Marital status

We found increased hair cortisol content in participants from the settlements who were divorced compared with those who were single, married, or widowed (Table 2). Divorce is generally discouraged in Kenya, with only 7.5% of current respondents in-

dicating that their marital status was divorced. Marriage in Kenya is often more than just the joining of a man and a woman, but a linking of 2 families. Although it has become more common to divorce in recent years, the couple is often pressured by their families and the community to resolve their differences (Sobania 2003; Makeni 2010). The current divorce laws in Kenya also make the process difficult and, often, insurmountably costly (Makeni 2010; Chigiti 2012).

One of the main reasons for a woman to divorce her husband is cruelty. Men divorce women mainly due to unfaithfulness, incompatibility, and infertility (Sobania 2003). Thus, those participants in our study who are divorced could be more stressed as a victim of violence or the inability to bear children. Participants who were divorced could have increased stress, as they are the sole caretaker for their children and must rely upon other kin for help with childcare.

Income

Income is an indicator of SES. Forty-two percent of Kenyans live in poverty, demonstrating the extent of the issue (Sobania 2003). A study of preschoolers in Vancouver, Canada, found that maternal and paternal education, but not parental income was linked with increased hair cortisol (Vaghri et al. 2013). However, the hair cortisol content in these preschoolers whose parents made < $20 000 (the poverty line in this setting) was significantly higher than in parents whose annual income was > $20 000 (Vaghri et al. 2013). This is sufficient evidence to indicate that poverty can be associated with increased hair cortisol content in children. As with this study, poverty is associated with increased stress due to many aspects of survival. These findings are in line with many previous studies utilizing other matrices to analyze biomarkers of cortisol secretion (saliva, serum, and urine) that have found a relationship between increased cortisol secretion and low income (Lupie et al. 2001; Evans and English 2002; Blair et al. 2005; Evans and Kim 2007; Blair et al. 2011).

Toilets and water collection

Many people in settlement communities, particularly women, have to travel considerable distances to go to the toilet and shower facilities and to collect water, putting them at greater risk for harassment and violence, particularly rape. Volunteers who responded that they felt unsafe collecting water or going to the toilet had increased hair cortisol content. The lack of safe and clean water and (or) sanitation is a violation of a recent (2010) United Nations (UN) resolution to the Universal Declaration of Human Rights, which also stated that access to water and sanitation is needed to realize all other human rights (UN 2010). Our findings of higher hair cortisol content in participants who responded "no" to whether they feel safe collecting water and going to the toilet could be reflective of a human rights violation manifesting as increased chronic stress. Distance (too far), no privacy, lack of security, and cleanliness were stated as reasons why volunteers in our current study reported not feeling safe going to the toilet or collecting water.

In 2009, a UN independent expert stated that access (and safe access) to sanitation should be considered a basic human right, as it "evokes the concept of human dignity." (UN 2010) The literature shows that individually perceived violations of dignity can be physically experienced (Mann 1998; Chilton 2006). Chilton (2006) describes the dignity approach, a framework to exploring and addressing human rights violations at the individual level that have been linked to chronic stress and poorer health.

Stress in Africa

There are few studies of stress outside of Western countries. The only other study we found that utilized analysis of hair cortisol in an African population found that participants with PTSD had higher hair cortisol content than traumatized controls from a civil war area in Uganda (Steudte et al. 2011), emphasizing the utility of the assay for Africans. A study that examined Kenyan elders caring for their orphaned grandchildren and the impact of this care-giving on perceived and physiological measures of stress found that caregivers have higher levels of perceived stress as determined by a culturally modified perceived stress scale than non-caregivers (Ice et al. 2012). A study of pastoralist women from Northern Kenya reported that culturally defined local idioms of stress were the most sensitive indicators of psychosocial experiences, showing high concordance with salivary cortisol levels (Pike and Williams 2006).

Although we did not find a difference in hair cortisol content between participants who responded "yes" or "no" to the question, "Have you ever been assaulted when collecting water or going to the bathroom" (Table 2), this does not indicate that trauma does not play a role in chronic stress. There are many manifestations of violence seen in this region of the world, particularly against women, such as wife abuse, rape, trafficking, and incest, which we did not investigate. A previous study conducted in Kenya reported that 42% of the women surveyed had been physically assaulted by a partner (Raikes, 1990). In our current study, there may have been an under-reporting of the trauma the female participants experienced that was not captured in the survey or the hair cortisol data.

However, owing to the lack of appropriate measures (other than SES correlates), we must be very cautious before concluding that chronic stress, as measured by cortisol content of hair, was the major reason for the differences we found.

Conclusions

The effect of hair-washing frequency and sweating on hair cortisol content should be studied in African hair, to validate the use of hair cortisol as a biomarker of chronic stress in this region of the world. The lack of information about the effect hair-washing frequency has on hair cortisol content in our volunteers prevents us from definitely concluding the differences found between males and females. However, the correlations we found between cortisol content and the responses from the health survey support the application of hair cortisol as a biomarker of stress in sub-Saharan Africa in spite of the possible confounders described above.

Unlike other research that portrays low socio-economic status as merely a product of life's circumstances, these findings represent both political and social issues that can and should be realistically addressed. The fact that people who make below the minimum wage in settlements in Kenya have significantly increased hair cortisol, even after controlling for all other factors, indicates they do not have sufficient income to meet basic needs. This should be an impetus to increase minimum wage in Kenya to ensure citizens are earning enough to survive. Almost a quarter of the participants surveyed did not feel safe collecting water or going to sanitation facilities, and also have increased hair cortisol content. This is an area where a solution could tangibly be implemented to decrease stress in the community.

Social and cultural factors that may be contributing to chronic stress were identified, particularly gendered aspects of social life in Kenya, which historically has been a patriarchal society. A challenge in public health research is the integration of cultural and social factors that can link the aggregate and the individual with a biomedical measure. In line with our current results, examining chronic stress by analysis of hair cortisol levels could be an appropriate and sensitive marker to measure differences in cultural factors.

When contrasting or comparing populations, particularly in the case of the subgroups presented here, measurement of hair cortisol allows us to simultaneously evaluate socio-economic factors, health status, environmental factors and their cumulative

contribution to chronic stress. Quantifying chronic stress through hair cortisol content could be used to identify both communities and specific members therein who could profit from stress management strategies and (or) make modifications in risk behaviours that contribute to poor health outcomes. Investigating community stress levels could prove to be a novel approach to addressing and proposing solutions to the health and socio-economic disparities seen in this region of the world.

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