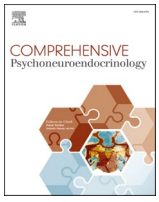
Comprehensive Psychoneuroendocrinology 18 (2024) 100231

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.sciencedirect.com/journal/comprehensive-psychoneuroendocrinology



Hair hormone data from Syrian refugee children: Perspectives from a two-year longitudinal study

Andrew K. May [a], Demelza Smeeth [b], Fiona McEwen [b,c], Patricia Moghames [d], Elie Karam [e], Michael J. Rieder [f], Abdelbaset A. Elzagallaai [f], Stan van Uum [g], Michael Pluess [a,*]

[a] Department of Psychological Sciences, School of Psychology, University of Surrey, Guildford, UK
[b] Biological and Experimental Psychology, School of Biological and Behavioural Sciences, Queen Mary University of London, London, UK
[c] Department of War Studies, King's College London, London, UK
[d] Médecins Du Monde, Beirut, Lebanon
[e] Department of Psychiatry and Clinical Psychology, Balamand University, St Georges Hospital University Medical Center, Institute for Development, Research, Advocacy and Applied Care (IDRAAC), Lebanon
[f] Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada
[g] Division of Endocrinology and Metabolism, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON, Canada

ARTICLE INFO

Keywords:
Hair
Cortisol
Testosterone
Dehydroepiandrosterone
War trauma
Forced displacement

ABSTRACT

For numerous issues of convenience and acceptability, hair hormone data have been increasingly incorporated in the field of war trauma and forced displacement, allowing retrospective examination of several biological metrics thought to covary with refugees' mental health. As a relatively new research method, however, there remain several complexities and uncertainties surrounding the use of hair hormones, from initial hair sampling to final statistical analysis, many of which are underappreciated in the extant literature, and restrict the potential utility of hair hormones. To promote awareness, we provide a narrative overview of our experiences collecting and analyzing hair hormone data in a large cohort of Syrian refugee children ($n = 1594$), across two sampling waves spaced 12 months apart. We highlight both the challenges faced, and the promising results obtained thus far, and draw comparisons to other prominent studies in this field. Recommendations are provided to future researchers, with emphasis on longitudinal study designs, thorough collection and reporting of hair-related variables, and careful adherence to current laboratory guidelines and practices.

1. Introduction

Scalp hair is an accessible and robust matrix for assaying several steroid hormone concentrations which may serve as objective biomarkers with research and clinical utility [1,2]. Compared to other sampling mediums such as saliva, blood, and urine [3,4], hair can be conveniently collected, transported, and stored without concern for decomposition, sterilization, cold-chain logistics, biohazard risk, and specialized skills and/or equipment [3,5–7]. Moreover, single hair samples afford longer-term assessment of circulating hormone levels, which otherwise requires repeated (and therefore, expensive and impractical) sampling of biofluids [5,6]. Considered together, these features suggest a wealth of potential utility for hair hormone data, particularly in the context of mental health, war trauma, and migration, where research on affected individuals must often cater for infrastructural limitations (e.g. within informal refugee camps) and sensitive ethical considerations [7–10].

Despite many apparent advantages, the use of hair hormone data remains clouded by a variety of issues, from a lack of standardization in methods and reference ranges for measured concentrations [2,6,11], to unclear mechanisms regarding how, and at what rate, hormones are sequestered into hairs [12]. Further research is needed to establish a uniform set of guidelines and references with which to harmonize hair hormone research, so that it can better inform clinical applications [11]. Here we provide a summary of our recent experience collecting hair and analyzing hormone concentrations in a large sample of Syrian refugee children and adolescents living in informal tented settlements in Lebanon, across two time points spaced 12 months apart. In addition to providing a further source of referenceable results to the literature, our aim is to offer useful perspectives that may assist future researchers

* Corresponding author: School of Psychology Faculty of Health & Medical Sciences, 05AC05 Lewis Carrol Building University of Surrey, Guildford, GU2 7XH, UK.
E-mail address: m.pluess@surrey.ac.uk (M. Pluess).

https://doi.org/10.1016/j.cpnec.2024.100231
Received 28 December 2023; Received in revised form 26 March 2024; Accepted 26 March 2024
Available online 8 April 2024

wishing to embark on similar endeavours. We begin with a brief overview of the current literature, before detailing our own study procedure for hair hormone collection and analysis, with emphasis on the unanticipated challenges. We then discuss our findings to date, comparing them to a selection of similar studies.

1.1. Background

Armed conflict has risen considerably in the last decade [13], subjecting an increasing number of individuals to substantial war trauma and forced migration, and thus exacerbating the burden of mental illness globally [14–16]. Providing physical and emotional support to affected individuals is challenging [17,18], requiring thorough awareness of their nested ecological systems [19,20] and how these can be positively influenced to improve well-being. To raise this awareness, concerted research efforts are needed, but these can be difficult to coordinate in migratory populations of vulnerable refugees with limited infrastructural access [21].

In recent years, hair hormone analysis has emerged as a convenient means of studying various biological-level systems [11] that lends itself well to the refugee context. Hair is easily and painlessly sampled [4,22] and provides a cumulative, retrospective summary [22,23] of the secretion of several steroid hormones [3], including corticosteroids (e.g. cortisol), sex steroids (e.g. testosterone), and precursors such as dehydroepiandrosterone (DHEA). Measures of these hormones serve as informative signatures of physiological processes pertinent to mental health [24], particularly stress response via the hypothalamic-pituitary-adrenal (HPA) and -gonadal (HPG) axes. The objectivity of these biomarkers helps to mitigate concerns surrounding language and recall bias [25], making them especially useful in research on children (40% of all forcefully displaced individuals are under 18 [26]), who aren't always capable of articulating their traumatic experiences of war and forced displacement [2,27].

Several studies on mental health in refugees and asylum seekers have already leveraged hair hormone data (Table 1), particularly with regard to cortisol [1,28]. For example, Steudte and colleagues [29] investigated severely traumatized young adults suffering from PTSD as a consequence of civil war in Northern Uganda. These individuals were discovered to have significantly elevated hair cortisol concentrations, positively correlated to the number of their traumatic stressors (the "building block" effect [30]), compared to traumatized controls. Etwel and associates [8] found that young women residing in Tripoli, Libya during a period of civil conflict, had significantly increased cortisol levels in scalp-near hair segments (reflecting a post-war period) than more distal segments mapping to pre- and peri-war periods (assuming scalp hair grows at 1 cm/month). Recent male Middle-Eastern asylum seekers (within the prior 12 months) in Germany evinced hair *hypercortisolism* compared to settled Turkish immigrants [23], who in turn displayed relative hypocortisolism compared to non-immigrant Germans. However, there was no difference in cortisol levels between asylum seekers with and without PTSD. In contrast, Buchmüller et al. [31] found *hypocortisolism* amongst Syrian asylum seekers compared to settled Kurdish immigrants in Germany, but asylum seekers in this instance were female, and had arrived in Germany, on average, two years prior. Dajani, Panter-Brick, and colleagues [9,32] assayed hair cortisol at three time points (baseline, 3 months later, 12 months later), in a gender-balanced sample of 733 adolescent Syrian refugees who were either current participants, or wait-listed controls, of a stress intervention program. Growth mixture modelling revealed three trajectories of cortisol production, namely hypersecretion, medium secretion, and hyposecretion. Adolescent refugees reporting more fear and insecurity were significantly likely to evince hypersecretion, but those reporting more PTSD symptoms were commonly hyposecretors. Among those individuals reporting the most frequent exposure to traumatic war events, hair cortisol levels seemed substantially dysregulated, with minimal correlation across time points. On balance, however, the intervention program helped to normalize cortisol production, down-regulating hypersecretion whilst upregulating hyposecretion. For Shaheen and colleagues [21], hair cortisol concentrations were positively associated with a preliminary PTSD diagnosis in war-exposed adolescents, but shared a negative relationship with sense of coherence [33], compared to non-exposed controls. Finally, in a sample of 91 male unaccompanied Middle-Eastern refugee minors, Sierau and associates [34] noted an inverse relationship between hair cortisol and total number, and number of different types, of lifetime traumatic stressors.

Taken together, current findings on hair cortisol in the context of war trauma appear to strengthen a known pattern. While hair cortisol is elevated after initial, dose-dependent traumatic exposure, concentrations tend to decline towards under-regulation (cortisol blunting) as trauma persists [1,24,34,37]. Consequently, hyposecretion of cortisol is a possible hallmark of refugees who have endured complex trauma (repeated traumatic events over a substantial period of time [38]). Beyond this pattern, however, there are few other conclusions that can be robustly drawn at present. Associations between hair cortisol and PTSD are mixed, and may depend on the temporal distance between traumatization and hair sampling (amongst many other methodological concerns; see Ref. [37] for review), while associations with psychopathologies aside from PTSD have been scarcely investigated in refugee

Table 1
Overview of studies examining hair hormones in the context of war trauma and forced migration.

Study	Sample	Sexes	Age range	Detection method	Hair hormone(s) analyzed	Estimated concentration range(s)
Stuedte et al., 2011 [29]	PTSD patients (n = 10) War-exposed controls (n = 17)	Females and males	16–26 years	Luminescence immunoassay	Cortisol	10–60 pg/mg
Etwel et al., 2014 [8]	War-exposed participants (n = 39)	Females only	19–42 years	ELISA	Cortisol	122–520 ng/g
Mewes et al., 2017 [23]	Asylum seekers (n = 56) Immigrants (n = 24) Non-immigrants (n = 28)	Females and males	20–49 years	Luminescence immunoassay	Cortisol	1–26 pg/mg
Dajani et al., 2018 [9]	War-exposed adolescents (n = 727)	Females and males	12–16 years	ELISA	Cortisol	1–30 pg/mg
Shaheen et al., 2018 [21]	War-exposed adolescents (n = 129) Non-trauma adolescents (n = 104)	Females and males	11–16 years	LC-MS/MS	Cortisol	0–12 pg/mg
Sierau et al., 2019 [34]	Unaccompanied refugee minors (n = 91)	Males only	14–19 years	LC-MS/MS	Cortisol	22–59 pg/mg
Schindler et al., 2019 [35]	War-exposed adolescents (n = 56) Non-traumatized controls (n = 36)	Females only	11–16 years	LC-MS/MS	Cortisol DHEA	0–6 pg/mg 4–16 pg/mg
Buchmüller et al., 2020 [31]	Internally displaced persons (n = 14) Asylum seekers (n = 37) Settled immigrants (n = 38)	Females only	22–46 years	LC-MS/MS	Cortisol Cortisone DHEA	0–16 pg/mg 2–47 pg/mg 0–45 pg/mg
de Graaff et al., 2024 [36]	Adult Syrian refugees (n = 115)	Females and males	18–69 years	LC-MS/MS	Cortisol DHEA	3–7 pg/mg 7–16 pg/mg

populations [1,21,34]. Most studies have been cross-sectional, but considering the pattern of cortisol secretion reverses over time [23,37,39], longitudinal studies are needed for clarity, especially in severely traumatized individuals [37].

Studies assaying hair hormones other than cortisol are even sparser in number [1]. Schindler and colleagues [35] discovered elevated hair DHEA in 92 female adolescents residing in the war-affected West Bank territory, compared to a control group. DHEA serves a regenerative role in the stress response system, helping to antagonise the effects of other stress hormones (like cortisol), thus elevated DHEA levels are suggestive of acute stress [40]. Interestingly, no significant differences in hair cortisol concentrations were evident between trauma-exposed and non-exposed groups, but the ratio of cortisol/DHEA was significantly lower following traumatization. Buchmüller and associates [31] discovered that a small sample ($n = 14$) of internally displaced women living in a refugee camp in Northern Iraq had marginally higher ($p = 0.05$) hair DHEA concentrations compared to settled Kurdish immigrants in Germany. De Graaff et al. [36] discovered that hair cortisol and DHEA levels in adult Syrian refugees based in the Netherlands ($n = 115$) were differentially related to the 20 PTSD symptoms outlined in the DSM-5, but neither hormone was related to the number of traumatic events experienced, or post-displacement stressors. A handful of studies have explored HPG axis hormones in hair, especially testosterone, and their relationship to mental health, but these studies have not focused on war trauma and migration, and have produced mostly null findings [1]. Thus, across all assayable hair hormones, considerable scope remains for additional research [41,42], ideally in larger, longitudinal cohorts [11].

As the number of studies incorporating hair hormone data expands [12,43], however, it is important to recall the assumptions on which this field is currently built [4]. Chief amongst these is the assumption that hair chronicles long-term hormone profiles, whereas available evidence only corroborates hair hormones as biomarkers of recent or ongoing stress [12]. Theoretically, longer hair samples should afford investigations of events further in the past, but assaying hormone levels beyond the scalp-near segment has been scarcely evaluated beyond clinically-relevant applications [44–46], and is prone to potential wash-out effects (whereby hormone levels diminish from scalp proximal to distal hair segments) [11,37]. A second common assumption is that hair hormones reflect central HPA axis activity, but hair follicles have local capacity to produce glucocorticoid hormones and respond to stressors independently of the rest of the body [2,12], which might have confounding effects, although no serious concerns were identified in a recent meta-analysis [12]. Additionally, exogenous sources of hormones (such as emollients and creams) may contaminate endogenous concentrations [3,47], but wash steps in the preparation of hair samples mitigate this risk [2]. Thirdly, unless appropriate controls are put in place, hair hormone analyses implicitly assume that hair grows (approximately 1 cm/month), and sequesters circulating hormones, at the same rate across individuals [2,5,6]. However, hair growth rate across ethnicities is known to differ [2,41], and hair hormone levels are non-trivially correlated to hair color [11]. This suggests an important role for genetic influences [48,49], the full scope of which remain unclear [1,50], however, Stalder and colleagues [5] provide evidence suggesting trait-based influences on hair cortisol account for more variance (59–82%) than state-based influences. Entry of hormones into hair, and the pace at which they are deposited is also subordinate to biochemical considerations, such as polarity, acidity, and intermolecular forces [1,7,11], adding further complexity. Lastly, it is assumed that hair hormone levels are free from the complications of circadian rhythms [7], but some studies suggest otherwise [51].

In addition to clarifying biological mechanisms, efforts must be made to converge the various methods and analyses of hair hormones towards a uniform standard [2,6]. The preparation of hair samples entails numerous considerations, including mass of hair used, washing method, pulverization, solvent volumes, incubation temperatures, and centrifugation and vortexing speeds (see Ref. [43]). Measuring hormone concentrations has mostly been achieved through immunoassay (enzyme-linked or luminescence) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods but results are inconsistent across these techniques, with immunoassay readings registering between 2.5 and 20-fold higher due, in part, to cross-reactivity with other components of the hair matrix [2,11,22,43]. Consequently, a correction factor, depending on immunoassay type and manufacturer, may need to be calculated to make measurements comparable to preferred LC-MS/MS readings [52]. Such method variability hinders the establishment of gold standard reference ranges [6,43,47], which are required across various population strata, especially sex and age groups [11,42]. There are also a plethora of covariates that need to be standardly reported, such as participant ethnicity, age, sex, BMI, pregnancy, smoking habits, alcohol intake, use of medications (oral contraceptives, painkillers, anxiolytics/antidepressants), hair color, hair washing frequency, hair treatments, any head coverings, date/season of sampling [5,37,42,53], and whether or not hair follicles were left attached [12]. Comprehensive reporting will facilitate the synthesis of independent studies in order to detect and validate key trends in the field. As contribution towards this effort, we detail below our recent experience collecting and analyzing hair samples in a large cohort of Syrian refugee children living in informal settlements in Lebanon.

2. The BIOPATH study

The biological pathways of risk and resilience in Syrian refugee children (BIOPATH) study was initiated with the aim of collecting psychological, physiological, and genetic level data on a sample of Syrian refugee youths and their caregivers in Lebanon to afford a broad assessment of the multisystemic consequences of war trauma and forced displacement [54]. Following ethical approval from the Institutional Review Board at the University of Balamand/Saint George Hospital University Medical Center, Lebanon (ref: IRB/O/024-16/1815), the Lebanese National Consultative Committee on Ethics and the Ministry of Public Health, child-caregiver dyads were recruited from informal tented settlements in the Beqaa region of Lebanon, and data were collected across two waves, spaced 12 months apart (2017–2018). At baseline (wave 1; 2017), 1594 child-caregiver dyads were enrolled into the study, of which 1001 successfully re-participated at wave 2. A description of the entire study is provided by McEwen and colleagues [54,55]. Here we focus on a detailed narration of the physiological arm of the study, from hair sample collection to hormone data analysis.

2.1. Acceptability of hair sampling

Given the focus on biological pathways, participants could only be enrolled into the BIOPATH study if they consented to hair sampling, thus gauging the acceptability of sampling was an important prerequisite to the initiation of the study. Although frequently touted as convenient and well-received by research participants, the acceptability of hair sampling may vary based on cultural factors, privacy concerns, and the clarity of communication about the research objectives [2]. For example, the small, temporary bald spot created following hair sampling is often undesirable to those of Chinese ethnicity because of traditional cultural and aesthetic concerns [1]. In an early feasibility study, the acceptability of hair sampling was explored with 54 child-caregiver dyads, of which 90% voiced support for the procedure. However, focus groups with the refugee children and interviews with field staff highlighted several reservations related to the look of the cut patch and being “made fun of” (embarrassment is a concern amongst adults as well [7]), or whether the hair sample may be used in “black magic” or to find out information that may lead to the family being expelled from Lebanon. To alleviate some of these concerns, we used a photo and, where necessary, pen and paper drawings to demonstrate how much hair would be cut, and the researchers taking the samples demonstrated on their own hair how the visibility of the cut patch could be minimized

through styling (especially for girls).

2.2. Consenting and sampling process

Despite mostly favorable views towards hair sampling, obtaining informed consent from refugee parents was still met with a number of important challenges. Parents, especially mothers, were concerned about the well-being of their children, and preferred to err on the side of caution by first consulting a male caregiver (either the father or the eldest son) to ensure mutual agreement. Where necessary, we rescheduled the consenting process to a day when the male caregiver could also be present to discuss the research and raise any concerns. We adjusted the process to match the literacy and education level of the caregiver(s), providing both a detailed information sheet and an "easy read" version that included photos, both of which were used to support an oral consent process. We checked caregivers' and children's understanding of the sampling process by asking them to re-explain it and, if they were unable to do so, we used pen and paper to draw pictures to explain where on the head the sample would be taken from and the size of the sample that would be taken. Families that related hair sampling to "black magic" were the most challenging to obtain consent from, as room to negotiate around this belief was limited; however, there were few communities where this was mentioned as a concern. Lastly, cultural factors were pertinent when attempting to enrol older female children, who commonly wore hair coverings. Although there was concern that access to scalp hair would be denied for religious reasons (the religion of most participants was Sunni Muslim), only one family refused hair sampling on these grounds.

The aforementioned challenges aside, we were fortunate to achieve a high participation rate, leading to a substantially larger sample size compared to other studies (Table 1). We attribute this compliance among refugee families to several factors. Firstly, before approaching individual families, we sought approval from the community leader ("chawich") at each tented settlement. Secondly, to assist with field work, we partnered with a non-government organization (NGO) that provided medical and mental health services within the Beqaa region and was widely trusted by families. The involvement of this NGO helped to soften the mostly cautious attitudes that parents had towards research participation, potentially in part because it seemed appropriate for a medical NGO to request the collection of biological samples and to take accompanying physical measurements (e.g., height and weight). Thirdly, it was made clear to families that regardless of whether they consented to participate or not, free mental health services would be available to them. For those families that did ultimately consent, we provided monetary compensation for their time (although this was only revealed to families *after* they expressed interest in the study). Fourthly, when requested by caregivers, female children were matched with female research assistants to respect cultural traditions surrounding gender. Lastly, when we re-visited families a year later, we contacted the community leader and some families within each informal settlement 1–2 weeks before visiting, asking them to alert other families to the visit and requesting that they refrain from shaving their child's head before the visit if they wished to take part in the study (shaving was a common practice to deal with headlice).

To commence with hair sampling from the posterior vertex of the scalp (where hair growth is most uniform [56]), research staff required minimal training, and only basic materials were needed. Yarn was used to isolate a small amount of hair, which was tied together as close to the scalp as possible and cut with clean scissors. Hair fragments were then packaged in aluminum foil with the scalp-end labelled (foil does not risk the introduction of moisture to the sample, unlike airtight plastic bags which are not recommended [7]). Because the timing of sampling was unimportant (unlike hormone measurements in biofluids that are strongly influenced by circadian rhythms [2,41]), multiple children could be sampled throughout the day, at flexible times convenient to each participating family (commonly between 09h00 and 16h00). Hair sampling took place from October to January at each wave, corresponding to the autumn and winter seasons.

Samples were sent for hormone extraction and measurement to the Drug Safety Lab at Western University, Ontario, Canada, which has a wealth of experience in hair hormone assays [8,56]. Cortisol, testosterone and DHEA concentrations were measured using enzyme-linked immunosorption assays 11-CORHU-E01-SLV, 11-TESHU-E01-SLV, and 20-DHEHU-E01 respectively, all supplied by ALPCO Diagnostics, USA.

2.3. Hair weight and length

In alignment with previous studies, we aimed to obtain hair samples with a target weight of 20 mg, a diameter of at least 3 mm, and a length of 2 cm, affording a summary of the past two months of hormone secretion. Consistency in sample weight is important to avoid biases in assay measurement, which can accumulate systematically when repeated measures are made, as in longitudinal studies [7]. To correct for any variation in sample weight, sample concentrations were normalized to the sample weight as standard. While a minimal number of children were unable to donate any hair sample (e.g. those with a clean shaven scalp), a significant number of children ($n = 208$) donated hair samples which fell short of the target length. Although this did not preclude the sample from being processed, as the resulting extracts still had detectable hair hormones, shorter samples represent a more recent time period, reducing comparability to longer hair samples. This issue was common in boys with shorter hair, potentially biasing our hormone readouts. Field notes by research staff revealed that head lice were common and children often had their heads shaved to combat the problem. Head lice may also be accompanied by, or indicative of, wider physical health issues, poor hygiene, and housing crowdedness, which could lead to further confounding in research on hair hormones [57,58].

2.4. Unusual characteristics

A small number of samples exhibited unusual characteristics during the extraction process. This included oiliness of the samples ($n = 3$) and discoloration of the extracts ($n = 36$). Oiliness was likely caused by build up of natural hair oils as field reports by research staff stated this was common. Discolored samples were often yellow or red and confirmed to be due to henna treatments. While colored extracts were associated with altered hormone levels in the BIOPATH cohort, this was likely driven by gender effects as girls commonly had colored extracts. Previous studies have indicated that hair dye may impact endocrinological measures [56, 59], however this is not consistently observed [7]. To our knowledge there has been no explicit investigation of henna dying effects on hair hormone measures. Lastly, some samples were not deemed to be of sufficient weight. In acknowledgement of these issues, we typically perform sensitivity analyses with problematic samples excluded to ensure they are not biasing results.

2.5. Immunoassay detection range

Each immunoassay has a range of concentrations that can be reliably detected. If hair extracts are too highly concentrated to be reliably measured, they can be diluted and re-measured, while, if they lie below the detection range, samples can be spiked with a known amount of the target to bring the concentration back into the assay's detection range [32]. Within the BIOPATH study, a significant number of cortisol concentrations fell below the detection range. Amongst such samples, whether by chance or unknown causality, young males were over-represented, and so their concentrations were not excluded to avoid bias.

2.6. Preliminary assessment

Raw hormone readouts were interrogated for issues prior to any

formal hypothesis testing. The distribution of all hormones exhibited a strong, positive skew, thus concentrations were logged (base 10) to mitigate skewing in analyses. Batch effects were also evident for all hormones. Although these were mostly random for DHEA and testosterone readings, cortisol concentrations notably decreased with increasing batch number. We found no other study variable that covaried with this batch effect, except for date of sample collection. Previous reports have suggested that hair samples stored for longer yield lower hormone concentrations [53], however, we detected the inverse relationship (older samples were in earlier batches that had higher cortisol concentrations than samples stored for a shorter duration). Consequently, all of our subsequent hormone analyses have included a control for batch effects, either by including batch number as a categorical predictor variable in regression models, or by centering readings with the mean concentration per batch. Lastly, a small number of participants were excluded (from the physiological arm of the study) because they had hormone concentrations >3 standard deviations from the sample mean of the logged concentrations ($n = 11$–16, depending on hair hormone). One participant was also excluded due to Klinefelter syndrome, which was detected from their DNA sample.

3. Hair hormone results to date

A selection of descriptive statistics for BIOPATH participants are summarized in Table 2. Children and adolescents were between 11 and 12 years old on average (estimated range: 6–20 years), and largely of Syrian nationality. Female children and adolescents were more likely to engage in hair alterations (chemical treatments, dyes, etc.) but washed their hair less frequently compared to males ($p < 0.01$ for all Chi-square tests). The majority of children and adolescents claimed to have never smoked, but field workers noted that they often observed youths smoking throughout the tented settlements. Smoking may blunt hormone secretion, particularly cortisol [49], and thus should be borne in mind as a confounding issue, although meta-analysis of hair cortisol did not reveal a significant effect of habitual smoking [22].

Reference ranges for all hormones (in ng/g, without adjustment for batch effects) are displayed in Table 2 and compared in Fig. 1 (figures are based on wave 1 data, where hormone ranges were at their widest). Concentration ranges were notably wide, even after the removal of outliers. Females had significantly higher cortisol concentrations, but lower testosterone and DHEA concentrations compared to males (all $p <$ 0.001). All hormone concentrations were substantially higher in adolescents compared to younger children (all $p < 0.001$, Fig. 1B), likely attributable to the effects of puberty, assessed via the Pubertal Development Scale (Fig. 1C [60]). Hormone concentrations tended to decrease with time since participants had left Syria (Fig. 2), and were significantly higher in black colored hair compared to brown (Fig. 3).

Beyond the descriptive summaries and simple comparisons provided above, we have conducted three more detailed analyses of hair hormones to date. Smeeth et al. [10] provides a comprehensive assessment of hair cortisol across both waves of data. In brief, we found that elevated hair cortisol significantly predicted higher PTSD symptoms, likely due to the recency of (and possibly, ongoing) traumatization in many children [31]. However, children that had fled from Syria more than 12 months prior exhibited lower concentrations, possibly supporting the reversal of cortisol trajectories over longer time periods. Hair cortisol correlated with the number of war events children were exposed to, but only meaningfully so in those 12 years and older at exposure, either because older children had more war exposure on average, and/or because of issues related to meaning-making [61] and memory formation [62]. However, particular types of war events (e.g. bombardment, bodily harm) were not correlated with hair cortisol, nor were measures of current living conditions within the informal tented settlements. Importantly, effect sizes for detected associations were small compared to the effects of demographic variables such as age and sex, which were far stronger predictors of hair cortisol variability. Further to this work, we also examined the role of hair cortisol in childhood resilience [63], finding decreased resilience (defined as meeting one or more diagnostic thresholds for common mental disorders) in children with higher hair cortisol. An interaction between hair cortisol and a polygenic score for depression (PGS001829) was also identified, whereby children with a higher genetic predisposition towards depression were more likely to be

Table 2
Descriptive statistics for BIOPATH participants in the physiological arm of the study.

Variable	Wave 1		Wave 2	
	Females N = 833[a]	Males N = 751[a]	Females N = 528[a]	Males N = 396[a]
Child Age	11.31 (2.47)	11.42 (2.35)	12.27 (2.48)	12.00 (2.16)
BMI	18.2 (3.6)	17.7 (3.5)	18.9 (3.8)	18.0 (2.9)
Nationality				
Iraqi	1 (0.12%)	0 (0.00%)	1 (0.19%)	0 (0.00%)
Lebanese	1 (0.12%)	7 (0.93%)	1 (0.19%)	2 (0.51%)
Other	0 (0.00%)	1 (0.13%)		
Palestinian	7 (0.84%)	5 (0.67%)	6 (1.14%)	2 (0.51%)
Syrian	824 (98.92%)	738 (98.27%)	520 (98.48%)	392 (98.99%)
Ever smoked				
No	830 (99.76%)	733 (97.60%)	526 (99.62%)	381 (96.21%)
Yes	2 (0.24%)	18 (2.40%)	2 (0.38%)	15 (3.79%)
Time since leaving Syria				
0–12 months	158 (18.99%)	137 (18.36%)	3 (0.57%)	1 (0.25%)
12–24 months	110 (13.22%)	117 (15.68%)	80 (15.15%)	66 (16.67%)
24–36 months	120 (14.42%)	99 (13.27%)	70 (13.26%)	49 (12.37%)
36–48 months	335 (40.26%)	257 (34.45%)	100 (18.94%)	67 (16.92%)
48–60 months	109 (13.10%)	136 (18.23%)	167 (31.63%)	102 (25.76%)
> 60 months			108 (20.45%)	111 (28.03%)
Frequent hair alterations				
No	483 (58.05%)	669 (89.08%)	333 (63.07%)	357 (90.38%)
Yes	349 (41.95%)	82 (10.92%)	195 (36.93%)	38 (9.62%)
Frequency of hair washing				
2–4 times per week	552 (66.35%)	414 (55.13%)	319 (60.53%)	205 (51.77%)
5–7 times per week	214 (25.72%)	280 (37.28%)	179 (33.97%)	178 (44.95%)
Once per week	66 (7.93%)	57 (7.59%)	29 (5.50%)	13 (3.28%)
Hair weight (mg)	22.70 (2.44)	23.03 (1.86)	22.22 (2.11)	22.37 (1.93)
Hair color				
Black	471 (56.54%)	582 (77.50%)	217 (41.10%)	304 (76.77%)
Brown	345 (41.42%)	167 (22.24%)	259 (49.05%)	91 (22.98%)
Other	2 (0.24%)	0 (0.00%)	1 (0.19%)	0 (0.00%)
Red	15 (1.80%)	2 (0.27%)	51 (9.66%)	1 (0.25%)
Cortisol (ng/g)				
N	828	746	527	396
Median (IQR)	108 (42, 246)	53 (27, 106)	105 (61, 221)	63 (38, 127)
Range	3 - 2013	4 - 2269	5 - 1999	5 - 1332
Testosterone (ng/g)				
N	821	749	526	396
Median (IQR)	1.08 (0.38, 2.59)	1.52 (0.80, 2.81)	0.62 (0.38, 1.36)	1.06 (0.60, 2.12)
Range	0.03–17.17	0.06–18.72	0.03–11.33	0.16–15.36
DHEA (ng/g)				
N	825	750	526	396
Median (IQR)	11 (7, 18)	26 (19, 38)	11 (7, 16)	21 (15, 30)
Range	2–148	4–144	2–76	5–99

[a] Mean (SD); n (%).

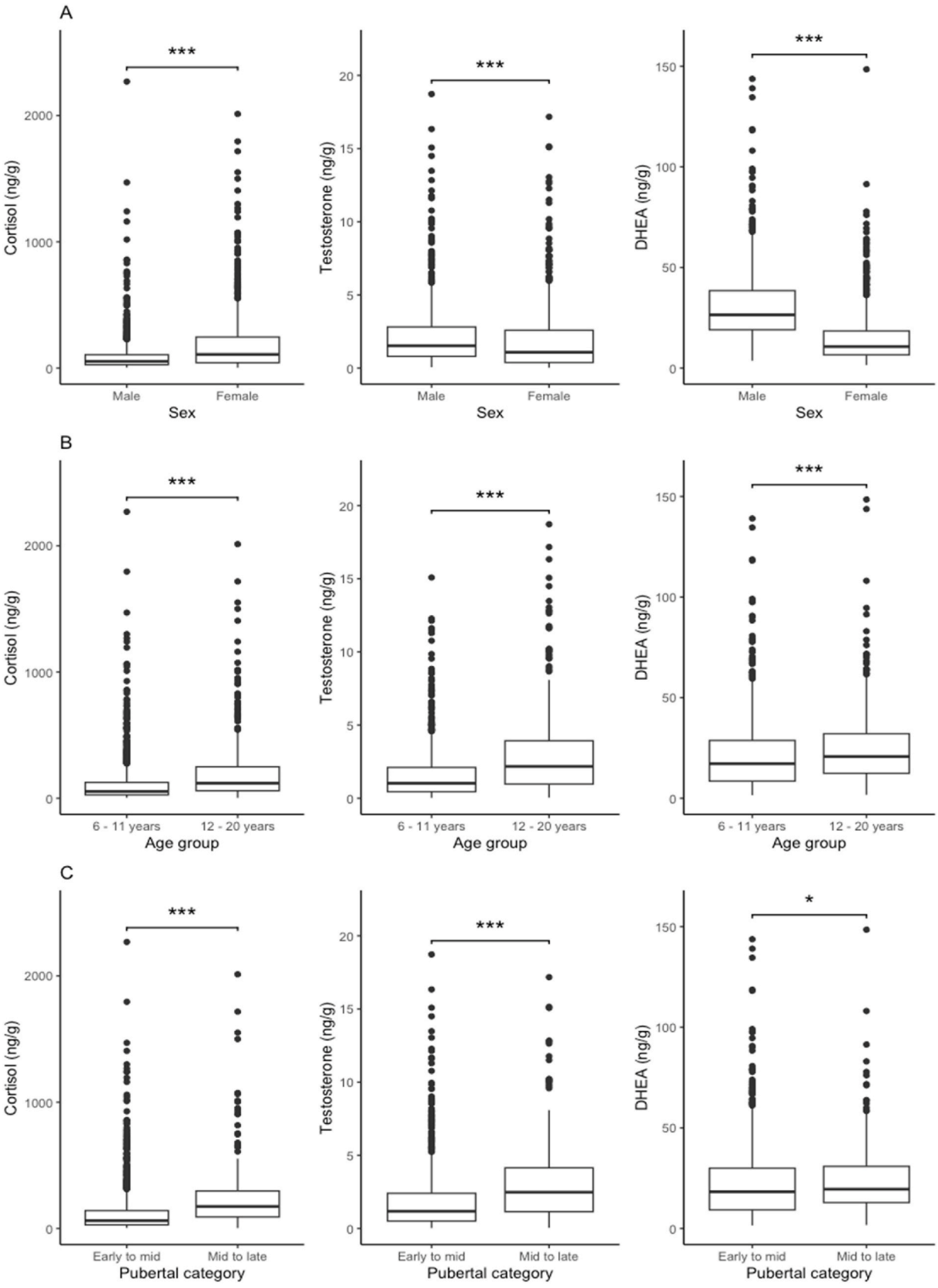


Fig. 1. Hair hormone level comparisons for BIOPATH study participants (wave 1). Levels of cortisol, testosterone and dehydroepiandrosterone (DHEA) are compared across A) sexes, B) age groups, and C) pubertal stages. Significant differences were observed in all comparisons. Wilcoxon rank sum test ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

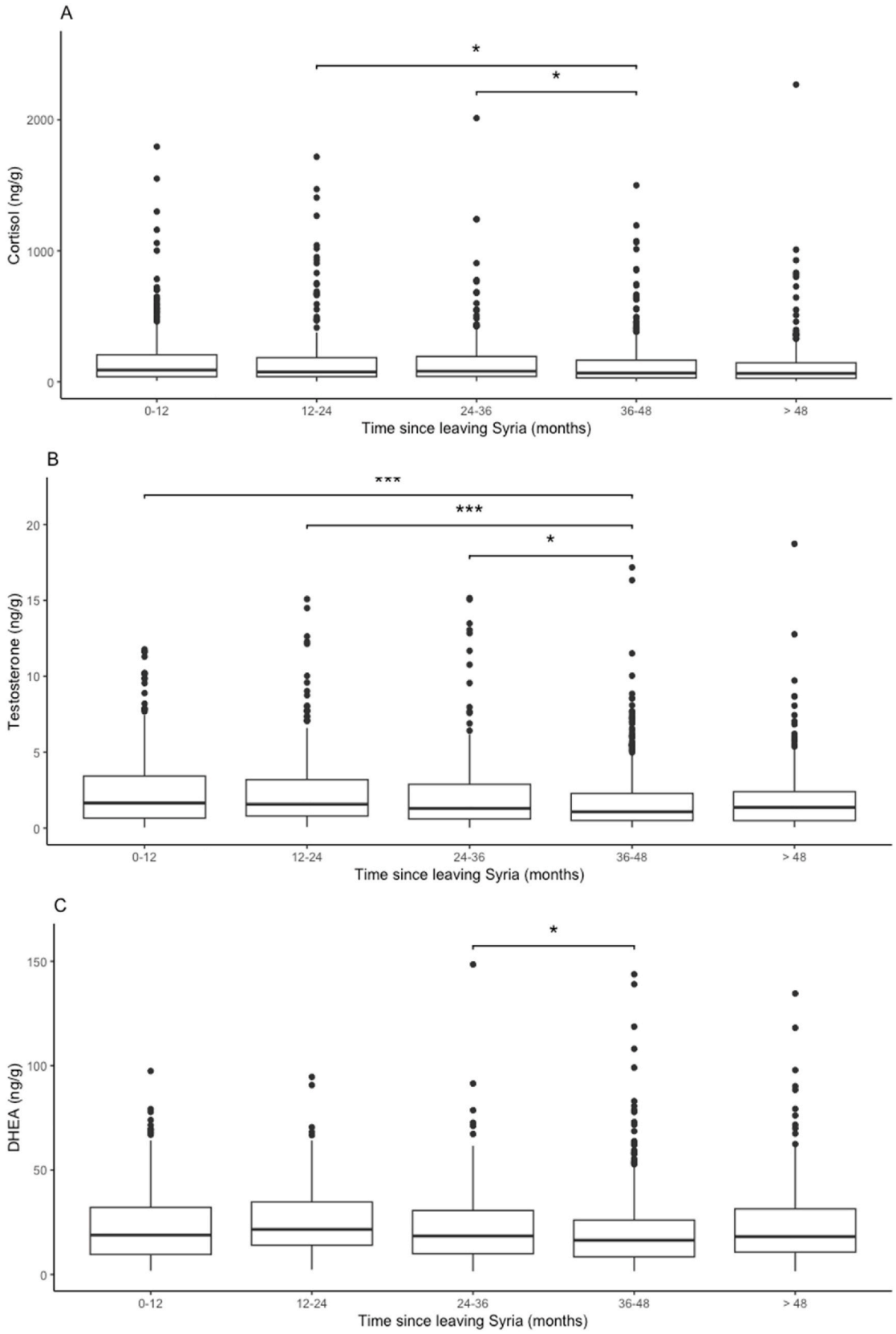


Fig. 2. Hair hormone levels as a function of time since leaving Syria (wave 1). Levels of A) cortisol, B) testosterone, and C) dehydroepiandrosterone (DHEA) are compared based on how many months prior to wave 1 data collection participants had fled from Syria. A subset of pairwise comparisons are indicated, revealing the general decrease in hormone levels with time. Wilcoxon rank sum test ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

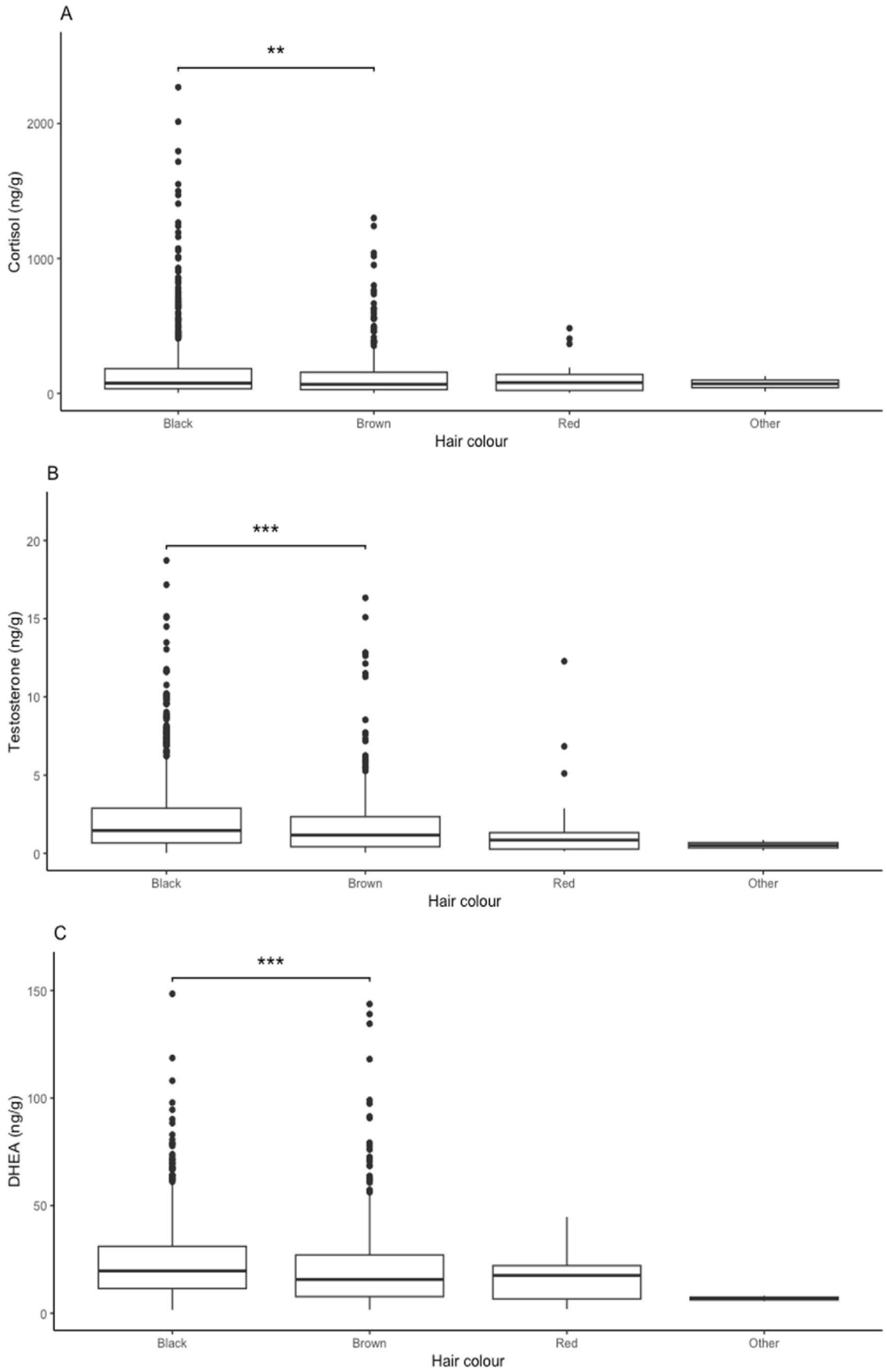


Fig. 3. Hair hormone levels as a function of hair color. For A) cortisol, B) testosterone, and C) dehydroepiandrosterone (DHEA), black hair had significantly higher concentrations compared to brown hair. Other hair colors were not well-represented. Wilcoxon rank sum test ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

categorized as resilient at low hair cortisol levels, but the probability of resilience decreased considerably as concentrations rose. In the context of investigating the environmental sensitivity [64] and mental health of BIOPATH participants, May et al. (under review [65]) noted that elevated hair DHEA concentrations were significantly associated with more depression and anxiety, whilst other hair hormones were relatively uninformative. In contrast to hair cortisol, our research efforts have not revealed any prominent effects yet for hair testosterone or the cortisol/DHEA ratio [6], but these variables have not been the primary focus of research questions investigated thus far.

4. Discussion

Hair hormone data have been an innovative and interesting addition to the refugee mental health literature, affording a glimpse into the physiological systems of individuals in the atypically stressful context of war trauma and forced displacement. However, implementing this method, from sample collection to statistical analysis, involves often overlooked complexities and challenges, many of which are not routinely reported yet [7], leading some to suggest the method is being used with undue confidence [12]. Within this paper, we have attempted to provide a fuller perspective on hair collection and analysis, raising awareness of the scope of strengths and weaknesses of the technique, as well examining a selection of results in our own and other studies.

Reflecting on our sample collection procedure highlights several important lessons. Assessing the acceptability of hair sampling with a smaller focus group, and demonstrating the technique via drawings, was pivotal to the overall success of participant consent, and reinforced the importance of clearly communicating research procedures to participants [2]. Unanticipated issues with hair sample length, oiliness, and discoloration were clarified through diligent field notes from research staff. Cultural traditions surrounding hair needed to be carefully observed and catered for - an issue which isn't commonly emphasized in the Western-dominated scientific literature [1,32]. However, hindsight also reveals a number of improvements that could have been made. Firstly, we did not screen for strenuous activity prior to hair sampling, which might affect hair hormone values via the release of exercise-induced cortisol, sweat, and sebum [2,66]. Aside from the general physical activity of young children [67], many participants were involved in some form of manual labour (often construction or farming) to help their families cope economically, suggesting that strenuous activity was not uncommon in our sample. However, Dajani and colleagues [9] found no difference in hair cortisol between refugee children engaged in some form of physical activity versus those who were not. Secondly, despite hair coverings not significantly impacting our sampling of hair, there remain questions regarding their effect on endocrinological measures. Sunlight and ultraviolet radiation exposure can influence hair hormone concentrations by triggering changes in the composition of the hair shaft, potentially breaking down or altering molecular structures within hair. A study in which hair was exposed to sunlight and artificial UV radiation highlighted reductions in hair cortisol concentrations by 32% and 50% respectively [68], but less clear effects were observerd in a naturalistic study [7]. Thus, it remains uncertain whether hair coverings have any significant impact, prompting several calls for future research [21,35]. Covering of hair was not systematically recorded within the BIOPATH study, resulting in a lost opportunity to investigate this further (although it could be reasonably assumed to apply to most adolescent female participants). Thirdly, we did not follow-up on the possible implications of the issue of head lice, which was unlikely to have affected hormone extraction (due to wash steps in sample preparation [52]), but may have symbolized other health and contextual factors bearing relevance to hormone functioning. Fourthly, the need for proper scientific classification of hair type (e.g. straight, wavy, curly, kinky) was recently noted, due to the surprisingly complex influence hair type has on endogenous hormones [7], highlighting an additional confounding variable missing from the BIOPATH dataset. Lastly, because black and brown were the overwhelmingly predominant hair colors in our cohort, and because refugees were largely of Syrian nationality, we did not need to consider seriously the role of minority stress on hormone secretion [7,48], but this may be an important consideration in more heterogeneous samples.

From the results we have obtained thus far, a number of comparisons can be drawn with similar studies. Dajani et al. [9] also found that refugee girls, and older children, had significantly higher hair cortisol compared to boys, and younger children respectively. This contrasts against general meta-analytic findings on adults, however, where men exhibit hair cortisol concentrations 21% higher than women on average [22]. There have been conflicting reports on the correlation between hair hormones and age [27,42], especially regarding children and adolescents [11] but, in this instance, our findings align with meta-analytic evidence demonstrating increasing hormone secretion with age [11,22]. Similar to Binz and colleagues [11], and Neumann et al. [48], we found black hair to predict signficantly higher cortisol concentrations compared to brown hair (after controlling for sex and batch effects). Black hair also predicted significantly higher testosterone levels, but no differences were noted for DHEA concentrations. Hair color likely intertwines with sunlight and UV exposure considerations, which will need to be unpacked in future [48]. Like Moody and colleagues [7], we did not find a relationship between hair cortisol and hair-washing or hair treatment (after controlling for batch effects and sex), despite small-effect relationships reported previously [22], although treatments may need to be more carefully delineated between different types (e.g. heat versus chemical) in future investigations [42].

Associations between hair hormones, war exposure, and mental health that we have explored are in keeping with much of the existing literature. Hair cortisol concentrations were elevated amongst participants with higher, and more recent war exposure, but steadly declined with time since leaving Syria [21,29]. Concuring with meta-analytic evidence (but possibly not with De Graaff and colleagues [36]), DHEA levels in our participants appeared elevated as a consequence of war trauma, especially in individuals self-reporting high anxiety and depression, but not necessarily PTSD [35,69]. In contrast, unlike Sierau and colleagues [34] ($r = 0.25, p = 0.026$), we did not find an association between the emotional problems subscale of the Strengths and Difficulties Questionnaire and hair cortisol in male participants ($r = -0.06, p = 0.13$), although we used the parent-report (rather than the self-report) version of the scale which demonstrated poor internal consistency reliability ($\alpha = 0.50$ - 0.65). The interaction between hair hormones and polygenic scores is a relatively new avenue of research, with mixed results to date [70–72], but ours appears to be the first such examination in the context of refugee mental health with adequate statistical power, and does suggest some interesting potential. In our studies and others overall, effect sizes have been somewhat underwhelming, but this is likely a realistic indicator that hair hormones are mechanistically complex [37]. Also, while the general assumption in this literature is that war exposure is the main causal factor underpinning hormone variation, refugees face an alarming magnitude of other stressors which contribute their own effects on variation, individually and collectively. A particular strength of the BIOPATH study is our recording of other sources of stress (such as abuse, bullying, and the general refugee environment [12,42]), which we have attempted to account for [10,73,74].

On balance, however, our ranges of hormone concentrations were notably wider than other studies (Table 1), even where hormone extraction methods were similar. Because we relied on immunoassay, and because we did not have any matching LC-MS/MS readings to estimate a correction factor [43], our readings may be up to 20 fold overestimated. Notably, our sample size was between 2 and 58 times larger than other studies, and involved participants of both sexes across a large age range that encompasses puberty. We also conducted sampling across autumn and winter (when hormone concentrations are assumed to be higher [5,53]), which may have introduced seasonal

variability in our own sample, and creates uncertainty when comparing with studies sampling in the summer and spring months [42]. Counter productively, such wide variation may be limiting our ability to detect clear signals against background noise, making it difficult to disentangle environmental effects on hormone secretion from interindividual biological differences. A control group would have helped in this regard, but such a group would have been difficult to define and obtain. Notwithstanding, our hormone measures still speak to important perturbations in hormone secretion, which is especially concerning during the developmentally sensitive periods of childhood and adolescence [28]. Miscalibration of hormone functioning in childhood tends to persist into adulthood, and cortisol research in particular has demonstrated the range of health-related consequences of atypical hormone regulation [22,49], strongly encouraging continued research into the biological sequelae for war-exposed refugees.

To advance the field, many have suggested prioritising multi-method study designs in future [22]. Since hair hormone results do not always mirror findings in bodily fluids [28,37,75], and are unlikely to be useful for research questions regarding hormone rhythmicity [22], it may be necessary to assess hormones in multiple matrices. Moreover, hormone levels are but singular outcomes of vastly more complex biological systems [27], and will, in time, need to be contextualized within broader understandings of individual physiology, neurology [76], genetics [41, 48], and epigenetics [22]. This would help to clarify many of the current uncertainties surrounding hair hormones [12], but multi-method studies are arguably more difficult to organise in refugee samples. Encouragingly, the BIOPATH dataset includes both genetic and epigenetic data, which can be mined in future to further investigate hair hormone variation.

4.1. Recommendations

In the interim, the following summarized recommendations should be borne in mind. During study design, researchers should plan for the collection of as many hair-related variables as possible [7,42,53], while restricting hair sample collection to a single season [5], but repeated longitudinally where feasible to understand hormone variability over time [37]. Arranging a focus group of target participants may help to gauge the acceptability of hair sampling, and identify any specific cultural and societal (e.g. minority stress based on hair color/appearance) factors relevant to the study procedure and hormone analysis. The focus group might also provide an opportunity to consider which hair types will likely be sampled (see Ref. [7] for an overview of types), and how hair type will be recorded (e.g. self- and/or observer-report). Because hair type has been historically associated with race [77], researchers must carefully consider any ethical implications. Hair sampling from the posterior vertex of the scalp should continue to be prioritised, with a target mass over 5 mg. Hair sample length will be dependant on the research question, bearing in mind wash-out effects [11], and the generally short hair of males [42]. If participants have aesthetic (or other cultural) concerns about the cut patch of hair, then the method of "tiny snips" may be beneficial, where several smaller samples are collected from different scalp positions [7]. Using professional hair styling scissors (e.g. thinning scissors) is also recommended to minimise how conspicuous the cut patch is. Collected hair should be packaged in tinfoil, with the scalp-near end clearly and securely labelled. For hormone extraction, researchers are advised to consult Russell and colleagues [43] for an interlaboratory comparison of procedures. Ideally, hormones should be assayed using liquid chromatography-tandem mass spectrometry where feasible, but immunoassay is sufficient particularly when a correction factor can be estimated to account for cross-reactivity [43]. When communicating the study results, thorough reporting of hair hormone concentration ranges, covariates, and technical issues (e.g. batch, technician, and/or seasonal effects) is strongly encouraged, in order to facilitate accurate comparison to other studies. Lastly, to maximize accountability and reproducibility, preregistration of the entire study is also advisable.

5. Conclusion

In conclusion, there is much to recommend hair hormone data in the context of war trauma and forced displacement, where other biophysiological investigations are considerably more challenging to implement. However, expectations should be tempered with the appreciation that hair analysis presents its own set of difficulties, and that hair hormones remain only single metrics of complex systems that vary interindividually. With only small effect sizes identified thus far, the utility of hair hormones remains restricted, and the clinical implications (for refugee wellbeing) are likely few, but as methodological issues are confronted and resolved, there may emerge greater usefulness in the future. We trust that perspectives from our own study help to suitably prepare those aiming to contribute to the burgeoning literature in this field.

Funding statement

This work was supported by the Eunice Shriver National Institute of Child Health & Human Development (MP, grant number R01HD083387) and the UKRI Postdoc Guarantee Fellowship (AKM, grant number EP/X028690/1).

CRediT authorship contribution statement

Andrew K. May: Writing – original draft, Formal analysis, Data curation. **Demelza Smeeth:** Writing – review & editing, Formal analysis, Data curation. **Fiona McEwen:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation. **Patricia Moghames:** Writing – review & editing, Project administration, Methodology. **Elie Karam:** Writing – review & editing, Funding acquisition, Conceptualization. **Michael J. Rieder:** Writing – review & editing, Methodology. **Abdelbaset A. Elzagallaai:** Writing – review & editing, Methodology. **Stan van Uum:** Writing – review & editing, Methodology. **Michael Pluess:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

No conflict of interest.

Acknowledgements

The BIOPATH study was funded by the Eunice Shriver National Institute of Child Health & Human Development (NICHD; R01HD083387) and sponsored by Queen Mary University of London. The funder and sponsor played no role in study design, in the collection, analysis or interpretation of data, in the writing of the report, or the decision to submit the article for publication. We warmly thank all participating families for their participation. We thank Cassandra Popham, Claudinei Biazoli, and all other members of the BIOPATH team (https://www.qmul.ac.uk/sbbs/about-us/our-departments/psychology/global-mental-health/meet-the-team/) for their dedication, hard work, and insights. We also thank Thu Chau and Meaghan Stolk for conducting the ELISA analyses.

References

[1] S. Ren, Z. Sun, J. Yang, The use of biochemical indexes in hair for clinical studies of psychiatric diseases: what can we learn about mental disease from hair? J. Psychiatr. Res. 158 (2023) 305–313, https://doi.org/10.1016/j.jpsychires.2023.01.004.

[2] M.J.E. Greff, J.M. Levine, A.M. Abuzgaia, A.A. Elzagallaai, M.J. Rieder, S.H.M. van Uum, Hair cortisol analysis: an update on methodological considerations and

clinical applications, Clin. Biochem. 63 (2019) 1–9, https://doi.org/10.1016/j.clinbiochem.2018.09.010.
[3] J. Karlén, J. Ludvigsson, A. Frostell, E. Theodorsson, T. Faresjö, Cortisol in hair measured in young adults - a biomarker of major life stressors? BMC Clin. Pathol. 11 (2011) 2–7, https://doi.org/10.1186/1472-6890-11-12.
[4] T. Stalder, C. Kirschbaum, Analysis of cortisol in hair - state of the art and future directions, Brain Behav. Immun. 26 (2012) 1019–1029, https://doi.org/10.1016/j.bbi.2012.02.002.
[5] T. Stalder, S. Steudte, R. Miller, N. Skoluda, L. Dettenborn, C. Kirschbaum, Intraindividual stability of hair cortisol concentrations, Psychoneuroendocrinology 37 (2012) 602–610, https://doi.org/10.1016/j.psyneuen.2011.08.007.
[6] S. Qiao, X. Li, S. Zilioli, Z. Chen, H. Deng, J. Pan, W. Guo, Hair measurements of cortisol, DHEA, and DHEA to cortisol ratio as biomarkers of chronic stress among people living with HIV in China: known-group validation, PLoS One 12 (2017) 1–15, https://doi.org/10.1371/journal.pone.0169827.
[7] S.N. Moody, L. van Dammen, W. Wang, K.A. Greder, J.M. Neiderhiser, P.A. Afulani, A. Willette, E.A. Shirtcliff, Impact of hair type, hair sample weight, external hair exposures, and race on cumulative hair cortisol, Psychoneuroendocrinology 142 (2022) 105805, https://doi.org/10.1016/j.psyneuen.2022.105805.
[8] F. Etwel, E. Russell, M.J. Rieder, S.H. Van Uum, G. Koren, Hair cortisol as a biomarker of stress in the 2011 Libyan war, Clin. Invest. Med. 37 (2014) 403, https://doi.org/10.25011/cim.v37i6.22245.
[9] R. Dajani, K. Hadfield, S. van Uum, M. Greff, C. Panter-Brick, Hair cortisol concentrations in war-affected adolescents: a prospective intervention trial, Psychoneuroendocrinology 89 (2018) 138–146, https://doi.org/10.1016/j.psyneuen.2017.12.012.
[10] D. Smeeth, F.S. McEwen, C.M. Popham, E.G. Karam, J. Fayyad, D. Saab, M. J. Rieder, A.A. Elzagallaai, S. van Uum, M. Pluess, War exposure, post-traumatic stress symptoms and hair cortisol concentrations in Syrian refugee children, Mol. Psychiatr. 28 (2023) 647–656, https://doi.org/10.1038/s41380-022-01859-2.
[11] T.M. Binz, L. Rietschel, F. Streit, M. Hofmann, J. Gehrke, M. Herdener, B. B. Quednow, N.G. Martin, M. Rietschel, T. Kraemer, M.R. Baumgartner, Endogenous cortisol in keratinized matrices: systematic determination of baseline cortisol levels in hair and the influence of sex, age and hair color, Forensic Sci. Int. 284 (2018) 33–38, https://doi.org/10.1016/j.forsciint.2017.12.032.
[12] O. Kalliokoski, F.K. Jellestad, R. Murison, A systematic review of studies utilizing hair glucocorticoids as a measure of stress suggests the marker is more appropriate for quantifying short-term stressors, Sci. Rep. 9 (2019) 1–14, https://doi.org/10.1038/s41598-019-48517-2.
[13] The armed conflict location & event data Project, global dashboard. , 2023. (Accessed 21 November 2023) https://acleddata.com/dashboard/{\#}/dashboard.
[14] K.E. Miller, A. Rasmussen, War exposure, daily stressors, and mental health in conflict and post-conflict settings: bridging the divide between trauma-focused and psychosocial frameworks, Soc. Sci. Med. 70 (2010) 7–16, https://doi.org/10.1016/j.socscimed.2009.09.029.
[15] J.S. Murray, War and conflict: addressing the psychosocial needs of child refugees, J. Early Child. Teach. Educ. 40 (2019) 3–18, https://doi.org/10.1080/10901027.2019.1569184.
[16] S. Arakelyan, A. Ager, Annual Research Review: a multilevel bioecological analysis of factors influencing the mental health and psychosocial well-being of refugee children, J. Child Psychol. Psychiatry Allied Discip. 62 (2021) 484–509, https://doi.org/10.1111/jcpp.13355.
[17] M. Stewart, J. Anderson, M. Beiser, E. Mwakarimba, A. Neufeld, L. Simich, D. Spitzer, Multicultural meanings of social support among immigrants and refugees, Int. Migrat. 46 (2008) 123–159, https://doi.org/10.1111/j.1468-2435.2008.00464.x.
[18] G. Laverack, The Challenge of Promoting the Health of Refugees and Migrants in Europe: A Review of the Literature and Urgent Policy Options, Challenges, vol. 9, 2018, p. 32, https://doi.org/10.3390/challe9020032.
[19] A.S. Masten, Resilience in developmental systems: principles, pathways, and protective processes in research and practice, in: M. Ungar (Ed.), Multisystemic Resilience: Adaptation and Transformation in Contexts of Change, Oxford University Press, New York, NY, 2021, pp. 113–134, https://doi.org/10.1093/oso/9780190095888.003.0007.
[20] U. Bronfenbrenner, The Ecology of Human Development, Harvard University Press, Cambridge, MA, 1979.
[21] M. Shaheen, L. Schindler, R. Saar-Ashkenazy, K. Bani Odeh, H. Soreq, A. Friedman, C. Kirschbaum, Victims of war—psychoendocrine evidence for the impact of traumatic stress on psychological well-being of adolescents growing up during the Israeli–Palestinian conflict, Psychophysiology 57 (2018) 1–11, https://doi.org/10.1111/psyp.13271.
[22] T. Stalder, S. Steudte-Schmiedgen, N. Alexander, T. Klucken, A. Vater, S. Wichmann, C. Kirschbaum, R. Miller, Stress-related and basic determinants of hair cortisol in humans: a meta-analysis, Psychoneuroendocrinology 77 (2017) 261–274, https://doi.org/10.1016/j.psyneuen.2016.12.017.
[23] R. Mewes, H. Reich, N. Skoluda, F. Seele, U.M. Nater, Elevated hair cortisol concentrations in recently fled asylum seekers in comparison to permanently settled immigrants and non-immigrants, Transl. Psychiatry 7 (2017) e1051–e1055, https://doi.org/10.1038/tp.2017.14.
[24] I. Ouellet-Morin, C.L. Odgers, A. Danese, L. Bowes, S. Shakoor, A.S. Papadopoulos, A. Caspi, T.E. Moffitt, L. Arseneault, Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children, Biol. Psychiatr. 70 (2011) 1016–1023, https://doi.org/10.1016/j.biopsych.2011.06.017.
[25] D. Pereg, R. Gow, M. Mosseri, M. Lishner, M. Rieder, S. Van Uum, G. Koren, Hair cortisol and the risk for acute myocardial infarction in adult men, Stress 14 (2011) 73–81, https://doi.org/10.3109/10253890.2010.511352.
[26] United Nations High Commissioner for Refugees, Global Trends: Forced Displacement in 2022, Copenhagen, Denmark, 2023. https://www.unhcr.org/global-trends-report-2022.
[27] V. Prado-Gascó, U. de la Barrera, S. Sancho-Castillo, J.E. de la Rubia-Ortí, I. Montoya-Castilla, Perceived stress and reference ranges of hair cortisol in healthy adolescents, PLoS One 14 (2019) 1–13, https://doi.org/10.1371/journal.pone.0214856.
[28] I. Schalinski, T. Elbert, S. Steudte-Schmiedgen, C. Kirschbaum, The cortisol paradox of trauma-related disorders: lower phasic responses but higher tonic levels of cortisol are associated with sexual abuse in childhood, PLoS One 10 (2015) 1–18, https://doi.org/10.1371/journal.pone.0136921.
[29] S. Steudte, I.T. Kolassa, T. Stalder, A. Pfeiffer, C. Kirschbaum, T. Elbert, Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD, Psychoneuroendocrinology 36 (2011) 1193–1200, https://doi.org/10.1016/j.psyneuen.2011.02.012.
[30] I.T. Kolassa, V. Ertl, C. Eckart, S. Kolassa, L.P. Onyut, T. Elbert, Spontaneous remission from PTSD depends on the number of traumatic event types experienced, Psychological Trauma: Theory, Research, Practice, and Policy 2 (2010) 169–174, https://doi.org/10.1037/a0019362.
[31] T. Buchmüller, H. Lembcke, J. Busch, R. Kumsta, O.T. Wolf, B. Leyendecker, Exploring hair steroid concentrations in asylum seekers, internally displaced refugees, and immigrants, Stress 23 (2020) 538–545, https://doi.org/10.1080/10253890.2020.1737008.
[32] C. Panter-Brick, K. Wiley, A. Sancilio, R. Dajani, K. Hadfield, C-reactive protein, Epstein-Barr virus, and cortisol trajectories in refugee and non-refugee youth: links with stress, mental health, and cognitive function during a randomized controlled trial, Brain Behav. Immun. 87 (2020) 207–217, https://doi.org/10.1016/j.bbi.2019.02.015.
[33] A. Antonovsky, Unraveling the Mystery of Health: How People Manage Stress and Stay Well, Jossey-Bass, San Francisco, CA, 1987.
[34] S. Sierau, H. Glaesmer, T. Klucken, T. Stalder, Hair cortisol, lifetime traumatic experiences and psychopathology in unaccompanied refugee minors, Psychoneuroendocrinology 104 (2019) 191–194, https://doi.org/10.1016/j.psyneuen.2019.03.003.
[35] L. Schindler, M. Shaheen, R. Saar-Ashkenazy, K.B. Odeh, S.H. Sass, A. Friedman, C. Kirschbaum, Victims of war: dehydroepiandrosterone concentrations in hair and their associations with trauma sequelae in palestinian adolescents living in the West Bank, Brain Sci. 9 (2019), https://doi.org/10.3390/brainsci9020020.
[36] A.M. de Graaff, P. Cuijpers, L. Boschloo, M. Elsawy, S. Hunaidy, S. Seedat, A. B. Witteveen, A.C. Huizink, M. Sijbrandij, The associations of hair cortisol and DHEA with posttraumatic stress disorder symptoms in refugees, Compr. Psychiatr. 129 (2024), https://doi.org/10.1016/j.comppsych.2023.152438.
[37] S. Steudte-Schmiedgen, C. Kirschbaum, N. Alexander, T. Stalder, An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: insight from recent hair cortisol findings, Neurosci. Biobehav. Rev. 69 (2016) 124–135, https://doi.org/10.1016/j.neubiorev.2016.07.015.
[38] U. de Silva, N. Glover, C. Katona, Prevalence of complex post-traumatic stress disorder in refugees and asylum seekers: systematic review, BJPsych Open 7 (2021) 1–11, https://doi.org/10.1192/bjo.2021.1013.
[39] H. Luo, X. Hu, X. Liu, X. Ma, W. Guo, C. Qiu, Y. Wang, Q. Wang, X. Zhang, W. Zhang, G. Hannum, K. Zhang, X. Liu, T. Li, Hair cortisol level as a biomarker for altered hypothalamic-pituitary- adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 Wenchuan earthquake, Biol. Psychiatr. 72 (2012) 65–69, https://doi.org/10.1016/j.biopsych.2011.12.020.
[40] F. Dutheil, S. de Saint Vincent, B. Pereira, J. Schmidt, F. Moustafa, M. Charkhabi, J. B. Bouillon-Minois, M. Clinchamps, DHEA as a biomarker of stress: a systematic review and meta-analysis, Front. Psychiatr. 12 (2021) 1–14, https://doi.org/10.3389/fpsyt.2021.688367.
[41] A.D. Grotzinger, D.A. Briley, L.E. Engelhardt, F.D. Mann, M.W. Patterson, J. L. Tackett, E.M. Tucker-Drob, K.P. Harden, Genetic and environmental influences on pubertal hormones in human hair across development, Psychoneuroendocrinology 90 (2018) 76–84, https://doi.org/10.1016/j.psyneuen.2018.02.005.
[42] S. Fischer, R. Duncko, S.L. Hatch, A. Papadopoulos, L. Goodwin, S. Frissa, M. Hotopf, A.J. Cleare, Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample, Psychoneuroendocrinology 76 (2017) 144–153, https://doi.org/10.1016/j.psyneuen.2016.11.011.
[43] E. Russell, C. Kirschbaum, M.L. Laudenslager, T. Stalder, Y. De Rijke, E.F.C. Van Rossum, S. Van Uum, G. Koren, Toward standardization of hair cortisol measurement: results of the first international interlaboratory round robin, Ther. Drug Monit. 37 (2015) 71–75, https://doi.org/10.1097/FTD.0000000000000148.
[44] V.L. Wester, M. Reincke, J.W. Koper, E.L.T. Van Den Akker, L. Manenschijn, C. M. Berr, J. Fazel, Y.B. De Rijke, R.A. Feelders, E.F.C. Van Rossum, Scalp hair cortisol for diagnosis of Cushing's syndrome, Eur. J. Endocrinol. 176 (2017) 695–703, https://doi.org/10.1530/EJE-16-0873.
[45] S. Thomson, G. Koren, L.-A. Fraser, M. Rieder, T.C. Friedman, S.H.M. Van Uum, Hair analysis provides a historical record of cortisol levels in cushing's syndrome, Exp. Clin. Endocrinol. Diabetes 118 (2009) 133–138, https://doi.org/10.1055/s-0029-1220771.
[46] C. Ibrahim, S. Van Uum, Hair analysis of cortisol levels in adrenal insufficiency, Can. Med. Assoc. J. 186 (2014), https://doi.org/10.1503/cmaj.140407, 1244–1244.

[47] R. Slominski, C.R. Rovnaghi, K.J.S. Anand, Methodological considerations for hair cortisol measurements in children, Ther. Drug Monit. 37 (2015) 812–820, https://doi.org/10.1097/FTD.0000000000000209.
[48] A. Neumann, G. Noppe, F. Liu, M. Kayser, F.C. Verhulst, V.W.V. Jaddoe, E.F.C. Van Rossum, H. Tiemeier, Predicting hair cortisol levels with hair pigmentation genes: a possible hair pigmentation bias, Sci. Rep. 7 (2017) 1–8, https://doi.org/10.1038/s41598-017-07034-w.
[49] D. Carroll, A.T. Ginty, A.C. Whittaker, W.R. Lovallo, S.R. de Rooij, The behavioural, cognitive, and neural corollaries of blunted cardiovascular and cortisol reactions to acute psychological stress, Neurosci. Biobehav. Rev. 77 (2017) 74–86, https://doi.org/10.1016/j.neubiorev.2017.02.025.
[50] H.M.C. Schreier, M. Bosquet Enlow, T. Ritz, B.A. Coull, C. Gennings, R.O. Wright, R.J. Wright, Lifetime exposure to traumatic and other stressful life events and hair cortisol in a multi-racial/ethnic sample of pregnant women, Stress 19 (2016) 45–52, https://doi.org/10.3109/10253890.2015.1117447.
[51] C.F. Sharpley, K.G. Kauter, J.R. McFarlane, Diurnal variation in peripheral (Hair) vs central (Saliva) HPA axis cortisol concentrations, Clin. Med. Insights Endocrinol. Diabetes 3 (2010) 9–16, https://doi.org/10.4137/cmed.s4350.
[52] W. Gao, C. Kirschbaum, J. Grass, T. Stalder, LC–MS based analysis of endogenous steroid hormones in human hair, J. Steroid Biochem. Mol. Biol. 162 (2016) 92–99, https://doi.org/10.1016/j.jsbmb.2015.12.022.
[53] J.G. Abell, T. Stalder, J.E. Ferrie, M.J. Shipley, C. Kirschbaum, M. Kivimäki, M. Kumari, Assessing cortisol from hair samples in a large observational cohort: the Whitehall II study, Psychoneuroendocrinology 73 (2016) 148–156, https://doi.org/10.1016/j.psyneuen.2016.07.214.
[54] F.S. McEwen, C. Popham, P. Moghames, D. Smeeth, B. de Villiers, D. Saab, G. Karam, J. Fayyad, E. Karam, M. Pluess, Cohort profile: biological pathways of risk and resilience in Syrian refugee children (BIOPATH), Soc. Psychiatr. Psychiatr. Epidemiol. 57 (2022) 873–883, https://doi.org/10.1007/s00127-022-02228-8.
[55] F.S. McEwen, C.E. Biazoli, C.M. Popham, P. Moghames, D. Saab, J. Fayyad, E. Karam, T. Bosqui, M. Pluess, Prevalence and predictors of mental health problems in refugee children living in informal settlements in Lebanon, Nature Mental Health 1 (2023) 135–144, https://doi.org/10.1038/s44220-023-00017-z.
[56] B. Sauvé, G. Koren, G. Walsh, S. Tokmakejian, S.H. Van Uum, Measurement of cortisol in human Hair as a biomarker of systemic exposure, Clin. Invest. Med. 30 (2007) 183, https://doi.org/10.25011/cim.v30i5.2894.
[57] M.I. Alberfkani, WijdanM.S. Mero, The incidence of scabies and head lice and their associated risk factors among displaced people in Cham Mishko Camp, Zakho City, Duhok Province, Iraq, Pol. J. Microbiol. 69 (2020) 463–469, https://doi.org/10.33073/PJM-2020-050.
[58] Y.T. Fu, C. Yao, Y.P. Deng, H.M. Elsheikha, R. Shao, X.Q. Zhu, G.H. Liu, Human pediculosis, a global public health problem, Infectious Diseases of Poverty 11 (2022) 1–15, https://doi.org/10.1186/s40249-022-00986-w.
[59] C.J. Gidlow, J. Randall, J. Gillman, G.R. Smith, M.V. Jones, Natural environments and chronic stress measured by hair cortisol, Landsc. Urban Plann. 148 (2016) 61–67, https://doi.org/10.1016/j.landurbplan.2015.12.009.
[60] M.A. Carskadon, C. Acebo, A self-administered rating scale for pubertal development, J. Adolesc. Health 14 (1993) 190–195, https://doi.org/10.1016/1054-139X(93)90004-9.
[61] K. Cox, D.P. McAdams, Meaning making during high and low point life story episodes predicts emotion regulation two years later: how the past informs the future, J. Res. Pers. 50 (2014) 66–70, https://doi.org/10.1016/j.jrp.2014.03.004.
[62] K.S. Dawson, R.A. Bryant, Children's vantage point of recalling traumatic events, PLoS One 11 (2016) 1–13, https://doi.org/10.1371/journal.pone.0162030.
[63] D. Smeeth, A.K. May, E.G. Karam, M.J. Rieder, A.A. Elzagallaai, S.V. Uum, M. Pluess, Risk and resilience in Syrian refugee children : a multisystem analysis, 1–13, https://doi.org/10.1017/S0954579423000433, 2023.
[64] M. Pluess, Individual differences in environmental sensitivity, Child Development Perspectives 9 (2015) 138–143, https://doi.org/10.1111/cdep.12120.
[65] A.K. May, D. Smeeth, F. McEwen, M.J. Rieder, A.A. Elzagallaai, S. van Uum, F. Lionetti, M. Pluess, The Role of Environmental Sensitivity in the Mental Health of Syrian Refugee Children: A Multi-Level Analysis, (n.d.), .
[66] C.D. Voegel, M. Hofmann, T. Kraemer, M.R. Baumgartner, T.M. Binz, Endogenous steroid hormones in hair: investigations on different hair types, pigmentation effects and correlation to nails, Steroids 154 (2020) 108547, https://doi.org/10.1016/j.steroids.2019.108547.
[67] C. Boreham, C. Riddoch, The physical activity, fitness and health of children, J. Sports Sci. 19 (2001) 915–929, https://doi.org/10.1080/026404101317108426.
[68] V.L. Wester, N.R.P. van der Wulp, J.W. Koper, Y.B. de Rijke, E.F.C. van Rossum, Hair cortisol and cortisone are decreased by natural sunlight, Psychoneuroendocrinology 72 (2016) 94–96, https://doi.org/10.1016/j.psyneuen.2016.06.016.
[69] M. van Zuiden, S.Q. Haverkort, Z. Tan, J. Daams, A. Lok, M. Olff, DHEA and DHEA-S levels in posttraumatic stress disorder: a meta-analytic review, Psychoneuroendocrinology 84 (2017) 76–82, https://doi.org/10.1016/j.psyneuen.2017.06.010.
[70] K.F. Ahrens, R.J. Neumann, N.M. von Werthern, T.M. Kranz, B. Kollmann, B. Mattes, L.M.C. Puhlmann, D. Weichert, B. Lutz, U. Basten, C.J. Fiebach, M. Wessa, R. Kalisch, K. Lieb, A.G. Chiocchetti, O. Tüscher, A. Reif, M.M. Plichta, Association of polygenic risk scores and hair cortisol with mental health trajectories during COVID lockdown, Transl. Psychiatry 12 (2022) 1–10, https://doi.org/10.1038/s41398-022-02165-9.
[71] K. Bolhuis, H. Tiemeier, P.R. Jansen, R.L. Muetzel, A. Neumann, M.H.J. Hillegers, E.T.L. van den Akker, E.F.C. van Rossum, V.W.V. Jaddoe, M.W. Vernooij, T. White, S.A. Kushner, Interaction of schizophrenia polygenic risk and cortisol level on pre-adolescent brain structure, Psychoneuroendocrinology 101 (2019) 295–303, https://doi.org/10.1016/j.psyneuen.2018.12.231.
[72] L. Rietschel, F. Streit, G. Zhu, K. McAloney, J. Frank, B. Couvy-Duchesne, S.H. Witt, T.M. Binz, J.L. Bolton, C. Hayward, N. Direk, A. Anderson, J. Huffman, J.F. Wilson, H. Campbell, I. Rudan, A. Wright, N. Hastie, S.H. Wild, F.P. Velders, A. Hofman, A. G. Uitterlinden, J. Lahti, K. Räikkönen, E. Kajantie, E. Widen, A. Palotie, J. G. Eriksson, M. Kaakinen, M.-R. Järvelin, N.J. Timpson, G. Davey Smith, S.M. Ring, D.M. Evans, B. St Pourcain, T. Tanaka, Y. Milaneschi, S. Bandinelli, L. Ferrucci, P. van der Harst, J.G. Rosmalen, S.J. Bakker, N. Verweij, R.P. Dullaart, A. Mahajan, C.M. Lindgren, A. Morris, L. Lind, E. Ingelsson, L.N. Anderson, C.E. Pennell, S. J. Lye, S.G. Matthews, J. Eriksson, D. Mellstrom, C. Ohlsson, J.F. Price, M. W. Strachan, R.M. Reynolds, H. Tiemeier, S. Ripke, M. Mattheisen, A. Abdellaoui, M.J. Adams, E. Agerbo, T.M. Air, T.F. Andlauer, S.-A. Bacanu, M. Bækvad-Hansen, A.T. Beekman, D.A. Bennett, K. Berger, T.B. Bigdeli, J. Bybjerg-Grauholm, E. M. Byrne, N. Cai, E. Castelao, T.-K. Clarke, J.R. Coleman, C. Consortium, N. Craddock, U. Dannlowski, G. Davies, G. Davies, EcoJ.C. de Geus, P. De Jager, I. J. Deary, F. Degenhardt, E.C. Dunn, E.A. Ehli, T.C. Eley, V. Escott-Price, T. Esko, H. K. Finucane, M. Gill, S.D. Gordon, J. Grove, L.S. Hall, T.F. Hansen, C. Søholm Hansen, T.F. Hansen, A.C. Heath, A.K. Henders, S. Herms, P. Hoffmann, G. Homuth, C. Horn, J.J. Hottenga, D. Hougaard, H. Huang, M. Ising, R. Jansen, E. Jorgenson, S. Kloiber, J.A. Knowles, W.W. Kretzschmar, J. Krogh, Z. Kutalik, M. Lang, G. Lewis, Y. Li, D.J. MacIntyre, P.A. Madden, J. Marchine, H. Mbarek, P. McGuffin, D. Mehta, A. Metspalu, C.M. Middeldorp, E. Mihailov, L. Milani, G. W. Montgomery, S. Mostafavi, N. Mullins, M. Nauck, B. Ng, M. Nordentoft, D. R. Nyholt, M.C. O'Donovan, P.F. O'Reilly, H. Oskarsson, M.J. Owen, S.A. Paciga, C. B. Pedersen, M.G. Pedersen, N.L. Pedersen, M.L. Pergadia, R.E. Peterson, E. Pettersson, W.J. Peyrot, D.J. Porteous, D. Posthuma, J.B. Potash, J.A. Quiroz, J. P. Rice, B.P. Riley, M. Rivera, D.M. Ruderfer, S. Saeed Mirza, R. Schoevers, L. Shen, J. Shi, E. Sigurdsson, G.C. Sinnamon, J.H. Smit, D.J. Smith, J.W. Smoller, H. Stephansson, S. Steinberg, J. Strohmaier, K.E. Tansey, A. Teumer, W. Thompson, P.A. Thomson, T.E. Thorgeirsson, J. Treutlein, M. Trzaskowski, D. Umbricht, S. van der Auwera, G. van Grootheest, A.M. van Hemert, A. Viktorin, H. Völzke, Y. Wang, B.T. Webb, M.M. Weissman, J. Wellmann, G. Willemsen, H.S. Xi, B.T. Baune, D.H. R. Blackwood, D.I. Boomsma, A.D. Børglum, H.N. Buttenschøn, S. Cichon, E. Domenici, J. Flint, H.J. Grabe, S.P. Hamilton, K.S. Kendler, Q.S. Li, S. Lucae, P. K. Magnusson, A.M. McIntosh, O. Mors, P. Bo Mortensen, B. Müller-Myhsok, B. W. Penninx, R.H. Perlis, M. Preisig, C. Schaefer, J.W. Smoller, K. Stephansson, H. Tiemeier, R. Uher, T. Werge, A.R. Winslow, G. Breen, D.F. Levinson, C.M. Lewis, N.R. Wray, P.F. Sullivan, J. McGrath, I.B. Hickie, N.K. Hansell, M.J. Wright, N. A. Gillespie, A.J. Forstner, T.G. Schulze, S. Wüst, M.M. Nöthen, M.R. Baumgartner, B.R. Walker, A.A. Crawford, L. Colodro-Conde, S.E. Medland, N.G. Martin, M. Rietschel, Hair cortisol in twins: heritability and genetic overlap with psychological variables and stress-system genes, Sci. Rep. 7 (2017) 15351, https://doi.org/10.1038/s41598-017-11852-3.
[73] C.M. Popham, F.S. McEwen, E. Karam, J. Fayyad, G. Karam, D. Saab, P. Moghames, M. Pluess, Predictors of psychological risk and resilience among Syrian refugee children, J. Child Psychol. Psychiatry Allied Discip. (2022) 1–9, https://doi.org/10.1111/jcpp.13670.
[74] C.M. Popham, F.S. McEwen, E. Karam, J. Fayyad, G. Karam, D. Saab, P. Moghames, M. Pluess, The dynamic nature of refugee children's resilience: a cohort study of Syrian refugees in Lebanon, Epidemiol. Psychiatr. Sci. 31 (2022), https://doi.org/10.1017/S2045796022000191.
[75] K. Pochigaeva, T. Druzhkova, A. Yakovlev, M. Onufriev, M. Grishkina, A. Chepelev, A. Guekht, N. Gulyaeva, Hair cortisol as a marker of hypothalamic-pituitary-adrenal Axis activity in female patients with major depressive disorder, Metab. Brain Dis. 32 (2017) 577–583, https://doi.org/10.1007/s11011-017-9952-0.
[76] K.M. Schulz, H.A. Molenda-Figueira, C.L. Sisk, Back to the future: the organizational-activational hypothesis adapted to puberty and adolescence, Horm. Behav. 55 (2009) 597–604, https://doi.org/10.1016/j.yhbeh.2009.03.010.
[77] M.W. Eddy, Hair classification, Proc. Pa. Acad. Sci. 12 (1938) 19–26.