DHEAS patterning across childhood in three sub-Saharan populations: Associations with age, sex, ethnicity, and cortisol

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Abstract

Objectives: Hormones have many roles in human ontogeny, including the timing of life history ‘switch points’ across development. Limited hormonal data exist from non-Western children, leaving a significant gap in our understanding of the diversity of life history patterning. This cross-sectional study examines dehydroepiandrosterone sulfate (DHEAS) production in relation to age, sex, ethnicity, and cortisol concentrations, as well as average age of adrenarche, among Aka and Ngandu children of the Central African Republic and Sidama children of Ethiopia.

Methods: Hair was collected from 480 children (160 per population) aged 3-18 years old. These samples were analyzed for DHEAS and cortisol concentrations using ELISAs. A generalized additive model was used to examine DHEAS patterning in relation to age, sex, cortisol, and ethnicity. The derivative of DHEAS as a function of age was used to identify average age of adrenarche in each population.

Results: DHEAS patterning in these three populations is distinct from Euro-American patterns of production. In all three groups, the population-level age at adrenarche onset occurs slightly later than Euro-American averages, with both Central African populations experiencing a later onset than the Ethiopian population.

Conclusions: DHEAS patterns and age at adrenarche vary across cultures, perhaps indicating adaptive life history responses in diverse eco-cultural environments. Delayed involution of the fetal zone and DHEAS patterning may offer both cognitive protection and immune defense in high-risk, nutritionally-poor environments. Additional research in the majority world is essential to improving our understanding of the diversity of hormonal development and timing of ‘switch points’ in life history trajectories.

KEYWORDS
adrenarche, cortisol, DHEAS, life history theory, sub-Saharan Africa

1 | INTRODUCTION

As mediators between biology, environment, and behavior, hormones have received significant attention in anthropological research (e.g., Bartz et al., 2010; Bribiescas, 1996; Burnham et al., 2003; Ellis & Essex, 2007; Ellison, 2001; Flinn, 1999, 2006; Flinn & Ward, 2005; Gettler, McDade, Agustin, Feranil, & Kuzawa, 2013; Gray & Campbell, 2009; Lee, Macbeth, Pagani, & Young, 2009; Nepomnaschy & Flinn, 2009; Worthman, 1999; Worthman & Konner, 1987). Hormones are essential to a wide range of biological processes—ranging from regulation of fetal development to the triggering of life history events to immune system maintenance—and are crucial to uncovering our plasticity in
ontogeny and life history (e.g., West-Eberhard, 2003; Worthman, 1993, 1999). It can often be a challenge, however, to collect these data from children or in diverse cultures and remote locations. Traditional methods (i.e., collection of saliva, urine, or serum) are not feasible in many geographies due to storage needs, collection requirements, or cultural restrictions. As a result, we largely lack data on the wide range of human diversity in hormones (Worthman, 1999), particularly among preadolescent children.

This cross-sectional study has two goals. The first is to examine the developmental trajectory of dehydroepiandrosterone sulfate (DHEAS) production and its relationship with age, sex, and cortisol among children aged 3–18 years in three sub-Saharan populations—Aka and Ngandu of the Central African Republic, and Sidama of southwestern Ethiopia. Although DHEAS has known correlations with age (e.g., de Peretti & Forest 1978; Orentreich, Brind, Rizer, & Vogelman, 1984; Sulcova, Hill, Hampf, & Starka, 1997) and cortisol (e.g., Goodyer, Herbert, & Altham, 1998, 2001; Phillips et al., 2010), we hypothesize that its patterning may manifest differently across populations, reflecting adaptive life history strategies within diverse ecocultural contexts. Our second goal is to estimate the average age of onset for adrenarche, the biological event underpinning postnatal DHEAS production. Adrenarche has known variation in timing of onset (Del Giudice, Ellis, & Shirtcliff, 2011; Pratt, Manatunga, & Li, 1994; Rotter, Wong, Lifrak, & Parker, 1985), yet little empirical research has been conducted outside of Euro-American populations (but see Worthman, 1993). We hypothesize that onset may be later in comparison to Euro-American populations, as found in other biological events such as menarche (Parent et al., 2003; Worthman, 1999). To our knowledge, this is the largest sample to date—both among children and in sub-Saharan populations—to use hair hormone analysis to these ends.

1.1 | Life history theory

Life history theory (LHT) is an evolutionary theoretical framework that centers on energy allocation (Charnov, 1993; Hill & Kaplan, 1999; Kaplan, Lancaster, & Robson, 2003; Stearns, 1976, 1992). The primary expectation of LHT is that a species’ (or an individual’s) strategy should reflect the best possible allocation of energy across the lifespan to maximize reproductive success. The two main categories of energetic expenditure are somatic and reproductive. Somatic effort broadly refers to growth and survival/maintenance, while reproductive effort includes mating, parenting, and inclusive fitness (Hill & Kaplan, 1999; Kaplan et al., 2003). Energy or effort allocated to one of these categories cannot be used for another. Thus, there are competing demands that, along with natural selection, extrinsic risk (e.g., morbidity/mortality rates), and the limiting factor of time, shape the life course of a species. This framework allows for predictions of the timing of important events across the lifespan, including: length of gestation; age at weaning; age at menarche; age at first reproduction; number of offspring; age at menopause; senescence; and the duration of the lifespan.

Developmental trajectories may be set early in life, during critical periods when experience with the environment, particularly harshness or unpredictability, provides cues as to the optimal reproductive strategy (e.g., Belsky, Steinberg, & Draper, 1991; Draper & Harpenden, 1982, 1988; Quinlan, 2007). Recent research characterizes human extended childhood as a phase of assessment, where children use their experience with risk and unpredictability, in both the social and physical environments, to shape their life history strategies (Del Giudice, Angeleri, & Manera, 2009; see also Belsky et al., 1991; Bogin, 2002). This framework suggests that life history stages allow an individual to evaluate local conditions and vary timing of transitions in order to generate optimal developmental and reproductive strategies. These transitions are coordinated by hormones, which trigger gene expression. As parents alone may not be capable of providing an accurate prediction of future conditions, adaptive plasticity is invaluable to human development (e.g., West-Eberhard, 2003). Thus, onset of adrenarche, the developmental event underpinning human juvenility, can also be thought of as a ‘switch point’ following the evaluative phase of early childhood—a transition point in human life history where hormones (in response to environmental cues) “turn on” the genes that lead to phenotypic variation (Del Giudice et al., 2009).

1.2 | DHEAS production and adrenarche

The adrenarche event is marked by rising post-natal production of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS; hereafter DHEA/S when referring to both), typically co-occurring with onset of middle childhood (generally between 6 and 8 years old; Campbell, 2006, 2011; Del Giudice et al., 2009; Parker, 1991). DHEAS, which is the most abundant steroid hormone in circulation, has several hypothesized functions relating to neurocognitive function, social and physical development, and protection against excessive stress (discussed below). Based on these functions, it appears that our adrenarcheal patterning, in comparison to other primates, reflects an adaptive extension of juvenility, one that allows for additional cognitive and behavioral development (Bernstein, 2016).

Onset of DHEAS production during childhood, an event known as adrenarche, is due to the development of the zona reticularis (ZR) in the adrenal cortex (Dohm, 1973; Parker, 1991). The fetal zone produces DHEA after birth but its subsequent postnatal involution is typically rapid (within 6 months); it is not until around 3–4 years of age that islands
of DHEA can sometimes be detected in circulation (Dohm, 1973; Hui et al., 2009). As these islands mature into the ZR layer, DHEA/S becomes more evident in circulation, and is almost always detectable by age 8 (Dohm, 1973; Parker, 1991). The layer continues to increase in thickness during development, reflected by increasing levels of DHEA/S (Dohm, 1973; Parker, 1991), reaching its terminal thickness around age 13 (Nakamura, Rainey, Kurotaki, Hui, & Sasano, 2010). Because it typically precedes puberty, adrenarche was formerly believed to be linked with gonadarche. It is now clear that these are not directly connected (although there is some evidence that the same genes control expression, with estimated heritability between 57% and 65%; see Parker, 1995), as one can proceed in the absence of the other (Campbell, 2006; Parker, 1991). DHEA/S levels appear to be associated with age, and continue to rise through the mid-20s (Worthman, 1999) and in some places as late as the mid-30s (e.g., Turkana men of northern Kenya; Campbell, Leslie, and Worthman, 1999) and in some places as late as the mid-30s.

The adrenarche event encompasses physical, cognitive, and social changes that mark a visible transition to middle childhood (Campbell, 2011; Del Giudice et al., 2009). Physically, onset of adrenarche triggers: increasing production of axillary hair, emergence of pubic hair, and rising oil production from sebaceous glands (Parker, 1991); growth (Parker, 1991; Zemel & Katz, 1986; but see Campbell, 2011); and development of the immune system, particularly as an antagonist against cortisol (Hechter, Grossman, & Chatterton, 1997). In addition, DHEAS can act not only as a DHEA reservoir, as can be back-converted into DHEA [which in turn can be converted to testosterone and dihydrotestosterone or estradiol via steroidogenic enzymes (White & Porterfield, 2013)], but DHEAS can also be converted directly into other androgens/estrogens from reservoirs in peripheral tissues (Labrie et al., 2005). Cognitively, DHEA/S is associated with neurological development, by facilitating learning and enhancing memory (Majewska, 1995). Given these neurological aspects of DHEA/S production, it is perhaps not surprising that the adrenarche event maps neatly onto the 5-to-7-year-old transition (White, 1996), a time when, cross-culturally, children start to “make sense” (Lancy & Grove, 2011; Rogoff, Sellers, Pirrotta, Fox, & White, 1975) due to their increased reasoning abilities. Socially, onset of DHEA/S production appears to lead to increased social interactions and reduced fearfulness (Campbell, 2006). Studies undertaken in western Kenya also indicate that elevated DHEAS concentrations are associated with decreased malaria parasite density among pubertal females (Leenstra et al., 2003) and increased malaria resistance, as well as reduced parasite density, among pubertal males (Kurtis, Mtlilib, Onyango, & Duffy, 2001).

There is individual variation in the timing of the adrenarche event (Del Giudice et al., 2011; Pratt et al., 1994; Rotter et al., 1985), and onset can be somewhat earlier among girls than boys (Sulcová et al., 1997). It is hypothesized that perhaps environmental cues, like increases in body mass index (Remer & Manz, 1999) or prenatal programming (Ong et al., 2004), set the timing of adrenarche but it may be that the onset of DHEA/S production is due solely to the maturation of the adrenal glands. However, both physical and social environments have known effects on the timing of hormonal events; it has been well-established, for example, that both nutritional (e.g., Kulín, Bwibo, Mutie, & Saniner, 1982) and social (e.g., Ellis & Garber, 2000) stress can affect pubertal timing. It has also been demonstrated among rhesus monkeys that calorie restriction can inhibit age-related declines in DHEAS (Lane, Ingram, Ball, & Roth, 1997), suggesting that environmental inputs may have important effects on DHEAS production. Ellis and Essex (2007), for example, found that low parental support is correlated with earlier onset of adrenarche. As a result, it appears likely that the timing of adrenarche, within the context of life history patterning, is tied to early social and environmental inputs (Del Giudice et al., 2011).

1.3 | Cortisol production

Cortisol is a steroid hormone produced in the zona fasciculata of the adrenal glands. It is the key glucocorticoid produced by humans, playing many important roles in both normal maintenance of the body via its metabolic function as well as in development, immune function, and maintenance of homeostasis. Perturbations to homeostasis can be characterized as stressors, stress, or allostatic, as the process by which an organism attempts to return to homeostasis (Chrousos, 1998; Sapolsky, 1994). These perturbations are often caused by risk or unpredictability in the physical or social environment (e.g., Charnov, 1993; Chisholm, Burbank, Coall, & Gemmiti, 2005; Ellis & Essex, 2007; Moore et al., 1997; Quinlan, 2007).

Effects of allostatics can vary depending on the timing, patterning, duration, and individual experience of stressors. For males, prenatal stress can lead to higher disease risk, whereas the greatest risk for females is associated with stress during peripubertal or pubertal maturation (Bale & Epperson, 2015). Allostatic load represents the cumulative effects of extended or repeated stress (McEwen, 1998; McEwen & Stellar, 1993) and is tied to a wide range of negative health impacts including, among others, immune deficiency, cognitive impairment, inhibited growth, delayed sexual maturity, damage to the hippocampus, sensitivity of amygdala fear circuits, and psychological maladjustments (e.g., Flinn, 2006; McEwen, 1998; McEwen & Stellar, 1993; Nepomnaschy & Flinn, 2009; Sapolsky, 1999). Individuals also vary in both responsiveness to (Ellis & Boyce, 2008; Ellis, Jackson, & Boyce, 2006; McEwen, 1998; McEwen & Stellar, 1993) and
perceptions of (McEwen, 1998; McEwen & Steller, 1993; Piko, 2002) stress and this, coupled with genetic differences, can result in distinct outcomes despite similar experiences. An alternative perspective on the allostatic load suggests that chronic stress provides children information during development that, in the context of adaptive plasticity, may inform appropriate life history strategies (Ellis & Del Giudice, 2014; Ellis et al., 2006). Regardless, stress clearly has significant impacts on life history trajectories, and cortisol is one measure of its effects.

1.4 | DHEA/S as a cortisol antagonist

In addition to its role in children’s social and physical development, DHEAS may also act as an antagonist to cortisol, mitigating some of the effects of allostatic load. High cortisol:DHEAS ratios are linked to persistent major depression among children 8–16 years old (Goodyer et al., 1998). Adequate DHEAS concentrations have been hypothesized to mitigate the detrimental effects of cortisol hypersecretion on the brain (Goodyer, Park, Netherton, & Herbert, 2001). Higher cortisol:DHEAS ratios are also associated with increased risk of metabolic syndrome (at least among adult males), while appropriate methodology may be preferable when collecting biomarker data, as it minimizes the potential for psychological harm or physical risk.

A single hair sample can be indicative of an individual’s “hormone phenotype” and concentration changes can reflect, for example, developmental inflection points or the experience of long term stress (Davenport et al., 2006; Russell et al., 2012). Hair does not reflect circulating hormone concentrations at the time of collection, but provides a picture of an individual’s physiological state during hair growth. This allows for examination of a time-averaged signal generated over the time of hair growth (especially cortisol—see Meyer & Novak, 2012; Sauvé et al., 2007; Stalder & Kirschbaum, 2012), in contrast to highly variable point samples (e.g., saliva or blood collection). By providing both a picture of an individual’s hormonal phenotype as well as a window into their experience over several months, hair hormone analysis extends the range of questions that can be examined within the constraints of anthropological fieldwork.

1.6 | Study populations

Research was undertaken among three sub-Saharan populations: Aka forest foragers, Ngandu horticulturalists, and Sidama agropastoralists. Aka forest foragers and Ngandu horticulturalists reside in the southwestern portion of the Central African Republic within the tropical rainforest of the Congo Basin. Aka live in association with Ngandu horticulturalists. This relationship is one of both economic exchange (with Aka exchanging forest goods for agricultural foods) and social and spiritual ties (Bahuchet & Guillaune, 1982; Hewlett, 1991; Takeuchi, 2005). Although the Aka remain mobile, time spent living in close proximity to the Ngandu village has increased in recent years (Meehan, Hagen, & Hewlett, 2017).
The Aka social environment is highly supportive. Aka practice demand sharing; this, along with cooperation and egalitarianism, is a core value (Bahuchet, 1990; Hewlett, 1992). Aka are indulgent and affectionate parents, instilling autonomy and independence at an early age (Hewlett, 1991). Infants are held most of the day by their mothers or another caregiver, and allomothers (nonmaternal caregivers) constitute an essential component to children’s social environments (Meehan, 2005). Aka children as young as 3 years old often act as caregivers, although infants would not be left for extensive periods with other children until the caregiver reached approximately 5 years of age. By the age of 10, children have already acquired many of the skills necessary to forest life (Hewlett & Cavalli-Sforza, 1986).

In contrast to their intimate rearing environment, the physical environment can be considered fairly harsh. Among the Aka, there are high rates of infant and childhood mortality (Hewlett, 1991). The leading causes of death at all ages are infectious and parasitic diseases (Hewlett, van de Koppel, & van de Koppel, 1986). Most Aka children have weight-for-age (60.63%) and height-for-age (80.95%) z-scores 2 SD below WHO reference means, suggesting that the population is severely underweight and stunted (Meehan, Helfrecht, & Quinlan, 2014). While nutritional stress is certainly an important issue in this population, it must also be noted that Aka are genetically distinct from other Central African peoples (Bozzola et al., 2009; Dietz, Marino, Peacock, & Bailey, 1989; Jarvis et al., 2012) and are considered a “pygmy” population, indicating that they will almost always fall below reference means (Meehan et al., 2014). Despite these environmental hardships, Aka view their environment—specifically, the forest—as giving and supportive (Hewlett, Lamb, Leyendecker, & Scholmerick, 2000).

As noted above, the Ngandu live in the same geographic region and frequently in close proximity to the Aka but differ ethnically, linguistically, and culturally. Ngandu parenting styles have been characterized as more authoritative than among the Aka (Hewlett, 1991). Infants are typically held less often than Aka infants and care is less intimate (Hewlett & Lamb, 2002; Meehan, 2008). Men and women work independently and in strongly sex-prescribed activities (Hewlett, 1991; Meehan, 2008). Although the Ngandu practice sharing, it occurs less frequently and is not as widespread (Hewlett, 1991; Hewlett & Lamb, 2002).

Ngandu are subject to similar disease risks as Aka, particularly malaria and other parasites. Additional diseases known to be problematic include bronchitis, sexually-transmitted diseases, and high blood pressure (Hewlett, 1991). Helfrecht and Meehan (2016) found that fully one-third of Ngandu children had weight-for-age (33.04%) and height-for-age (34.51%) z-scores 2 SD below CDC reference means, indicating that nutritional stress is relatively high in this population.

Sidama agropastoralists reside in the Sidama Zone of the Southern Nations, Nationalities, and Peoples’ Region of southwestern Ethiopia. Sidamaland, part of the Great Rift Valley, lies in the area between Lake Awassa and Lake Abaya, the northern and southern boundaries, respectively (Brögger, 1986; Hamer, 1987). Both agriculture and cattle herding are important components of Sidama life but concentration may vary depending on residence elevation (i.e., low-, mid-, or high-land environments; Hamer, 1987). The data here come from Sidama residing in a lowland, peri-urban environment.

Similar to the Ngandu, the Sidama practice a strict sexual division of labor (Asefach & Nigatu, 2008; Hamer, 1987). Children assist their parents in all tasks and, as they mature, they segregate into sex-proscribed activities (Hamer, 1987). Due to increased school attendance, however, it was noted that many children are spending less time obtaining these skills from their parents. The gerontocracy is male-dominated (although women gain influence when they become mothers and particularly when their sons attain elderhood; Brögger, 1986) and infiltrates parenting style—men are considered responsible for the actions of their wives and children (Hamer, 1987). Participation in community life, while generally desirable and pleasant, is obligate; cooperation is essential to social and economic success (Brögger, 1986).

In a survey of disease risks that affect child health, parents frequently noted malaria, bacteria, parasites (particularly helminths; Ashenafi, Techalew, Mulugeta, Asrat, & Berhanu, 2011), and pneumonia, as well as influenza and typhoid (Helfrecht, 2016). They additionally stated that the prevalence of these is worsened by food shortages. Although the majority of Sidama children fall within normal ranges for height- and weight-for-age, 26.8% had height-for-age and 36% had weight-for-age z-scores 2 SD below CDC reference means (Helfrecht, 2016; see also Yewelsew, Kennedy, Gates, & Stoecker, 2008). These scores, like those among Aka and Ngandu, clearly indicate serious potential for increased morbidity and mortality (Martorell & Ho, 1984; Pelletier & Frongillo, 2003; Scrimshaw, Taylor, & Gordon, 1968).

Below, we present the methods and results of our examination of DHEAS patterning and onset of adrenarche among these three sub-Saharan populations. Based on the diverse ecocultural contexts, we hypothesize that DHEAS patterning will manifest differently across populations, reflecting adaptive plasticity in human life history trajectories. We additionally hypothesize that onset of adrenarche, like other biological events, will occur later than among Euro-American populations.

2 | METHODS

The protocol and procedures presented below were reviewed and approved by Washington State University’s Institutional
Review Board. Following explanation of the proposed research to participants and prior to hair collection, informed assent was obtained from child participants and parent permission was obtained from all adults.

### 2.1 Hair collection

Hair was collected from 480 children (80 male, 80 female in each population), between the ages of approximately 3 and 18 years. Hair samples were obtained by shaving or snipping a small portion (~3 cm diameter) from the posterior vertex of the participant’s head (left side). Consistency in where hair is sampled is important as there is research indicating variation in hormone concentrations in different areas of the scalp, with the posterior vertex demonstrating the least intra-sample variation (Sauvé et al., 2007). If a sample was collected by shaving, a new razor blade was used for each participant; scissors, when used, were thoroughly cleaned with alcohol swabs and allowed to dry between collections. Hair was collected directly into a labeled paper envelope and sealed.

As this study was noninvasive, it was not possible to obtain blood in order to compare circulating hormone to hair hormone. In addition, blood (or urine/saliva) would have required several repeated samples to make such a comparison. It should be noted, however, that such studies have been previously done with cortisol and found that hair cortisol concentrations (HCC) are significantly correlated with those found in saliva (D’Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Sauvé et al., 2007), urine (Sauvé et al., 2007), and serum (Sauvé et al., 2007; Yang, Lan, Meng, Wan, & Han, 1998).

### 2.2 Age

Aka parents do not keep track of children’s birthdates, but ages of children were accurately calculated by relative aging to other children in camp and by using seasonal or local events to determine the child’s birth month and year (see Helfrecht & Meehan, 2016; Meehan et al., 2014). In addition, two of the authors (CH & CM) spent 5 and 13 years, respectively, working with this population, allowing for a cumulative acquisition of birth dates and demographic data; several, but not all, of the children participating in this study have been involved in previous research so their birth month and year were previously known. As research among this population of Aka has been ongoing for 30+ years, both participants and researchers are aware of significant events that have occurred in the village. This allows for year of birth to be determined with relative ease. From there, seasonal events—such as when caterpillar season occurred or corn was planted—can be used to determine the month of birth. Among the Ngandu and the Sidama, families typically know the age and date of birth for their children and, if not, birth certificates are usually available. When this information was not available, ages were determined to month and year of birth using relative aging based on season of birth, other family members whose birth date was known, and known birth dates of nearby neighbors. Age is reported here in years.

### 2.3 Hair hormone analysis

Prior to starting extraction, it was necessary to remove foreign matter from the hair samples. When working with populations who live, for example, in mobile camps or mudbrick homes, such as forest foragers, horticulturalists, and agropastoralists, hair collections often include extraneous material. Some samples had significant quantities of such matter (e.g., lice/lice eggs, dirt, or leaf debris) that could affect weights or interfere with hormone analysis (Cooper, Kronstrand, & Kintz, 2012) if not inspected and removed prior to the extraction process. While some researchers are concerned that washing the hair (especially with water) may remove cortisol (Hamel et al., 2011), others note that the exterior of the hair shaft could have been exposed to exogenous sources of hormone and that all samples should undergo decontamination procedures (Society of Hair Testing, 1997).

After visual examination and debris removal, approximately 30 mg of hair was weighed into a glass vial. Hair was then washed twice in 3 ml of isopropanol for 3 min. Isopropanol has been previously determined to be the best choice of wash medium as it does not extract cortisol from the interior of the hair shaft (e.g., Davenport et al., 2006) and additionally serves to remove any remaining debris. Hair was dried completely (3–5 days minimum) under a fume hood.

More hormone can be recovered from ground than milled hair (Davenport et al., 2006). Thus, the complete sample of dry hair was next placed into a stainless steel microvial (1.8 ml) along with 4 chrome steel beads (size 3.2 mm) and sealed with a silicon rubber cap. These vials were placed in liquid nitrogen for 1 min and then pulverized for 1 min at a high speed using a BioSpec (Bartlesville, OK) mini-beadbeater-16 (set between 3.0 and 3.5). The liquid nitrogen-pulverization cycle was repeated two additional times (3 cycles total). The use of liquid nitrogen is not essential, but freezing the hair led to the greatest consistency in the processed samples. Following the third cycle, hair samples had a uniform powder-like consistency.

Ten milligrams (or less) of powdered hair was weighed into a plastic tube and extracted in 4 ml of methanol. Quantities greater than 10 mg did not serially dilute consistently, but dilutions of samples ≤10 mg were proportional. Samples were next sonicated at 45°C for 30 min at 50 KHz using a Branson 3800 sonicator. Sonication, like milling, can increase the amount of hormone that can be extracted from hair (Fourie et al., 2016). Samples were then placed on a plate.
shaker at room temperature for 23.5 h at 470 RPM (started at 550 RPM to get the powder into solution, then reduced to 470). At the end of the incubation period, samples were centrifuged at 2620G/3000 RPM for 10 min at 4°C to separate the powder from the methanol, and 3.5 ml of the extract was aliquotted into a polypropylene tube. Samples were dried in a vacuum concentrator at 37°C until completely evaporated (5 h), and then stored at 4°C if not immediately removed. The dry extracts were reconstituted in 500 μl of assay buffer and sonicated for 20 min at 37°C.

To determine hair hormone concentrations, the samples were assayed using commercially-available ELISA kits (for development of this procedure and subsequent analyses, ALPCO [Salem, NH] Cortisol ELISA [Catalog no. 11-CORHU-E01-SLV] and Salimetrics [State College, PA] Salivary DHEA-S [Catalog No. 1-1252] kits were used). Cross-reactivity for the ALPCO cortisol ELISA is 13.6% for prednisolone, 7.6% for corticosterone, 7.2% for deoxycorticosterone, 7.2% for progesterone, 6.2% for cortisone, 5.6% for deoxycortisol, 5.6% for prednisone, and 1.6% for dexamethasone. Crossreactivity for the Salimetrics DHEA-S ELISA is 0.0844% for androsterone and 0.0268% for transandrosterone.

Results for cortisol are returned as ng/ml, while DHEAS results are returned in pg/mg. These were converted to pg/mg by dividing the result in half (as only 500 μl of buffer is used to reconstitute the sample) and then dividing by 0.875 (because only 3.5 ml of the original 4 ml extraction was dried down). This was then divided by the initial weight of the powdered hair to determine ng/mg for cortisol and pg/mg for DHEAS. For cortisol, this was then multiplied by 1000 in order to determine pg/mg. Both cortisol and DHEAS results are presented here in pg/mg.

2.4 Statistical methods

The hair hormone concentrations for DHEAS were strongly skewed and were therefore log-transformed to improve normality; while both transformed and untransformed data are presented below, transformed data were used for analyses. Generalized additive models (GAM) were used to evaluate the associations of age, sex, cortisol, and population with DHEAS hair hormone concentrations, as well as in examining the relationship between DHEAS and age by population to estimate derivatives. GAMs can be a preferable alternative to linear analyses as they employ a local scoring algorithm that allows smoothing of predictor variables and identification of nonlinear covariate effects (Hastie & Tibshirani, 1986). A one-way ANOVA was used to examine mean population differences in cortisol concentrations and a t-test was used to evaluate mean differences between the sexes. The GAM analyses and plots were generated using R 3.3.2 and the Mixed GAM Computation Vehicle with GCV/AIC/REML Smoothness Estimation package v. 1.8-1.5; all other analyses were conducted in Stata 10.1.

3 RESULTS

Of the initial 480 participants, we were able to successfully analyze 449 (221 male; 228 female) for hair DHEAS concentrations (Table 1). Across the entire sample, hair DHEAS concentrations ranged from a minimum of 11.46 pg/mg to a maximum of 1372.57 pg/mg, with a mean of 231.34 pg/mg (SD = 231.34) and a median of 163.98 pg/mg. Log10-transformation of the data reduces the skew.

Of the 480 participants, 479 (240 male, 239 female) were included in the cortisol analyses (Table 2). One Sidama girl had an extremely high HCC (>7000 pg/mg). At the time of collection, this almost 11-year-old had a skin infection on her head. Because her HCC well-exceeds the range identified within the rest of the sample and is likely due to something external (e.g., the skin infection itself increasing local cortisol and/or a topical treatment that was not fully eliminated during the two wash cycles), she was removed from the analyses. Hair cortisol concentrations across the entire sample ranged from a minimum of 24.82 pg/mg to a maximum of 3405.42 pg/mg, with a mean of 535.27 pg/mg (SD = 440.01) and a median of 451.94 pg/mg. Log10-transformation of the data reduces the skew.

Based on loess regression plots, each population appears to have its own DHEAS and cortisol patterning (Figure 1). Surprisingly, very high DHEAS levels were found among the youngest in all three samples, but especially in the Aka and Sidama populations. In general, DHEAS patterning does not reflect expectations based on the literature cited above; the high concentrations among young children coupled with the relatively gradual rise after the adrenarche event deviate from previously identified trajectories. For the most part, cortisol concentrations mirrored the DHEAS trajectory, albeit with visible variation at the tails of the age distribution.

Results from a one-way ANOVA indicated that there are significant differences in cortisol concentrations between populations [F(2,236)=58.52, P < .001]. Post hoc comparisons using the Scheffe test indicate that there is no significant difference between Aka and Ngandu, but both of these populations have significantly (P < .001) higher HCC than Sidama children (see Table 2 for means and SD). Sex differences among children were not expected but it has been noted in some other studies that males have higher cortisol responses to stress than females (see Kudielka & Kirschbaum, 2005 for a review) so this was tested here. Among the Aka and the Sidama, but not the Ngandu, males have higher mean cortisol concentrations. This only reaches significance for the Sidama (t = −1.88, P = .031).
A GAM was used to evaluate the effects of age, sex, cortisol, and population on DHEAS concentrations; based on the loess regression plots, an interaction term for sex and population was also included (Table 3). The intercept for this model represents Aka females. Controlling for age within each population, results indicate that Aka have significantly higher DHEAS concentrations than Sidama, but not Ngandu (although there does appear to be a trend in this direction as well). In general, there is no difference in DHEAS concentrations between males and females. Sidama children have the lowest hair DHEAS concentrations, controlling for age, sex, cortisol, and population. Within populations, there is no difference between Aka males and females or between Sidama males and females, but Ngandu males have significantly lower DHEAS concentrations than females. For both Aka and Sidama children, age is a significant predictor of DHEAS hair concentrations. Because GAMs allow the data to determine the fit of the curve, the estimated degrees of freedom (edf) allow interpretation of the variation that exists across the sample. Here, the relatively high edf indicate that there are evident changes across the age range of the sample. Age is not a significant predictor for Ngandu children, and the regression line is relatively flat in comparison to Aka and Sidama, albeit somewhat curvilinear (Figure 2). HCC also has a significant (though relatively minor) effect on DHEAS concentrations, with higher cortisol being associated with higher DHEAS concentrations. The model accounts for 47% of the variance in DHEAS hair concentrations.

### Table 1
Summary statistics for age, DHEAS, and log<sub>10</sub>-transformed DHEAS by population and sex

<table>
<thead>
<tr>
<th></th>
<th>Aka</th>
<th></th>
<th>Ngandu</th>
<th></th>
<th>Sidama</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls 71</td>
<td>Boys 63</td>
<td>Girls 77</td>
<td>Boys 79</td>
<td>Girls 80</td>
<td>Boys 79</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>9.82</td>
<td>8.69</td>
<td>7.89</td>
<td>7.99</td>
<td>8.18</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>3.88</td>
<td>4.02</td>
<td>3.48</td>
<td>3.38</td>
<td>3.63</td>
</tr>
<tr>
<td>DHEAS (pg/mg)</td>
<td>Mean</td>
<td>359.65</td>
<td>356.03</td>
<td>293.56</td>
<td>184.54</td>
<td>109.11</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>78.19-853.93</td>
<td>89.94-905.26</td>
<td>43.80-1372.57</td>
<td>33.08-736.52</td>
<td>24.07-598.46</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>226.26</td>
<td>209.99</td>
<td>222.64</td>
<td>131.07</td>
<td>108.74</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; DHEAS</td>
<td>Mean</td>
<td>2.46</td>
<td>2.48</td>
<td>2.37</td>
<td>2.18</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1.89-2.93</td>
<td>1.95-2.96</td>
<td>1.64-3.14</td>
<td>1.52-2.87</td>
<td>1.38-2.78</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.30</td>
<td>0.25</td>
<td>0.29</td>
<td>0.27</td>
<td>0.32</td>
</tr>
</tbody>
</table>

### Table 2
Summary statistics for age, cortisol, and log<sub>10</sub>-transformed cortisol by population and sex

<table>
<thead>
<tr>
<th></th>
<th>Aka</th>
<th></th>
<th>Ngandu</th>
<th></th>
<th>Sidama</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls 80</td>
<td>Boys 80</td>
<td>Girls 80</td>
<td>Boys 80</td>
<td>Girls 79</td>
<td>Boys 80</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>9.83</td>
<td>9.41</td>
<td>7.94</td>
<td>8.05</td>
<td>8.14</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.99-18.00</td>
<td>3.06-18.00</td>
<td>3.02-18.26</td>
<td>3.13-17.10</td>
<td>2.76-18.12</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.25</td>
<td>4.33</td>
<td>3.53</td>
<td>3.40</td>
<td>3.64</td>
</tr>
<tr>
<td>Cortisol (pg/mg)</td>
<td>Mean</td>
<td>577.65</td>
<td>624.07</td>
<td>681.91</td>
<td>641.59</td>
<td>279.03</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>112.30-1488.00</td>
<td>97.32-2728.16</td>
<td>97.67-1957.35</td>
<td>34.23-3072.48</td>
<td>24.82-1921.89</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>342.02</td>
<td>447.36</td>
<td>383.86</td>
<td>451.96</td>
<td>313.14</td>
</tr>
<tr>
<td>Log&lt;sub&gt;10&lt;/sub&gt; cortisol</td>
<td>Mean</td>
<td>2.68</td>
<td>2.70</td>
<td>2.76</td>
<td>2.72</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2.05-3.17</td>
<td>1.99-3.44</td>
<td>1.99-3.29</td>
<td>1.53-3.49</td>
<td>1.39-3.28</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.28</td>
<td>0.29</td>
<td>0.26</td>
<td>0.30</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Average age of adrenarcheal onset was determined by taking the derivative of the curve for age regressed on log\textsubscript{10}-transformed DHEAS concentrations using a GAM. The zero point of the curve is the age at which DHEAS production changes from declining concentrations to rising production and is illustrated for each population in Figure 3. Based on these samples, the age at which the early childhood decline in DHEAS transitions to an increase in DHEAS concentrations—representing onset of adrenarche—is approximately 9 years among Aka and Ngandu children, and around 8 years for Sidama children.

**4 | DISCUSSION**

We were successfully able to use hair hormone analysis to examine patterns of DHEAS and cortisol production among children in three sub-Saharan populations, providing an alternate methodology for life history research in areas where traditional methods are challenging. We additionally provide baseline data for these two biomarkers, useful to both intra- and cross-cultural analyses. Most importantly, the DHEAS patterns presented here reflect distinct variation from known patterns of development, with implications for studies of human ontogeny and life history trajectories. That differences exist was expected, but the high levels observed across early childhood and the comparatively flat pattern observed among Ngandu children were both surprising. Although all three populations appear to have delayed involution of the fetal zone in comparison to Euro-American populations, the Sidama and Aka both reflect a decline across early childhood followed by a rise across middle childhood and adolescence, whereas Ngandu only trend towards a positive association between DHEAS and age. We also found that while there was, in general, no sex difference in DHEAS concentrations, Ngandu males had significantly lower DHEAS than Ngandu females.

Two factors of the physical environment, malnutrition and disease burden, may be of relevance here in relation to both DHEAS and cortisol patterning. Although previous sample sizes for hair cortisol analyses using ELISAs and conducted among children are smaller, potentially obscuring

**FIGURE 1** Scatter plot and loess regression of age (in years) on cortisol and DHEAS hair concentrations (pg/mg) by population and sex. Each dot represents an individual data point.

**TABLE 3** Significance values of the GAM predictors (population, sex, log\textsubscript{10}-transformed cortisol, population × sex, and age by population) for log\textsubscript{10}-transformed DHEAS concentrations ($n = 449$; adj. $R$-sq $= 0.471$).

<table>
<thead>
<tr>
<th>Parametric coefficients</th>
<th>Estimate</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.191</td>
<td>.000</td>
</tr>
<tr>
<td>Ngandu</td>
<td>-0.083</td>
<td>.075</td>
</tr>
<tr>
<td>Sidama</td>
<td>-0.511</td>
<td>.000</td>
</tr>
<tr>
<td>Male</td>
<td>0.009</td>
<td>.845</td>
</tr>
<tr>
<td>Log\textsubscript{10}-transformed cortisol</td>
<td>0.096</td>
<td>.016</td>
</tr>
<tr>
<td>Ngandu × male</td>
<td>-0.198</td>
<td>.002</td>
</tr>
<tr>
<td>Sidama × male</td>
<td>0.055</td>
<td>.395</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smooth terms</th>
<th>edf</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age × Aka</td>
<td>2.606</td>
<td>.000</td>
</tr>
<tr>
<td>Age × Ngandu</td>
<td>1.863</td>
<td>.258</td>
</tr>
<tr>
<td>Age × Sidama</td>
<td>3.632</td>
<td>.000</td>
</tr>
</tbody>
</table>
extant variation, the concentrations reported are also generally much lower than those found here (see Boesch et al., 2015; Groenveld et al., 2013; Grunau et al., 2013; Karlén, Frostell, Theodorsson, Faresjö, & Ludvigsson, 2013; Karlén, Ludvigsson, Frostell, Theodorsson, & Faresjö, 2011; Noppe et al., 2014; Steudte et al., 2011; Vaghri et al., 2013). It is possible that our methodology is more effective at extracting hair cortisol, as we both milled and sonicated our samples, methods known to improve extraction (see Fourie et al., 2016). It is also probable that our results are a reflection of the greater environmental stress in these geographies. In all three populations, the majority of children fall below international reference means for height- and weight-for age, suggesting extensive experience with both acute and chronic nutritional stress (Helfrecht, 2016; Helfrecht & Meehan, 2016; Meehan et al., 2014). Malnutrition is associated with elevated cortisol (e.g., Alleyne & Young, 1967; Fernald & Grantham-McGregor, 1998; Jaya Rao, Srikantia, & Gopalan, 1968; Smith et al., 1981), and this is a likely explanation for the high levels of cortisol seen across all three populations. Given the comparatively lower rates of malnutrition among the Sidama in this study (Helfrecht, 2016), it is not surprising that their cortisol concentrations are significantly lower than among Aka and Ngandu children.

Disease burden is also high in both these geographies and some risks, such as malaria and other parasitic diseases, are shared. This also likely contributes to the high HCC observed, but may also be a factor in DHEAS patterning. As noted above, higher concentrations of DHEAS are associated with reduced parasite density and increased resistance to malaria among adolescents in Kenya (Kurtis et al., 2001; Leenstra et al., 2003). The coupled impact of disease and malnutrition has potentially led to an allostatic load requiring modification of DHEAS production, particularly among Aka and Ngandu children.

FIGURE 2  GAM plots by population (A, Aka; B, Ngandu; C, Sidama). Each tick on the x-axis represents an individual data point.
children, who face a greater risk of death from malaria than Sidama children (WHO, 2010). The loess regression plots suggest that this is a feasible explanation, as the DHEAS trajectories largely mirror cortisol concentrations.

Little data on DHEAS patterning currently exist for majority world populations (but see Worthman, 1993), but fairly extensive data exist on puberty. Although puberty and adrenarche are not related events, these data may be informative for interpreting the timing and patterning of DHEAS production observed here. For example, examination of age at menarche in nonindustrial populations allowed for an understanding of the historically-novel trend toward earlier puberty (e.g., Parent et al., 2003). It is possible that the “delayed” adrenarcheal onset observed in these three populations is actually more reflective of timing throughout our evolutionary history, and earlier onset in minority world populations is due to greater environmental stability/less nutritional stress, biasing our previous interpretations.

**FIGURE 3** Derivatives (dotted lines) of the GAM for DHEAS by age (years)
This lens may also aid in our understanding of the patterning of DHEAS production. Worthman (1999) found that the amount of time between onset of puberty (as marked hormonally) and the menarche event is greater among Kikuyu girls than among British girls; Kikuyu girls exhibited onset of puberty an average of 2.9 years prior to reaching menarche, whereas this difference was only 2.3 years among British girls. The slow rise of DHEAS production following adrenarche observed here may reflect a comparable pattern among British girls. The stress of malnutrition may be impacting DHEAS production, as nutritional status is a hypothesized factor influencing DHEAS production (Ong et al., 2004; Remer & Manz, 1999; Shi, Wudy, Buyken, Hartmann, & Remer, 2009). Although Ngandu girls experience significant declines in their HAZ and WAZ scores between certain developmental phases, Ngandu boys start and remain lower across development (Helfrecht & Meehan, 2016). This may, in part, explain girls’ higher DHEAS concentrations, as their HAZ scores (a measure of chronic nutritional stress) average higher than those of boys. In contrast, however, Ngandu girls have higher HCC, which would suggest the potential for greater nutritional stress. It is possible that their HCC reflects more acute nutritional stress, as their WAZ scores are more comparable to those of boys, and girls often do more allocate and resource acquisition while boys are in school. Additional research including anthropometric and health measures, as well as allostatic load, is necessary to better evaluate this outcome.

Although this is the largest sample to date making use of hair hormone analysis among children and in sub-Saharan populations, the age range of the sample is not evenly distributed, which necessitates some caution when drawing conclusions surrounding the youngest (<5 years old; n = 92 for DHEAS) and oldest (12 and above; n = 75 for DHEAS) participants (see also Figures 1 and 2). A wider age range is needed to fully understand why DHEAS concentrations are so high in young children, as well as to complete the picture of DHEAS patterning in non-Western populations. Future research should be longitudinal, commencing data collection in infancy and extending into early adulthood in order to better evaluate adrenal maturation and DHEAS patterning across development. In addition, anthropometrics were not collected in all three populations, preventing a deeper exploration into the relationship between environment and genetics at this time. Explorations of HAZ, WAZ, and BMIZ in relation to hair DHEAS concentrations among Sidama children, however, indicated that these measures may be less predictive than HCC (Helfrecht, 2016). We have here provided evidence of the usefulness of this non-invasive method to explore questions related to biomarkers and life history, but it should be noted that cultural or religious restrictions might make this approach challenging in some regions, particularly in areas where hair is associated with sorcery (such as in the Central African Republic). Despite their beliefs in sorcery, Aka and Ngandu parents and children were willing to participate and no concerns were expressed by the communities.

This study offers a significant contribution towards uncovering the full range of “normal” DHEAS and cortisol patterning among populations where it has traditionally been difficult to collect biomarkers. The variation from expected trajectories of DHEAS production provides some of the first empirical data on adaptive responses to the local environments during early and middle childhood. These data further demonstrate the importance of situating development within local ecocultural contexts and the need for continued research in majority world populations.

**AUTHOR CONTRIBUTIONS**

CH designed the study and collected the data with input from CM, RB, EH, and SD. CM and SD provided logistical support. DDA and CH conducted the hair hormone analyses. EH and CH conducted the statistical analyses. CH authored the manuscript. CM, RB, EH, SD, and DDA provided feedback on the manuscript.

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**REFERENCES**


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