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**Measurement of cortisol, DHEA and testosterone in the hair of children: preliminary results and promising indications**

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## Measurement of androgens in the hair of children

### Abstract

Hormone analysis is a valuable tool for understanding how physiology and behavior interact. Cortisol in hair has recently been examined as a measure of longer-term hormone output. The aim of this study was to investigate the relationships between other androgens in hair and anthropometric measures. In a child sample (n=114, mean age 8.5 years, 66 females) levels of cortisol, DHEA and testosterone were assayed in the 0-3cm section proximal to scalp. The 3-6cm segment within a subsample of female participants (n=35) was examined and compared. Results showed that testosterone strongly correlated with DHEA, and moderately correlated with cortisol (0-3cm only). Higher hormone concentrations were present in the 3-6cm segment. Finally, there was a weak positive association between DHEA and height. The replication of previously identified associations between androgens, particularly testosterone-DHEA, and with developmental measures suggests hair may offer a valid method of hormone measurement for DHEA and testosterone.

### Key words

hair; enzyme-immuno-assay; testosterone; DHEA; androgens; measurement

## 1. Introduction

Hormone analysis is a valuable tool for understanding the interaction between physiology, development, stress, behavior, and psychopathology. The main methods of hormone measurement sampling have been via analysis of saliva and blood samples. These biospecimens provide a momentary assessment of hormone levels, influenced by a range of endogenous and exogenous factors, including circadian rhythms, exercise, diet, metabolism, and stressors within the wider and immediate environment (Hansen, Garde, & Persson, 2008; Matchock, Dorn, & Susman, 2007). These sources of short-term variation are reduced with a relatively new method of measurement - hormone levels in hair from the scalp. Research suggests that hair provides a retrospective marker of free steroid hormone levels, with concentrations reflecting systemic levels integrated over months (Stalder et al., 2017). The principal analyte assayed in hair to date has been cortisol (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Recently, research has begun to assess other stress responsive hormones, such as the androgens dehydroepiandrosterone (DHEA) and testosterone, which also serve a developmental role. A number of studies have been conducted, with a range of age groups, including adults (Dettenborn et al., 2016; Gao et al., 2013; Pereg et al., 2013; Qiao et al., 2017; Thomson, Koren, Ven Steen, Rieder, & Van Uum, 2009) adolescents (Grotzinger, Mann, et al., 2018; Grotzinger, Briley, et al., 2018), children (Helfrecht et al., 2017), and infants (Schury et al., 2017). Hair hormone concentrations (Stalder et al., 2017) are not directly comparable with momentary hormone biomarkers (e.g., blood and saliva), highlighting a strong requirement for further investigations into hair as a longer-term index of hormone levels – particularly for androgens. Here we will evaluate the validity of hair as a measure of DHEA and testosterone in a sample of children, using the enzyme-linked immunosorbent assay (ELISA) method.

In children who have commenced adrenarche (6-8 years of age), but not yet gonadarche (9-12 years), DHEA and testosterone are primarily adrenal in source<sup>1</sup> and positively associated, as shown by salivary measures (Matchock et al., 2007). After gonadarche, gonadal androgen production alters the hormonal milieu, particularly in boys as testosterone output from the testes increases markedly (Udhane & Fluck, 2015). In the absence of significant increases of gonadal testosterone, adolescent girls have been found to continue to demonstrate a significant positive association between DHEA and testosterone in

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<sup>1</sup> Testosterone is metabolised in peripheral tissue from adrenal DHEA.

saliva (Marceau et al., 2013; Matchock et al., 2007). The influence of gonadal hormones is reflected in different patterns of hormonal association across adolescence (Ruttle, Shirtcliff, Armstrong, Klein, & Essex, 2015). Adrenarche, therefore, represents an ideal phase in which to examine DHEA and testosterone relationships in hair in boys and girls without the influence of the gonadal androgens, and where a positive relationship between hormones has been reliably found in saliva.

DHEA and testosterone are integral hormones in pubertal development (Havelock, Auchus, & Rainey, 2004; Styne & Grumbach, 2011). The early stages of adrenarche are associated with few external physical markers (Byrne et al., 2016), however anthropometric measures can be used as an index of maturation (Biro et al., 2003; Rogol, Clark, & Roemmich, 2000) and androgen levels (DHEA) (Rege & Rainey, 2012). To date, only cortisol concentrations in hair have been measured against anthropometric maturational indices. Having a higher BMI and being male was associated with higher hair cortisol concentrations (HCCs) during childhood, assayed via liquid chromatography tandem mass spectrometry (LC-MS/MS (Rippe et al., 2016) and ELISA (Noppe et al., 2014; Simmons, Badcock, et al., 2015; Veldhorst et al., 2014). HCCs assayed via ELISA were also positively related to waist circumference in obese children (Veldhorst et al., 2014), but this result is yet to be repeated in normative weight-range children.

The most efficacious and valid assay methodology for measuring hormone levels in hair is contended. Currently, LC-MS/MS and ELISA techniques are considered to be the most accessible and viable (Gow, Thomson, Rieder, Van Uum, & Koren, 2010). While some research suggests LC-MS/MS as the superior method of analysis (Gao, Kirschbaum, Grass, & Stalder, 2016), both methods have been validated for hair hormone extraction within adult (Deshmukh, Hussain, Barker, Petroczi, & Naughton, 2010; Noppe, de Rijke, Dorst, van den Akker, & van Rossum, 2015; Sauv e, Koren, Walsh, Tokmakejian, & Van Uum, 2007), and child populations (Slominski, Rovnaghi, & Anand, 2015). Prior research has identified high correlations between LC-MS/MS and immunoassay (including ELISA) for cortisol levels (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009; Russell et al., 2015; Slominski, Rovnaghi, & Anand, 2015). Further research validating each respective method has been called for, with an emphasis on within-study comparisons (Russell, Koren, Rieder, & Van Uum, 2012), the extension to other hormones (such as androgens), and the exploration of differences across brands of ELISA kits, where sensitivity and cross-reactivity can vary widely (Miller, Plessow, Rauh, Groschl, & Kirschbaum, 2013). Recent research measuring androgens in hair assayed via LC-MS/MS reports that testosterone and DHEA levels were

below the rate of detection for many child and some adolescent participants (Grotzinger, Mann, et al., 2018; Grotzinger, Briley, et al., 2018). Grotzinger, Briley et al. (2018) reported non-detectable levels of testosterone (28.5%) and DHEA (9.9%) in a cohort of 7.80 – 19.47 year old twins ( $M = 12.34$ ,  $SD = 2.77$ ), which was associated with younger age and earlier pubertal status.

The ELISA method has a lower limit of analyte detection compared with LC-MS/MS (Gow et al., 2010; Slominski et al., 2015), and therefore may prove useful in the analysis of hair androgen concentrations prior to gonadal maturation. To date, only one other study reports the extraction of androgens (DHEAS) in hair using ELISA in a child sample (Helfrecht et al., 2017). Combined, this highlights the importance of further analysis of androgens in hair using the ELISA method of extraction in child samples. A within-study comparison of assays or ELISA kits is beyond the scope of this report; instead we will provide a preliminary examination of the assay of DHEA and testosterone, as well as relationships with cortisol and anthropometric measures, in hair using ELISA.

In sum, the use of hair as a longer-term hormone index is a promising compliment to momentary indices, however further investigation of methods is required, particularly for hormones other than cortisol. Here we report the first study of DHEA and testosterone levels in hair within a child sample, which will allow us to evaluate whether the positive relationship typically observed between these hormones during adrenarche in saliva and blood is replicated in hair. In a subsample of female participants ( $n=35$ ), assays were also conducted on multiple segments of hair, i.e., 0-3 and 3-6cm from the scalp. The section most proximal to the scalp (0-3cm) is considered the most reliable marker for cortisol, with HCCs reducing down the hair shaft (Stalder et al., 2017). Comparisons across segments (0-3 and 3-6cm) will therefore help to evaluate the stability of DHEA and testosterone across hair lengths.

The following hypotheses were tested:

- 1.) There will be a positive association between DHEA and testosterone in hair (as demonstrated in saliva and blood samples obtained in peri-adrenarcheal and adolescent female samples).
- 2.) These associations will be consistent across hair segments, 0-3 & 3-6cm in length (only examined in girls).
- 3.) Hormone levels will be lower in the distal segment (3-6cm) compared with the proximal segment (0-3cm) (only examined in girls).
- 4.) Hair hormones will be positively associated with physical maturation measures.

Sex differences were examined, as well as the relationship of cortisol with testosterone and DHEA, however, no specific hypotheses were proposed due to the limited studies in this area.

## **2. Method**

### *2.1 Participants*

Participants were 128 children (mean age 8.44 years, SD 0.34, 87 females) participating in the Families and Childhood Transitions Study (FACTS). Participants were recruited from lower socioeconomic (SES) areas within the Melbourne metropolitan area. Approximately 25% of families had incomes that were at, or below, the poverty line, according to Poverty Lines Australia, June 2016 (UoM, 2016)<sup>2</sup>. Participants were assumed to be in the early stages adrenarche, based upon the age range of the sample (mean age 8.44 years, SD 0.34), the parent report of Tanner stage physical maturation, where 94% of females and 73% of males showed no sign of secondary sexual characteristics, and no females had reached menarche. The Tanner parent-report measure has shown high congruence with a physician report of developmental stage (Coleman & Coleman, 2002).

Written consent was obtained from the primary caregiver and verbal consent obtained from the participating child. Exclusion criteria included a history of head trauma, clinically significant developmental or intellectual disorders, clinically significant endocrine abnormalities, and immediate or long-term use of steroidal or amphetamine based medications (reviewed on a case by case basis). Recruitment, methods and study protocol have been described previously in greater detail (Simmons et al., 2017). Of the 128 hair samples provided, 125 samples were viable (n=2 medical exclusions and multi-hormone outliers including ingestion of steroidal medication and illnesses across the last several months; n=1 not enough to assay). With the addition of socioeconomic and anthropometric indices the complete available dataset was n=114. A sub-sample (n=35) of 3-6cm lengths of girls' hair samples (due to longer lengths) were randomly selected and assayed (mean age= 8.5 years).

### *2.2 Measures*

Hair samples were collected from the posterior vertex of each child's scalp, approximately 1cm<sup>2</sup> in diameter. Assays were conducted by Stratech Scientific (Sydney, Australia), using an ELISA methodology where samples were processed and assayed as described previously in Sharpley, Kauter, and McFarlane (2010) as well as D'Anna-Hernandez, Ross, Natvig, and Laudenslager (2011). ELISA assay methodology has been

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<sup>2</sup> Non-parametric t-test comparisons indicated no differences in hormones or anthropometric measures according to status above or below the poverty line.

tested and described in studies measuring hair cortisol and androgen concentrations in animal samples (Koren, Mokady, & Geffen, 2006; Koren et al., 2002), cortisol in adult samples (D'Anna-Hernandez et al., 2011; O'Brien, Tronick, & Moore, 2013; Pereg et al., 2013), as well as cortisol (Ouellette et al., 2015; Slominski et al., 2015) and DHEAS (Helfrecht et al., 2017) across childhood samples. In brief, samples were cut down to 3cm lengths (from scalp end), and washed (this step is in contrast to Sharpley et al. (2010)). Hair was left to dry at room temperature (RT) and minced prior to extraction. Samples of 50mg were extracted via the addition of methanol at RT for 36 h. After extraction, methanol was evaporated to dryness at 45 °C under airstream. The remaining residue was reconstituted in the ELISA sample buffer (Salimetrics), vortexed for 10 seconds, incubated at RT for 60 min and vortexed again prior to assay using commercially available Cortisol (sensitivity <0.007 µg/dL), Testosterone (sensitivity 1 pg/ml) and DHEA (sensitivity 5 pg/ml) ELISA kits (Salimetrics, Carlsbad, CA). All samples were assayed in duplicate. Salimetrics assay kits are validated for parallelism, and Stratech further validates kits for parallelism for the analytes and matrices (sample origin) used. This process ensures that the sample dilution response curve is parallel to the standard concentration response curve. The inter-assay coefficients of variation (CV) for the samples assessed were: DHEA – 6.5%; testosterone – 6.7%; and, cortisol – 6.4%. The intra-assay CVs were DHEA – 5.1%; testosterone – 4.9%; and, cortisol – 4.7%<sup>3</sup>. All CVs are within acceptable ranges.

Anthropometric measures included weight, height, waist circumference, and BMI (weight/height<sup>2</sup>). To reduce measurement error, two measurements of each were taken and the mean calculated, however if they were not within a specified range (0.5 cm for height, 0.1 kg for weight, 0.5 cm for waist) a third measurement was collected and the median used. Height was measured via a portable rigid Invicta stadiometer, weight via calibrated Tanita THD 382 digital scales, and waist circumference was measured by non-stretch anthropometric tape.

### 2.3 Statistical Analyses

*Missing data.* Outliers were corrected (winsorized) within one relevant unit (hair n=7; waist n=1; height=1). Combined, 113 participants (female n=65) had complete data across all hair and developmental measures. The following were recorded as missing due to error in initial measurement (weight n=1, BMI=n=2, height n=1).

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<sup>3</sup> Sensitivity and CVs are values that assess the specificity and accuracy of the assay method.

*Normality.* Raw hair hormone variables were positively skewed and demonstrated substantial kurtosis. Log transformations resulted with approximately normal distributions. Log transformations are considered common practice when dealing with non-normally distributed hormone data (Sollberger & Ehlert, 2016). Anthropometric measures demonstrated adequate normality; BMI and waist circumference showed minor positive skew however this was not a sufficient deviation from normality that warranted transformation or non-parametric analysis.

#### *Analytical approach*

Pearson Correlations were conducted across complete data (list wise deletion). Sex differences were analyzed by independent samples comparisons. Differences across hair segments were analyzed through paired samples t-tests. As a preliminary study within a novel area with a high number of exploratory comparisons where independence of associations was unlikely (hormones and anthropometrics) (Keppel & Wickens, 2004; Narum, 2006), a modified False Discovery Rate (FDR) correction was used (Benjamini & Yekutieli, 2001). For 51 possible comparisons between hormones within and across hormone segments, anthropometrics, and sex an adjusted p-value of  $p=.011$  was used.

### **3. Results**

Analyses indicated adequate normality, linearity, and homoscedacity for log transformed hair variables. For ease of interpretation, both raw and log-transformed values are presented in Table 1 and 2, however transformed variables were used in analyses. First, associations between cortisol, testosterone, and DHEA were analyzed (see Table 3). For the 0-3cm segment, testosterone strongly correlated with DHEA, and moderately correlated with cortisol, while DHEA and cortisol were not correlated. In the 3-6cm segment ( $n=35$ ; see Table 3) the only identified association was a strong correlation between testosterone and DHEA (all  $p$ 's  $< .001$ ).

Correlations across hair segments revealed strong correlations within each hormone (see Table 3). Furthermore, DHEA in the 0-3cm segment positively correlated with testosterone in the 3-6cm segment, and testosterone in the 0-3cm segment was positively correlated with DHEA in the 3-6cm length (see Table 3). Finally, DHEA in the 0-3cm segment showed a weak negative correlation with cortisol in the 3-6cm segment, however this relationship was not significant ( $p > .011$ ).

Paired sample comparisons across the segments demonstrated significantly higher hormone concentrations in the 3-6cm lengths compared with the 0-3cm lengths for cortisol  $t(36)=-5.961$ ,  $p < .001$ , and DHEA  $t(36)=3.989$ ,  $p < .001$ , but no difference for testosterone  $t(36)=.0102$ ,  $p = .687$ .

Four anthropometric measures were assessed - waist, height, weight, and BMI. Parametric analyses identified a weak positive association between DHEA (0-3cm) and height ( $r=.223$ ,  $p=.017$ ) (see Table 3). The relationship between DHEA and height became significant after controlling for the effect of age in days ( $r=.260$ ,  $p=.005$ ). While age was an important covariate, evaluation of interaction effects showed that age did not moderate the association between DHEA and height, for regression values and a visual depiction of this interaction please refer to the supplementary materials.

Independent t-tests indicated sex differences in hormone levels in the 0-3cm, where all hormone levels in hair were higher in males compared to females, see Table 2.

#### **4. Discussion**

In this study we examined hair as biosample for measurement of basal levels of DHEA and testosterone, and relationships with cortisol, using ELISA in an adrenarcheal child sample. First, a positive association between levels of DHEA and testosterone was predicted based on the developmental stage of our sample and prior studies using saliva and blood samples. This hypothesis was supported in the proximal (0-3cm) hair segment. Second, it was predicted that the association between DHEA and testosterone would be consistent across hair segments (only tested in girls). This was also supported in the distal (3-6cm) hair segment, as well as across segments (i.e., proximal testosterone correlated with distal DHEA, and vice versa). Furthermore, there were strong correlations for each hormone across the proximal and distal sections. Third, it was predicted that hormone levels would be lower in the distal (3-6cm) segment of hair (only tested in girls). Unexpectedly, testosterone levels were stable while cortisol and DHEA levels were *higher* in the distal segment. Fourth, hair hormone levels were predicted to relate to anthropometric indices. Although this hypothesis was supported, only one association between DHEA and height was identified. Finally, sex differences were identified where all hormone levels were higher in males compared with females (only tested in 0-3cm segment). Overall, this is the first study to examine levels of testosterone and DHEA in hair within a childhood sample using the ELISA assay method, and has replicated previously identified positive relationships between testosterone and DHEA in other blood and saliva.

The levels of cortisol reported in this study (2.53pg/mg, median) were lower than values reported in one younger pre-school childhood sample 20pg/mg (Vaghri et al., 2013), but consistent with another study of children of a similar age (3.51pg/mg) utilizing the same methods and laboratory for assays (Simmons, Badcock, et al., 2015). Salivary cortisol levels have been shown to remain stable from early to middle childhood (Kiess et al., 1995), while

HCCs have been shown to decline (from 1, 3, 5 and 8 years old; (Karlén, Frostell, Theodorsson, Faresjö, & Ludvigsson, 2013). Levels of testosterone (.5pg/mg) were lower than, while levels of DHEA (3.53pg/mg) were comparable with, Grotzinger, Briley, et al. (2018) (testosterone 2.45pg/mg and DHEA 3.89pg/mg). This difference is important, in that Grotzinger, Briley et al. utilized LC-MS/MS and reported undetectable levels of testosterone (28.5%) and DHEA (9.9%), which was associated with younger age and earlier pubertal status. Their cohort ranged in age between 7.80 and 19.47 years ( $M = 12.34$ ,  $SD = 2.77$ ), suggesting levels were undetectable at the lower end of this age range, which is specifically around the age of the cohort examined in the current study (i.e., an adrenarcheal, pre-gonadarcheal cohort). The relatively poor sensitivity of LC-MS/MS approaches has been previously noted for cortisol, in a study directly comparing LC-MS/MS and ELISA assays (Slominski et al., 2015)<sup>4</sup>.

As expected for this developmental period testosterone and DHEA were strongly and positively associated (Matchock et al., 2007). This pattern of association, combined with the age of this sample (approximately 8 years), is consistent with an adrenarcheal profile that is yet to undergo gonadarche, where DHEA levels increase and testosterone is primarily produced via peripheral conversion of DHEA (Matchock et al., 2007; Rege & Rainey, 2012; Soma, Rendon, Boonstra, Albers, & Demas, 2015; Styne & Grumbach, 2011). This is an important, if basic, validity check for hair hormone indices for DHEA and testosterone, and replicates previous findings with other biosamples in children pre-gonadarche. Consistent with prior literature (Matchock et al., 2007), there was no association between DHEA and cortisol, reflecting divergent patterns of secretion between cortisol with androgens (i.e., DHEA) during adrenarche (Rege & Rainey, 2012). Surprisingly, cortisol and testosterone were positively related in the proximal, but not distal hair segment. The positive association between cortisol and testosterone has mixed support within literature that has used salivary indices shown by positive coupling<sup>5</sup> during late childhood (Ruttle et al., 2015), positive covariation for females during adolescence (Simmons, Byrne, et al., 2015), and a negative association during middle childhood to middle adolescence (Matchock et al., 2007). This relationship, however, was not consistent across hair segments, reflecting that this may not be a strong association and thus requires further study.

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<sup>4</sup> Slominski et al. (2015) provide methods for a hair hormone extraction technique that results in significantly higher yields than other methods, and should be considered in future work.

<sup>5</sup> Coupling refers to the relationship between hormones within a person across several time-points.

Across hair segments, the strong association between testosterone and DHEA was consistent, however the association of cortisol with testosterone was not. Furthermore, only hormones in the proximal hair segment (0-3cm) were associated with anthropometric measures. It is possible that weaker associations, such as the relationship between cortisol and testosterone, and between hormones with anthropometrics, were only detectable with greater power from the larger sample size available for the proximal segment (n= 114 male and female participants versus n=35 female participants). Instead, this finding may reflect decreased reliability of measurement down the hair shaft beyond 3cm (Stalder et al., 2017), potentially due to increased influence from extraneous factors, such as sweat. Alternatively, this may relate to hair washing frequency (Rippe et al., 2016), however this is unlikely where a recent review found that over 80% of research studies failed to identify a significant effect of hair washing frequency on hair cortisol concentrations (Gray et al., 2018). Unexpectedly, cortisol and DHEA levels were higher in the distal segment compared with levels in the proximal segment. This finding is not consistent with prior literature on hair cortisol that found a reduction in cortisol levels from 0-3cm to 3-6cm in a meta-analysis in both teenage and adult samples (Stalder et al., 2017), or no difference in an adult sample (Manenschijn, Koper, Lamberts, & van Rossum, 2011). Strong associations between hormone levels across hair segments (i.e., 0-3cm cortisol with 3-6cm cortisol) were identified, consistent with prior research on rhesus macaques (Davenport, Tiefenbacher, Lutz, Novak, & Meyer, 2006). We are not aware of previous studies examining cortisol (or other hormone) levels along hair segments in children, therefore developmental, lifestyle, and assay between the age groups may influence the outcome. Moreover, the results are based on a small sub-sample of female participants (n=35) with a novel approach to multiple hormone assay of hair samples, and therefore conclusions should be tentative until results are replicated. Future studies might examine these relationships in a larger sample including boys and girls, while assessing other potential covariates such as age, puberty, and activity levels.

In a novel contribution to the literature, we found an association between DHEA and height. This may reflect the role of DHEA in development and maturation during childhood, particularly adrenarche. Premature adrenarche has been positively associated with androgens including testosterone and DHEA (Ibanez, Dimartino-Nardi, Potau, & Saenger, 2000), height (Leung et al. 2008), and waist circumference (Ibanez et al., 2003). It is therefore possible that the positive association between DHEA and height may reflect the role of DHEA in development. Further supported by the finding that the association between DHEA and height strengthened after the addition of age as a covariate. In contrast, other research has

found no association between DHEA in saliva and anthropometrics from middle childhood to early adolescence (Bond, Vella, Kiparissis, & Wynne-Edwards, 2006). It should also be noted that the previously shown positive association between hair cortisol and waist circumference (Veldhorst et al., 2014) and BMI (Rippe et al., 2016) was not replicated in this sample. It is possible that as a healthy sample with a restricted age range there was limited variability in maturational indices, where prior research focussed on obese children across a wider developmental range (8-12 years) (Veldhorst et al., 2014), or included widely diverse and large samples (n=2, 484) (Rippe et al., 2016). While the restricted age range allowed for the evaluation of hormones during a restricted developmental window, it meant that we were not able to evaluate developmental differences likely to affect hormone levels (or visa versa). Therefore, further research measuring these associations with hormone levels in hair is required.

Sex differences were identified, in which hormone levels were higher in males compared to females. Higher HCCs in males is consistent with prior research using LC-MS/MS (Rippe et al., 2016) and ELISA (Simmons, Badcock, et al., 2015). Higher levels of DHEA and testosterone in males is inconsistent with prior salivary research on a sample from middle childhood to early adolescence (Matchock et al., 2007). It is possible that males were more advanced in adrenarcheal development than the females in this sample, however there were no differences in age between the sexes, and females are typically more pubertally advanced than males of the same age (Rosenfield, Lipton, & Drum, 2009). Sex differences here may not be due to underlying biological differences, but rather exogenous integration of hormones, such as variation in exercise and sweat (Kirschbaum et al., 2009; Pragst & Balikova, 2006). Alternatively, contrasting sex differences across hair and salivary indices may suggest that each measure indicates different aspects of endogenous hormone levels. The cause of this difference remains unknown, indicating the need for within-study comparisons between methods, recognising developmental factors and sex.

There are several limitations to this study that should be considered. First, inconsistent with expectations and prior research strong associations between hormones (particularly cortisol) and anthropometric measures were not identified. It is possible that the inability to identify further relationships between androgens and developmental indices (anthropometrics) reflects poor validity of the hair hormone index used in this study. This explanation however, is unlikely given that we identified several associations consistent with the developmental stage of this sample. Such associations include: the identification of higher HCCs in boys, comparable HCC levels to prior study samples of a similar age range, and the

positive association between DHEA and testosterone. Alternatively, it is possible that the inability to identify more associations between androgens and developmental indices was due to the limited variability in developmental indices due to the restricted age range of this sample. Another limitation was the small sub-sample of female participants (n=35) used to compare across hair segments 0-3cm and 3-6cm. Consequently findings are limited in their extension and power, requiring further investigation. Finally, it should be recognised that the sample was recruited from lower SES areas within the Melbourne Metropolitan area. Although no relationships were identified with status above or below the poverty line, there are other ways of measuring SES and statistically modelling its effects. Hormones and development can be influenced by the surrounding environment where lower SES status is related to higher HCCs (Essex, Klein, Cho, & Kalin, 2002; Marsman et al., 2012; Palmer et al., 2013) and body composition (increased obesity) in childhood (Danielzik, Czerwinski-Mast, Langnase, Dilba, & Muller, 2004). These relationships warrant further investigation in future studies.

## **5. Conclusions**

This is the first study that examines DHEA and testosterone in children's hair utilising the ELISA methodology. A preliminary assessment of the validity of these hair measures was conducted via the identification of hormone associations that were reflective of the developmental stage of our sample, as well as the ability to show comparable levels of cortisol with prior research, and an association with anthropometric measures. This study represents a first step toward validating hair as a measure for endogenous levels of DHEA and testosterone, and demonstrates that hair has the potential to be a useful index of cortisol, DHEA, and testosterone levels in the study of behavior, development and psychopathology, and particularly in an adrenarcheal (and pre-gonadarcheal) sample. Further validation of hair hormone indicators can be achieved via within-study comparisons between methods of hormone extraction from hair (ELISA versus LC-MS/MS), as well as comparisons between hormone concentrations in hair with widely used measures, such as saliva and blood. A focus on validating stress and developmental hormones measured by hair would prove useful in further developing the field of psychoneuroendocrinology research.

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Table 1. Descriptive statistics for hormones in hair *untransformed* (0-3cm n=114, 3-6cm n=35), cortisol (cort), testosterone (TST), DHEA (DHEA), in pgmg, averaged across sex

	Min	Max	Mean	Median	SD
Cort (0-3)	0.46	17.99	4.21	2.52	4.09
TST (0-3)	0.18	1.79	0.50	0.40	0.33
DHEA (0-3)	0.71	15.93	3.53	2.35	3.38
Cort (3-6)	0.75	16.88	0.53	3.93	3.91
TST (3-6)	0.13	0.76	0.36	0.30	0.17
DHEA (3-6)	0.59	9.89	3.55	2.96	2.16

*Table 2.* Descriptive statistics for variables used in analyses, untransformed hormones in pg/mg, log transformed hormones in hair  $\text{pgmg}^{-1}$  (0-3cm n=114; 3-6cm n=35), cortisol (cort), testosterone (TST), DHEA (DHEA), and anthropometric measures, averaged across and separated by sex

	Mean (SD)	Mean (SD) Males	Mean (SD) Females	Mean Diff.
Cort (0-3)	0.47 (0.35)	0.59 (0.38)	0.39 (0.31)	0.20**
TST (0-3)	-0.37 (0.23)	-0.25 (0.21)	-0.45 (0.21)	0.20***
DHEA (0-3)	0.42 (0.31)	0.53 (0.32)	0.35(0.27)	0.14***
Cort (3-6)	0.64 (0.34)		0.63 (0.4)	
TST (3-6)	-0.48 (0.18)		-0.47 (0.19)	
DHEA (3-6)	0.47 (0.26)		0.47 (0.27)	
Height	132.8 (6.10)	133.02 (6.50)	132.64 (5.83)	0.38
Weight	30.17 (5.42)	29.75(5.27)	30.47 (5.54)	-0.71
Waist	60.7 (6.63)	60.67 (6.70)	60.82 (6.61)	-0.15
BMI	17.02 (2.55)	16.76 (2.39)	17.23 (2.43)	-0.47
Age days	3101.33 (120.71)	3090.27 (123.08)	3109.36 (119.26)	-19.09

\* significant at the 0.05 level (2-tailed), \*\* significant at the 0.01 level (2-tailed),

\*\*\*significant at a .001 level (2-tailed).

Table 3. Pearsons correlations between cortisol (cort), testosterone (TST), DHEA (DHEA), 0-3cm (n=114) and 3-6cm (n=35) segments, log transformed, and anthropometrics (height, waist, weight, BMI)

	1	2	3	4	5	6	7	8	9	10
1. l_cort (0-3)	-									
2. l_TST (0-3)	.366**	-								
3. l_DHEA (0-3)	.024	.544**	-							
4. l_cort (3-6)	.605**	-.303	-.405*	-						
5. l_TST (3-6)	.049	.657**	.558**	.152	-					
6. l_DHEA (3-6)	-.202	.582**	.760**	-.147	.778**	-				
7. Height	.047	.171	.223*	-.240	.202	.250	-			
8. Weight	.121	.130	.061	-.175	.118	.146	.618**	-		
9. Waist	.148	.041	-.052	.015	.070	.084	.364**	.876**	-	
10. BMI	.121	.054	-.055	-.047	.014	.019	.159	.871**	.884**	-

\* significant at the 0.05 level (2-tailed), \*\* significant at the 0.01 level (2-tailed), \*\*\*significant at the .001 level (2-tailed)