English Longitudinal Study of Ageing

Biomarker assays from hair

Background

The English Longitudinal Study of Ageing (ELSA) includes measurement of a number of biomarkers from blood such as cholesterol, C-reactive protein, fibrinogen, haemoglobin, ferritin and glycated haemoglobin (see http://www.elsa-project.ac.uk/). There are, however, several potentially interesting biomarkers that fluctuate diurnally so cannot be accurately assessed from single blood samples, or which have not previously been measured in ELSA. Hair has emerged as a valuable source of biomarker data over recent years, since it provides information about secretion of hormones and other substances integrated over several weeks (Russell et al., 2012; Stalder and Kirschbaum, 2012). While much of the research to date has been carried out on cortisol, other substances such as cortisone, dehydroepiandosterone (DHEA), melatonin, testosterone and progesterone can be assayed and are informative.

Data collection procedure

Hair samples were collected in wave 6 (2012/13) of ELSA during the home visits that study nurses made to participants. Details of the procedure followed by nurses can be found in Appendix 1 (abstracted from the nurse instructions at http://www.elsa-project.ac.uk/). The characteristics of the participants who provided hair samples and the reasons why samples were not collected from some individuals can be found in the ELSA Wave 6 nurse data file available from the UK data service (https://www.ukdataservice.ac.uk/, accession GN 33368). Briefly, participants were excluded if they were breast feeding, had a scalp condition that prevented collection of the sample, were unable to sit with head remaining still, or had less than 2cm hair at the posterior vertex. Of the 8,054 participants in the wave 6 nurse assessment, 1,989 were excluded for these reasons, leaving 6,061. There were 555 refusals, and hair could not be obtained from a further 55, resulting in 5,451 with hair samples. Steroid hormones were successfully extracted from 5,328 individuals. Note that this includes a small number of non-core ELSA participants such as partners aged less than 50. The ELSA Wave 6 nurse data file also includes information about hair colouring, hair dying, and chemical treatments. It is recommended that these variables are considered as potential confounders in all analyses.

Hormone assays

Samples were stored at room temperature before transfer to the laboratory at the Technische Universität Dresden (Germany), under the direction of Professor Clemens Kirschbaum. The steroid hormones were assayed using high performance liquid chromatography-mass spectrometry (LC/MS) following a standard wash and steroid extraction procedure (Gao et al., 2013). The inter- and intra-assay variation was around 15% for all hormones.

Hormone extraction was carried out in two phases:
Phase 1, 2015, n = 2,695
Phase 2, 2018, n = 2,633
Total n = 5,328
It is recommended that analyses of these data include assay phase as a covariate.

Six steroid hormones were measured in pg/ml

Hormone	Detectable	Undetectable	Missing	
Cortisol	4,911	0	417	
Cortisone	5,263	0	65	
DHEA	1,316	1,369	9	assessed in phase 1 only
Melatonin	891	1,788	16	assessed in phase 1 only
Testosterone	1,234	4,094	0	
Progesterone	3,069	2,259	0	

Hair weight
The file includes a variable weight_mg that indicates the weight of the hair sample. Hair weight was 7.50 mg for 5,184 (97.6%) of respondents. Samples with a hair mass <4.0 mg may become inaccurate. The target length of hair for steroid extraction was 3 cm most proximal to the head. Longer strands of hair were therefore cut to 3 cm before analysis.

Data distribution and trimming
Values for all hormones are negatively skewed, owing to a few very high values that are not physiologically credible. There are as yet no norms and ranges for these hair hormones in the age range of ELSA participants. The ELSA research group at UCL has employed the following criteria, but other investigators may prefer different methods:

Hormone	Exclusion of high values	Log transformation
Cortisol	Yes, >660.0 pg/ml	✓
Cortisone	No	✓
DHEA	Yes, >150.0 pg/ml	✓
Melatonin	Yes, >6.00 pg/ml	✓
Testosterone	Yes, >50.0 pg/ml	✓
Progesterone	Yes, >100.0 pg/ml	✓

References

Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., Kirschbaum, C., 2013. Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. J Chromatogr B Analyt Technol Biomed Life Sci 928, 1-8.

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