Psychoneuroendocrinology (2008) 33, 1257—1268

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Levels and confounders of morning cortisol collected from adolescents in a naturalistic (school) setting

Shona J. Kelly [a,*], Robert Young [b,1], Helen Sweeting [b,1], Joachim E. Fischer [c,2], Patrick West [b,1]

[a] Division of Epidemiology and Public Health, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2RD, UK
[b] MRC Social & Public Health Sciences Unit, 4 Lilybank Gardens, Glasgow G12 8RZ, UK
[c] Mannheim Institute of Public Health, Mannheim Medical Faculty, University of Heidelberg, Ludolf-Krehl-Strasse 7-11, D-68167 Mannheim, Germany

Received 9 April 2008; received in revised form 27 June 2008; accepted 28 June 2008

KEYWORDS
Hydrocortisone;
Adolescent;
Saliva;
Stress;
Cross-sectional studies;
Social environment

Summary Salivary cortisol is widely used in research but little is known about the typical, or expected, functioning of the HPA-axis in adolescents in naturalistic settings, nor whether the extensive array of confounders documented in the literature is applicable in this situation.

In a school-based study, 2995 15-year-old pupils provided two saliva samples, 30 min apart, in morning sessions timed to capture peak cortisol decline. The collection protocol was a balance between the large sample size obtainable in a school situation and a limited number of samples, constrained by the school timetable. In addition, pupils completed a questionnaire containing items previously shown to be associated with cortisol levels (e.g. time since awakening and life events), and their height and weight were measured. Outcome measures were cortisol levels at Times 1 and 2, and change (per minute) in cortisol between the two time points.

Median (IQR) cortisol levels for males and females were 10.5 (8.1) and 11.6 (9.3) nmol/L at Time 1, and 8.2 (6.0) and 8.1 (6.5) nmol/L at Time 2. 73% had a decline in cortisol level of more than 10% across the two time points, compatible with the expected diurnal pattern. In bivariate analyses, cortisol sampled on Monday, times of measurement and since awakening, prior smoking and several life events were associated with cortisol levels at Times 1 and 2 in both sexes. However, in multivariate analysis, few of these variables remained after controlling for times of measurement and since awakening and, in addition, the final models differed between the sexes. Two events (friend dying and splitting with a boy/girlfriend) predicted cortisol levels in both sexes while age, maturity, recent eating and smoking were predictors only in males. Several factors associated with cortisol change differed from those observed for absolute levels. Further adjustment for school clustering affected some associations, particularly time of measurement.

* Corresponding author. Tel.: +44 115 823 0449; fax: +44 115 823 0464.
E-mail addresses: shona.kelly@nottingham.ac.uk (S.J. Kelly), robert@sphsu.mrc.ac.uk (R. Young), helen@sphsu.mrc.ac.uk (H. Sweeting), jfischer@medma.uni-heidelberg.de (J.E. Fischer), patwest@sphsu.mrc.ac.uk (P. West).
[1] Tel.: +141 357 3949.
[2] Tel.: +49 621 383 9910; fax: +49 621 383 9920.

0306-4530/$ — see front matter
doi:10.1016/j.psyneuen.2008.06.010

This study managed many of the problems found in naturalistic research on cortisol and provides norms for morning cortisol levels in 15-year-old adolescents.

1. Introduction

With the advent of its measurement in saliva, cortisol has become widely used in research on stress. However, with a few notable exceptions (e.g. Rosmalen et al., 2005; Power and Hertzman, 2006) studies of cortisol have been conducted on relatively small samples, often including fewer than 100 individuals. In addition, there are little data on whether measured levels are within the expected (i.e. 'normal') range for community-living people (Patel et al., 2004). Despite a number of studies in relation to cortisol and adolescent depression, anxiety or chronic fatigue (e.g. Goodyer et al., 1998), it has been suggested that ''little is known about the typical or expected functioning of the HPA-axis in adolescents. This lack of research on cortisol activity in normal adolescents in their everyday environments is surprising'' (Adam, 2006).

While the assessment of salivary cortisol has been well validated (e.g. Kirschbaum and Hellhammer, 2000), and is suitable for large-scale studies (Rosmalen et al., 2005), it is complicated by a quirk of cortisol secretion; namely, that in addition to responding to stressors, cortisol follows a daily circadian rhythm in most people. The lowest levels generally occur around midnight and usually begin to rise before waking, continuing for 30—45 min thereafter (Schmidt-Reinwald et al., 1999; Wust et al., 2000), with a daily peak between 05:00 h and 08:00 h. This is followed by a rapid decline for the next few hours, then a gradual decline over the remainder of the day (Kirschbaum and Hellhammer, 1989). Although one approach to this variation within naturalistic settings is to collect repeated measures and use the mean, this is very crude and fails to capture changes in the daily circadian rhythm (Hruschka et al., 2005). A range of more sophisticated methods have therefore been used to characterise this, including indices of the slope of the decline, assessments in the morning and evening, and area under the curve measures (Ranjit et al., 2005).

Previous studies, focusing on either natural decline or reactivity, have identified a number of factors likely to impact on cortisol levels collected in the field setting. Firstly, because of its circadian pattern, time of day and time since awakening have a significant effect on measured levels (Kudielka and Kirschbaum, 2003). Secondly, studies have found the morning pattern to differ between males and females. Compared with men, women show a delayed decrease (Pruessner et al., 1997), and have higher morning levels (Steptoe et al., 2000). Morning salivary cortisol levels have also been found to be higher in girls, particularly post-pubertally, than boys (Netherton et al., 2004; Rosmalen et al., 2005). A third factor is day of the week, in respect of which there have been inconsistent findings. Thus, while some studies of working adults have found increased cortisol excretion (Maina et al., 2007) on work compared with weekend days, others have found no differences (Vidovic et al., 2007). A fourth possible factor is age and body size. However, although there is some evidence of positive associations between cortisol levels and age, body mass and pubertal stage in children and adolescents (Tornhage, 2002), results are inconclusive (Rosmalen et al., 2005; Netherton et al., 2004). In addition, there are a number of activities and states which have been found to impact on cortisol levels. These include the use of corticosteroid medication (Ricciardolo, 2007), eating (Rosmond et al., 2000), caffeine in dietary doses (Lovallo et al., 2005), smoking (Kirschbaum et al., 1992; Steptoe and Ussher, 2006), exercise (Ben-Aryeh et al., 1989; Filaire et al., 2001; Urhausen et al., 1995), and the experience of aggression, stress, distress and negative emotion (Haller et al., 2005; Adam, 2006). Serious physical illness also results in increased cortisol levels (Van den Berghe et al., 1998). Finally, levels are increased among those reporting work strain or more general stress (Steptoe et al., 2000), although results in respect of the relationship between life events and cortisol are inconsistent. Thus, while some studies find life events to be associated with higher cortisol levels (Strickland et al., 2002), others find no relationship (Goodyer et al., 1998).

Against this background, this paper is based on a study of 2995 adolescents (age 15), who provided two samples in a school setting, approximately 30 min apart, starting around 09.00 h, thus capturing the period of most rapid daytime decline. The aims are: (1) to present salivary cortisol levels among a large sample of community-living adolescents; and (2) to examine differences in respect of factors previously found to be associated with salivary cortisol measures. In addition, we describe our methodology for collecting samples in a naturalistic environment.

2. Methods

2.1. Study participants and procedures

Data are drawn from a survey conducted among pupils within 22 schools situated in the Central Clydeside Conurbation, a predominantly urban area in and around Glasgow city in the West of Scotland. The study received approval from the relevant Glasgow University Ethics Committee, participating local authorities and schools. The sampling scheme aimed to obtain a representative sample by selecting schools within strata based on geographical location (within Glasgow City or not), religious status (Catholic/Non-denominational) and deprivation (represented by proportion of pupils in receipt of a clothing grant). Subsequent analyses found that participating schools did not differ significantly from the remainder in the Central Clydeside Conurbation in respect of these dimensions, nor for total pupil roll or exam achievement by the end of statutory schooling (Sweeting et al., 2008). Within selected schools, all pupils in (Scottish) Secondary 4, the final statutory year of schooling, were invited to participate via letters sent to parents, including opt-out consent forms. In addition, participating pupils positively consented to all measures.