Psychoneuroendocrinology 66 (2016) 118–124

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Hypothalamic-pituitary-adrenal axis activity under resting conditions and cardiovascular risk factors in adolescents

Chi Le-Ha[a,*], Carly E Herbison[b], Lawrence J Beilin[a], Sally Burrows[a], David E Henley[a,c], Stephen J Lye[d], Stephen G Matthews[e], Craig E Pennell[b,1], Trevor A Mori[a,1]

[a] School of Medicine and Pharmacology, University of Western Australia, Australia
[b] School of Women's and Infant's Health, University of Western Australia
[c] Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Australia
[d] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada
[e] Department of Physiology, University of Toronto, Canada

ARTICLE INFO

Article history:
Received 22 March 2015
Received in revised form
29 November 2015
Accepted 5 January 2016

Keywords:
HPA axis activity
Cortisol
Corticosteroid binding globulin
Cardiovascular risk factors
Adolescence

ABSTRACT

Background: Activation of the hypothalamic-pituitary-adrenal (HPA) axis has been associated with higher levels of cardiovascular (CVD) risk factors in adults. This study aimed to assess the relation between measures of HPA axis activity under resting conditions and CVD risk factors in a general population of adolescents at 17 years.
Methods: A total of 1134 adolescents from the Western Australian Pregnancy Cohort (Raine) Study had phenotypic and socio-demographic data. The associations between HPA axis measures (plasma ACTH, total cortisol, calculated free cortisol, corticosteroid binding globulin (CBG), and salivary cortisol) and a range of cardiovascular risk factors were examined using multivariable linear regression models, with adjustment for gender, adiposity, birth weight, gestational age, and socio-behavioural factors.
Results: Plasma total cortisol was positively associated with systolic blood pressure (SBP) ($p = 0.011$), total cholesterol, HDL-cholesterol, and triglycerides (all $p < 0.001$), and hs-CRP ($p = 0.047$). Salivary cortisol was associated positively with HDL-C ($p = 0.033$) and negatively with LDL-cholesterol ($p = 0.016$); plasma calculated free cortisol was positively associated with triglycerides ($p = 0.006$); plasma CBG was positively associated with total cholesterol and HDL-cholesterol (both $p < 0.001$), LDL-cholesterol ($p = 0.022$), and hs-CRP ($p = 0.001$). After correction for multiple comparisons, significant associations remained for total cortisol with total cholesterol, HDL-C, and triglycerides; for calculated free cortisol with triglycerides; and for CBG with HDL-C, total cholesterol, and hs-CRP. Plasma ACTH was not associated with any cardiovascular risk factor. There was no association between BMI and any measure of HPA axis activity.
Conclusion: In an adolescent population, HPA axis measures under resting conditions are associated with a range of CVD risk factors. Clarification of the mechanisms underlying these associations in adolescence would be an important step in understanding the evolution of adult CVD.

1. Introduction

Heightened hypothalamic-pituitary-adrenal (HPA) axis activity has been considered a risk factor for cardiovascular disease (CVD) (Hamer et al., 2010). Altered HPA axis function may adversely affect the cardiovascular system by promoting atherosclerosis, resulting in a higher risk of CVD (Brotman et al., 2007). Cortisol, the end product of the HPA axis, may have a relevant role in cardio-metabolic regulation through its effects on inflammatory mechanisms (Walker, 2007). In adults, HPA axis hyperactivity has been associated with elevated blood pressure, insulin resistance, dyslipidaemia, and higher levels of central adiposity (Anagnostis et al., 2009; Rosmond and Björntorp, 2000). Inflammatory processes, which underlie the pathogenesis of atherosclerosis, have been linked to HPA axis activation (Turnbull and Rivier, 1999). In this respect, higher levels of the inflammatory marker IL-6 have been associated with a less pronounced cortisol awakening response and a less steep cortisol daily decline (DeSantis et al., 2012). Population data in adults have demonstrated that elevated plasma cortisol increases the prevalence of ischaemic heart disease (Reynolds et al., 2010); high long-term cortisol levels in hair are related to a history of CVD (Manenschijn et al., 2013); and high

* Corresponding author at: Royal Perth Hospital Unit, School of Medicine and Pharmacology, GPO Box X2213, Perth, 6847, Australia. Fax: +61 8 92240243.
E-mail address: cleha@meddent.uwa.edu.au (C. Le-Ha).
[1] Equal senior authors.

http://dx.doi.org/10.1016/j.psyneuen.2016.01.002
0306-4530/

urinary cortisol levels strongly predict cardiovascular mortality (Vogelzangs et al., 2010).

During adolescence, HPA axis maturation reflects the on-going maturation of the adolescent forebrain (McCormick and Mathews, 2010). Alterations in the activity of the biological stress systems and HPA function during this critical developmental period may have permanent effects on brain development, and further on cardio-metabolic risk (Pervanidou and Chrousos, 2012). The question arises as to whether HPA axis activity measured under resting conditions associates with an adverse cardiovascular risk profile in a general adolescent population. Reynolds et al. (2013) have previously reported in the Western Australian Pregnancy Cohort (Raine) Study adolescents, gender specific sensitivity of the HPA axis. Plasma levels of ACTH were lower, but total cortisol and corticosteroid binding globulin (CBG) were higher in females compared with males. In girls, the relative higher levels of salivary cortisol measured at awakening were significantly reduced by the use of oral contraceptives (OCs).

The present study aimed to investigate, in 17-year-old adolescents from the Raine Study, the associations between HPA axis measures of plasma ACTH, CBG, total cortisol, calculated free cortisol, and salivary cortisol, with a range of cardiovascular risk factors under resting conditions. Our *a-priori* hypothesis was that upregulation of HPA functions at 17 years of age would be associated with an adverse CVD risk factors profile including blood pressure, lipids, and the inflammatory marker C-reactive protein.

2. Methods

2.1. Study population

Participants were adolescents from the 17-year review of the Raine Study. This longitudinal study began as a pregnancy cohort, with 2900 women attending antenatal clinics and the tertiary obstetric King Edward Memorial Hospital in Perth, serially enrolled at 18 week's gestation between 1989 and 1991. Details of the cohort at birth were previously described (Newnham et al., 1993). A total of 2868 live births resulted and children were reviewed at ages 1, 2, 3, 5, 8, 10, 14 and 17. All aspects of the study were approved by the Human Ethics Committee at King Edward Memorial Hospital and/or Princess Margaret Hospital in Perth. The adolescents and their parents or guardian provided written informed consent for data collection.

At the 17-year review of the Raine Study, 1248 adolescents completed comprehensive questionnaires for socio-behavioural information, including nutrition, physical activity, smoking, alcohol consumption, OC use in females and drug use. Participants underwent assessments for height, weight, body mass index (BMI); cardiorespiratory fitness; blood pressure and arterial stiffness; and had fasting blood collected for biochemical analyses. At 17 years of age, Raine Study adolescents were representative of the current Western Australian population (Robinson et al., 2010). The participants with completed data for analysis in the present study were comparable to non-participants from the original cohort, with respect to age, gender, birth weight, gestational age, family income, and maternal smoking and alcohol consumption (Le-Ha et al., 2013a,b).

2.2. Assessments

2.2.1. Hormonal

The details of the protocol for hormone measures and analysis have previously been described by Reynolds et al. (2013). In brief, a fasting blood sample (no food or beverage except water was allowed from 10:00pm the night before) was obtained by a phlebotomist under non-arousing conditions during a home visit in the morning before 10:00am. On three consecutive weekdays, the adolescents used Salivette saliva collection tubes (Sarstedt, Germany) to obtain saliva 15 minutes after spontaneous awakening, and recorded date and time of collection. The saliva samples were stored at −80 °C until analysis.

Measurements of plasma total cortisol and CBG were carried out by ^{125}I radioimmunoassay (GammaCoat cortisol RIA – DiaSorin, MN USA and CBG RIA 100–BioSource Europe S.A., Belgium). Plasma ACTH was measured by ^{125}I immunoradiometric assay (ACTH-IRMA, DiaSorin, MN, USA). All samples were assayed in duplicate, and analyses were repeated for sample duplicates that differed by >20%. Multiple saliva samples of each adolescent were analysed in the same assay. Plasma free cortisol was calculated using Coolen's equation: $U = \sqrt{(Z^2 + 0.0122T)} - Z$, where $Z = 0.0167 + 0.0182\,(G - T)$. In this formula, U, G and T represent free cortisol, CBG and total cortisol, respectively (Coolens et al., 1987).

2.2.2. Clinical, biochemical, and anthropometric

Fasting serum glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and high sensitivity C-reactive protein (hs-CRP) were analysed in the PathWest Laboratory at Royal Perth Hospital. Homeostasis model of assessment for insulin resistance (HOMA-IR) was computed using the equation insulin (mU/L) X glucose (mmol/L)/22.5. Supine systolic (SBP) and diastolic (DBP) blood pressure were measured on a different day to venipuncture for blood samples. After 5 minutes of quiet rest, SBP and DBP were recorded using an oscillometric sphymomanometer (Dinamap Pro Care 100; Soma Technology, Bloomfield, Connecticut) with an appropriate cuff size. The average of the last 5 of 6 sequential BP readings was calculated. A Holtain Stadiometer was used to measure height (nearest 0.1 cm), and a Wedderburn Chair Scale to measure weight (nearest 100 g). Pubertal development was estimated with self-reported Tanner stage. Birth weight (g) was obtained from the participant's medical records. Gestational age (weeks) was calculated from the date of the last menstrual period, unless there was discordance of more than 7 days with ultrasound measurements as of less than 18 weeks; in those cases, the estimate was based on ultrasound biometry at 18 weeks gestation.

2.2.3. Socio-behavioural

The adolescents' relevant socio-behavioural information was obtained via a computer-based questionnaire. The number of cigarettes consumed each day in the last 7 days was calculated from the questions: "Have you ever smoked cigarettes in the past 12 months?" and "Have you smoked cigarettes in the past 4 weeks." Participants were also asked about the amount (can, glass, stubby, nip, or standard drink) and type of alcoholic beverage (beer, wine or spirits) consumed daily in the past week. An alcohol drinker was defined as consuming any alcohol during the last 7 days. OC use in girls was recorded from the question "In the last 6 months, have you taken any prescription medication(s), e.g., the Pill?" (if yes, "which medication(s), and are you still taking it?"). Two dietary patterns, "Healthy" and "Western", were identified from a 212-item food frequency questionnaire using factor analysis with varimax rotation, and scores for each pattern were generated (Ambrosini et al., 2009). Annual family income was categorised as Australian dollars ≤$35000, $35001 to ≤$78000, and >$78000. To assess cardiorespiratory fitness, the PWC $_{170}$ aerobic fitness test was used; the test predicts physical working capacity at a heart rate of 170 beats per minute, and has been validated in this adolescent population (Hands et al., 2009).