THE INFLUENCE OF GENDER AND LIFESTYLE ON CARDIOVASCULAR RISK FACTORS IN LATE ADOLESCENCE

A thesis presented for the degree of
Doctor of Philosophy
of The University of Western Australia

by

Chi Le-Ha

Doctor of Medicine,
Physician Fellow of the Royal College of Physicians and Surgeons of Glasgow

School of Medicine and Pharmacology
Royal Perth Hospital Unit
2014
Declaration

I declare that the work presented in this thesis is my own. The thesis has been substantially accomplished during my enrollment at the University of Western Australia, and no part of it has previously been submitted for any other academic degree in this or another institution. I have designed the studies in this thesis, developed research questions, prepared the datasets and conducted data analysis, interpreted the results, and written all chapters of this thesis, with the guidance from my academic supervisors Professor L J Beilin and Professor T A Mori, and with statistical advice from Mrs S Burrows. Where appropriate, the relevant authors whose published works have been referred to in this thesis are clearly acknowledged.

This thesis includes my published papers and a manuscript in preparation for publication; all have been co-authored. I have detailed the contribution of each co-author in the section ‘Publications, Presentations, and Awards.’

____________________________________________
Chi Le-Ha, candidate

____________________________________________
Professor Trevor A Mori, coordinating supervisor


Thesis abstract

Risk factors and risk behaviours that promote the development of atherosclerotic cardiovascular disease (CVD) begin early in life. Adolescence is an important period in the developmental course of the natural history of CVD, because it is during this period that lifestyle and behaviours that may adversely affect long-term cardiovascular risk are adopted. Obesity, the use of oral contraceptives (OC) in females, and other lifestyle factors such as dietary patterns, salt intake, alcohol consumption, cigarette smoking and physical activity are known to influence blood pressure (BP) and CVD risk in adults. In addition, the harmful effects of passive smoking exposure and maternal smoking in pregnancy on cardiovascular risk later in life have been reported.

Although the influence of gender on CVD risk has been extensively studied, little is known about how gender interrelates with clinical, biochemical and behavioural risk factors in late adolescence. Adolescence is also a period of emotional development and brain plasticity, during which both lifestyle and neuroendocrine mechanisms can influence the development of cardio-metabolic disorders.

This thesis examined data from adolescents that participated in the 17-year review of the Western Australian Pregnancy Cohort (Raine) Study, a population-based pregnancy cohort. Participants were the 17-year-old offspring of pregnant women recruited between May 1989 and November 1991. I aimed to investigate (i) the relationship between lifestyle factors and BP at 17 years, with particular reference to sex differences and their interaction with adiposity; (ii) the effects of long-term passive smoking exposure since birth and maternal smoking during pregnancy on HDL-cholesterol (HDL-C) at 17 years; (iii) the influence of active smoking on high-sensitivity C-reactive protein (hs-CRP) at 17 years; and (iv) the link between basal hypothalamus-pituitary-adrenal (HPA) axis activity and cardiovascular risk factors at 17 years.

I have shown that boys had 8.97 mmHg higher systolic BP compared with girls. OC use in girls, alcohol consumption in boys, increasing body mass index (BMI) and the urinary sodium-potassium ratio were associated with higher systolic BP. I showed that there is a continuous relationship between BMI and systolic BP in both genders, but the relationship shows a steeper gradient in boys, compared with girls not using OCs.
HDL-C levels were significantly lower in girls exposed to passive smoking since birth, compared with girls not exposed to passive smoking. There was no association between passive smoking and HDL-C in boys. Unexpectedly, there was no evidence of an association with HDL-C in girls whose mother smoked in pregnancy and subsequently were exposed to passive smoking.

Active smoking at 17 years was associated with significantly higher levels of hs-CRP in girls not using OCs, but not in girls using OCs, or in boys. OC use in non-smoking girls was the strongest factor associated with higher hs-CRP levels.

In this adolescent population, I observed significant associations between basal HPA axis activity and a range of conventional CVD risk factors. Specifically, basal HPA hyperactivity was associated with higher levels of systolic BP, total cholesterol, and hs-CRP.

In summary, I have demonstrated associations between a number of behavioural and lifestyle factors, as well as altered neurobehavioural function through the biological stress systems, and an adverse CVD risk profile in adolescents at 17 years of age. These findings are important in the context that adolescence is a time when unhealthy lifestyle behaviours that influence long-term CVD risk tend to become entrenched, and also a time of critical development in emotional and neurobehavioural function that may impact cardio-metabolic risk.
Table of contents

Thesis abstract v
Table of Contents vii
Publications, presentations and awards xi
Communication originating from this thesis in major media and scientific organisations xiv
Acknowledgements xvi
List of tables xvii
List of figures xix
Abbreviations and acronyms xx

Chapter 1:
Cardiovascular risk factors in adolescence – a review of the literature 1

1.1 The developmental origins of cardiovascular health and disease 2
  1.1.1 The hypothesis 2
  1.1.2 Animal models and the developmental origins of health and disease 4
  1.1.3 A life-course perspective of cardiovascular risk 5
1.2 Major studies examining the development of cardiovascular risk in children and adolescents 7
  1.2.1 The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study 7
  1.2.2 The Bogalusa Heart Study 9
  1.2.3 The Cardiovascular Risk in Young Finn Study 10
  1.2.4 The Generation R Study 11
  1.2.5 The Western Australian Pregnancy Cohort (Raine) Study 12
1.3 Cardiovascular risk factors in adolescence 14
  1.3.1 Overweight and obesity 14
  1.3.2 Hypertension 17
1.3.3 Biochemical risk factors 20
   1.3.3.1 Dyslipidaemias 20
   1.3.3.2 Glucose and Insulin resistance 22
   1.3.3.3 Inflammatory biomarkers 23
1.3.4 Lifestyle and health behaviours 25
   1.3.4.1 Tobacco smoking 25
   1.3.4.2 Alcohol consumption 27
   1.3.4.3 Oral contraceptive use 28
   1.3.4.4 Diet and dietary nutrients 29
   1.3.4.5 Physical activity and fitness 31
1.3.5 The metabolic consequences of stress in adolescence 32
1.3.6 Sex differences in cardiovascular risk 34
   1.3.6.1 Sex differences and the CVD burden in adults 34
   1.3.6.2 The emergence of sex differences in cardiovascular risk factors in early life 36

The scope of this thesis 37

References 38

Chapter 2

Oral contraceptive use in girls and alcohol consumption in boys are associated with increase blood pressure in late adolescence 67

Abstract 68

Introduction 69

Methods 69

Results 73

Discussion 80

References 83

Chapter 3

Gender difference in the relationship between passive smoking exposure and HDL-cholesterol levels in late adolescence 90
Chapter 4
Gender and the active smoking and high-sensitivity C-reactive protein relation in late adolescence

Chapter 5
Basal hypothalamic-pituitary-adrenal axis activity associates with increased levels of cardiovascular risk factors in an adolescent population

Chapter 6
An overview of the main findings and future directions
Appendix A
The Western Australian Pregnancy Cohort (Raine) Study – Participants from birth to the 17-year review 176

Appendix B
Published papers originating from this thesis 177
Publications, Presentations and Awards

Publications originating from this thesis:

The estimated contribution of each author is indicated in parentheses.

Chapter 2

(1) Oral contraceptive use in girls and alcohol consumption in boys are associated with increased blood pressure in late adolescence
Chi Le-Ha (80%), Lawrence J. Beilin (5%), Sally Burrows (5%), Rae-Chi Huang (3%), Wendy H Oddy (1%), Beth Hands (1%) and Trevor A Mori (5%)

*European Journal of Preventive Cardiology*, December 2013, vol 20, no 6, 947-955

Chapter 3

(2) Gender difference in the relationship between passive smoking exposure and HDL-cholesterol levels in late adolescence
Chi Le-Ha (80%), Lawrence J. Beilin (5%), Sally Burrows (5%), Rae-Chi Huang (3%), Wendy H. Oddy (1%), Beth Hands (1%) and Trevor A. Mori (5%)

*The Journal of Clinical Endocrinology & Metabolism*, May 2013, vol 98, issue 5, 2126-2135

Chapter 4

(3) Gender and the active smoking and high-sensitivity C-reactive protein relation in late adolescence
Chi Le-Ha (80%), Lawrence J. Beilin (6%), Sally Burrows (6%), Wendy H. Oddy (1%), Beth Hands (1%) and Trevor A. Mori (6%)


Chapter 5

(4) Basal Hypothalamic-pituitary-adrenal Axis Activity Associates with Increased Levels of Cardiovascular Risk Factors in an Adolescent Population
Chi Le-Ha (80%), Carly E Herbison (2%), Lawrence J Beilin (4%), Sally Burrows (4%),
David E Henley (2%), Stephen J Lye (2%), Stephen G Matthews (2%), Craig E Pennell
(2%), Trevor A Mori (4%)

[Manuscript submitted for publication]

**Publication not originating from this thesis:**

(5) Sex dimorphism in the relation between early adiposity and cardiometabolic
risk in adolescents

Rae-Chi Huang, Trevor A. Mori, Sally Burrows, Chi Le-Ha, Wendy H. Oddy,
Carly Herbison, Beth H. Hands, and Lawrence J. Beilin

*The Journal of Clinical Endocrinology & Metabolism*, June 2012, vol 97, E1014-E1022

**Conference presentations:**

(1) Determinants of blood pressure in late adolescence

Chi Le-Ha, 2011 *Research Symposium*, School of Medicine and Pharmacology,
University of Western Australia, Perth, Western Australia, 30 September, 2011

(2) Gender differences and the effects of BMI and lifestyle on blood pressure in late
adolescence

Chi Le-Ha, *The Western Australian Pregnancy Cohort (Raine) Study Annual Scientific
Meeting*, Perth, Western Australia, 18th November, 2011

(3) Determinants of blood pressure in late adolescence and the effects of BMI and
lifestyles factors

Chi Le-Ha, *High Blood Pressure Research Council of Australia Annual Scientific Meeting*,
Perth, Western Australia, 7-9 December 2011

(4) Long-term passive smoking exposure and HDL cholesterol in late adolescence

Chi Le-Ha, *The Western Australian Pregnancy Cohort (Raine) Study Annual Scientific
Meeting*, Perth, Western Australia, 17th August 2012

(5) Gender difference in the relationship between long-term passive smoking exposure
and HDL-cholesterol in late adolescence

(6) The relationship between long-term passive smoking and HDL-C in late adolescence

Chi Le-Ha, *Royal Perth Hospital Medical Research Foundation Young Investigators Day*, Perth Western Australia, 31st October 2012

(7) The relation between hypothalamic-pituitary-adrenal activity and cardiovascular risk factors in an unselected adolescent population

Chi Le-Ha, *High Blood Pressure Research Council of Australia Annual Scientific Meeting*, Melbourne, Victoria, 7-9 December 2013

*Awards:*

(1) Endeavour Postgraduate Award, Australian Government, 2009-2013

(2) Raine Study PhD Scholarship, The Raine Foundation, Western Australia, 2012-2013

(3) Young Investigator Award, Royal Perth Hospital Medical Research Foundation, Western Australia, 2012

(4) Foundation for High Blood Pressure Research Young Investigator Travel Award, 24th Scientific Meeting of the International Society of Hypertension, Sydney, New South Wales, 2012
Communication originating from this thesis in major media and scientific organisations

   http://www.dailymail.co.uk/health/article-2172303/High-blood-pressure-risk-later-life-teenage-girls-Pill.html


(5) **European Society of Cardiology.** Oral contraceptive use in girls and alcohol consumption in boys are associated with increased blood pressure in late adolescence – Press release. 11 July 2012. 


(8) **Time Magazine, USA.** Alexandra Sifferlin. Secondhand Smoke is More Damaging For Teen Girls Than Boys. May 1, 2013. 
   http://healthland.time.com/2013/05/01/secondhand-smoke-is-more-damaging-for-teen-girls-than-boys/

(9) **University of Western Australia.** University News. Passive smoking more risky for teen girls than boys. 7 May, 2013. 
(10) *The Daily Mail, UK.* Teenage girls are at a higher risk of health problems from passive smoking than boys. 30 April, 2013.  
http://www.dailymail.co.uk/health/article-2317290/Teenage-girls-higher-risk-health-problems-passive-smoking-boys.html

http://www.huffingtonpost.co.uk/2013/05/01/higher-passive-smoke-risk-teenage-girls_n_3191411.html

Acknowledgements

First and foremost, I am grateful to my academic supervisors, Professor Lawrence J Beilin and Professor Trevor A Mori, for their strong support and advice towards all aspects of my PhD studies. My special thanks to Mrs Sally Burrows; her enthusiastic consultancy in statistics and methodology has been instrumental in my analyses of data throughout the PhD project. I would like to express my deep gratitude to the families participating in the Raine Study, and acknowledge the support of the Raine Study Executives, the Raine Study team and manager Jenny Mountain.

I would like to acknowledge the support from the Australian Government’s Endeavour Awards Program. It has been a great honour to receive an Endeavour Postgraduate Award for my PhD studies in Australia. I am also honoured to have been granted a Raine Study PhD Scholarship. I acknowledge the support from the High Blood Pressure Research Council of Australia, who has contributed towards my travel expenses to conferences.

I would like to thank my colleagues in the Raine Study and co-authors of my four papers that contribute to this thesis, and thank particularly Dr Rae-Chi Huang for her encouragement. It has been an enjoyable experience to have worked at the Royal Perth Hospital Campus of the UWA School of Medicine and Pharmacology over the past four years, during which I have had great emotional support from colleagues and fellow PhD students.

Finally, I thank my wife Phong Hoa for her encouragement, without which this period of study might not have been realised. My special thanks to my extended families in Vietnam for their support. And I thank especially my daughter Dao Anh, who every day has accompanied her daddy on his PhD journey.
List of tables

Ch 2 – Table 1  Demographic, anthropometric and socio-behavioural characteristics of the study population  74
Ch 2 – Table 2  Clinical and biochemical characteristics, and comorbidities of the study population  75
Ch 2 – Table 3  Hypertensive status by weight categories  76
Ch 2 – Table 4  Multivariable models for adolescent systolic and diastolic blood pressure  78
Ch 2 – Table S1  Comparing the 17-year sample to non-participants at 17-year survey  87
Ch 2 – Table S2  Univariate models of lifestyle and socio-demographic factors affecting systolic and diastolic blood pressure  88
Ch 2 – Table S3  Sex and BMI adjusted models of lifestyle and socio-demographic factors affecting systolic and diastolic blood pressure  89
Ch 3 – Table 1  Demographic and socio-behavioural characteristics of the study population  100
Ch 3 – Table 2  Relationship between biomedical and socio-demographic factors and HDL-C in the whole adolescent sample  103
Ch 3 – Table 3  Multiple effects of sex, BMI, passive smoking, and maternal smoking in pregnancy on HDL-C levels in non-smoking adolescents  105
Ch 3 – Table 4  Multivariable models for the effects of passive smoking and maternal smoking in pregnancy on HDL-C in non-smoking adolescent girls  106
Ch 3 – Supplemental Table 1  Average daily cigarettes consumed in the household in passive smoking (with versus without maternal prenatal smoking) categories  115
Ch 3 – Supplemental Table 2  Multiple effects of sex, BMI, passive smoking and alcohol consumption on HDL-C in non-smoking adolescents  116
Ch 3 – Supplemental Table 3  Multiple effects of sex, BMI, and active smoking on HDL-C levels in all adolescents  117
Ch 3 – Supplemental Table 4  Multivariable models for the effects of active smoking on HDL-C in girls  118
Ch 4 – Table 1  Anthropometric, phenotypic and behavioural characteristics of the study samples  128
Ch 4 – Table 2  Serum biochemistry features of the study samples  130
Ch 4 – Table 3  Univariate associations between cardiovascular risk factors and hs-CRP  132
Ch 4 – Table 4  Multivariable model for the effects of active smoking on hs-CRP in the whole adolescent sample  133
Ch 4 – Supplemental Table I  Comparing the 17-year sample to non-participants at 17-year survey  141
Ch 4 – Supplemental Table II  Multivariable model for the effect of smoking on hs-CRP – adjusting further for leptin  142
Ch 5 – Table 1  Clinical and socio-demographic characteristics of the 17-years-old participants  152
Ch 5 – Table 2  HPA axis measures, by sex and the use of oral contraceptives in girls  154
Ch 5 – Table 3  Significant associations between HPA axis parameters and cardiovascular risk factors at 17 years – final multivariable regression models adjusted for BMI, sex, and the use of oral contraceptives  156
Ch 5 – Supplemental Table I  Final multivariable models of the associations between plasma total cortisol and cardiovascular risk factors  165
Ch 5 – Supplemental Table II  Final multivariable models of the associations of salivary cortisol and free calculated plasma cortisol with cardiovascular risk factors  167
Ch 5 – Supplemental Table III  Final multivariable models of the associations between corticosteroid binding globulin and cardiovascular risk factors  168
List of figures

Ch 1 – Figure 1  Periods of vulnerability to environmental influences  4
Ch 1 – Figure 2  Risk of chronic disease increases along a trajectory
through the life course  6
Ch 1 – Figure 3  The mean (+SE) extent of fatty streaks and raised lesions by 5-y age groups
of men and women in the thoracic aorta, abdominal aorta, and right
coronary artery  9
Ch 1 – Figure 4  Prevalence of fibrous plaques at autopsy examination in individuals
by age group in the Bogalusa Heart Study  10
Ch 1 – Figure 5  SBP and DBP tracking correlation coefficients against baseline age  18
Ch 1 – Figure 6  Changes in lipid levels and blood pressure in boys and girls
from age 11 to 19 years  21
Ch 1 – Figure 7  Relative risk of having a CRP of >1.0 mg/L in overweight/obese
children compared with healthy-weight children  24
Ch 1 – Figure 8  Biologic and behavioral pathways linking stress to obesity
and cardio-metabolic disorders  34
Ch 2 – Figure 1  Relationship of BMI and systolic BP or diastolic BP in boys versus
girls using or not using OC  79
Ch 3 – Figure 1  CONSORT diagram of adolescents attending the Raine Study
17-year follow-up  94
Ch 4 – Figure 1  Predicted log hs-CRP levels by smoking status in the three adolescent
groups, girls not using OCs, girls using OCs, and boys  127
Ch 4 – Supplemental Figure I  Fractional polynomial plot showing the relationship
between the number of cigarettes consumed in the last 7 days
and log hs-CRP levels  143
Ch 5 – Figure 1  The significant associations of time of blood sample collection and time of
Sunrise with plasma total cortisol  153
### Abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CBG</td>
<td>corticosteroid binding globulin</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>CIMT</td>
<td>carotid intima-media thickness</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
</tr>
<tr>
<td>DOHaD</td>
<td>developmental origins of health and disease</td>
</tr>
<tr>
<td>Fourth Report</td>
<td>Fourth Report on Blood Pressure in Children and Adolescents</td>
</tr>
<tr>
<td>HDL-C</td>
<td>high density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>homeostasis model of assessment for insulin resistance</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>high-sensitivity C-reactive protein</td>
</tr>
<tr>
<td>IHD</td>
<td>ischaemic heart disease</td>
</tr>
<tr>
<td>IL-1β</td>
<td>interleukin – 1 beta</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin 6</td>
</tr>
<tr>
<td>KEMH</td>
<td>King Edward Memorial Hospital</td>
</tr>
<tr>
<td>LDL-C</td>
<td>low density lipoprotein cholesterol</td>
</tr>
<tr>
<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>n</td>
<td>sample size</td>
</tr>
<tr>
<td>Na/K ratio</td>
<td>sodium to potassium ratio</td>
</tr>
<tr>
<td>NHANES</td>
<td>US Nutrition Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>OC</td>
<td>oral contraceptive</td>
</tr>
<tr>
<td>PDAY Study</td>
<td>Pathobiological Determinants of Atherosclerosis in Youth Study</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PWC$_{170}$</td>
<td>physical working capacity at a heart rate of 170 beats per minute</td>
</tr>
<tr>
<td>Raine Study</td>
<td>Western Australian Pregnancy Cohort Study</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SEIFA</td>
<td>Socio-Economic Indexes for Areas</td>
</tr>
<tr>
<td>TG/HDL-C ratio</td>
<td>the triglyceride to HDL-C ratio</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor alpha</td>
</tr>
</tbody>
</table>
Atherosclerotic cardiovascular disease (CVD) is a major public health burden and a leading cause of mortality in many industrialized countries.\textsuperscript{1} CVD is also responsible for approximately 80% of deaths in low or middle income countries worldwide.\textsuperscript{2} Major cardiovascular events such as stroke, myocardial infarction or aortic dissection are the outcomes of a long-term pathological vascular process known as atherosclerosis, which starts in childhood and progresses through adolescence into adulthood.\textsuperscript{3} The process starts with the accumulation of lipids in the intima of the vasculature, progressing to the development of an extravascular lipid core covered by a fibromuscular cap that leads to thrombosis, vascular rupture, and acute coronary, aortic or cerebral ischaemic syndromes. Coronary atherosclerosis starts at a young age.\textsuperscript{4} Epidemiological studies have shown that conventional CVD risk factors such as dyslipidaemias, hypertension, diabetes mellitus, and obesity are present early in life.\textsuperscript{5} Moreover, CVD risk factors have also been associated with fatty plaques, a form of transitional stages of atherosclerotic lesions, as early as in the teenage years.\textsuperscript{6}
1.1 The developmental origins of cardiovascular health and disease

1.1.1 The hypothesis

Health and disease have conventionally been studied and interpreted based on gene-based models. Adaptation of the organism was assessed as its genetic fitness to the environment.\(^7\) James Neel in the early 1960s proposed that changes in diet or exercise affect health because humans exceeded a genetic limit of adaptation.\(^8\) Some populations that earlier were selected for ‘thrifty genes’ to survive a period of famine would later be of higher health risk in an environment that is rich in nutrition. Despite its major limitations, this ‘thrifty genotype’ hypothesis has still been the basis of the current intensive genetic research of cardiovascular disease (CVD) and diabetes. Nonetheless, genome-wide association studies (GWAS) have shown strong associations in some cardiovascular diseases, but only account for a small component of the risk.\(^9\)

The developmental origins of health and disease concept is based on the assumption that early life experience has effects on vulnerability to disease later in life.\(^10\) Health risk increases across the normal range of development, in studies that use birth weight as a proxy measure.\(^11\) In 1989, for the first time birth weight was shown to be inversely related to blood pressure;\(^12\) and the increase in blood pressure in this study population continued to amplify through time.\(^13\) An inverse association between birth weight and serum cholesterol was also reported.\(^14\) David Barker and Nicholas Hales in 1992 proposed that the fetus in a difficult environment would trade off growth in order to survive in the uterus, but it might suffer adverse consequences.\(^15\) Their ‘thrifty phenotype’ hypothesis was that the fetus in nutritional deprivation would limit its growth, via developing insulin resistance, to survive to birth. However, the Barker-Hales model has several limitations: it assumes that fetal growth is on the causal pathway; induced by nutritional deprivation; and the presence of a severe insult or stress to the fetus.\(^16\) The explanation of the developmental origins of health and disease (DOHaD) concept based on this model is inadequate.\(^16\) For example, the model assumed that the developmental change was always induced by a severe stress to the fetus.\(^16\) Furthermore, the focus on low birth weight in this model has been widely criticized.\(^17\)
A new evolutionary model, suggested by Bateson and Gluckman, considers DOHaD as the expression of normal processes of developmental plasticity. Bateson and Gluckman proposed that “the embryo, fetus or infant draws information from its environment and adjusts its developmental trajectory accordingly, but that in doing so, it could suffer from longer term consequences.”\textsuperscript{16, 18, 19} Plasticity is the capacity of the organism to respond to environmental changes, on a time scale in between selection processes and homeostasis.\textsuperscript{16} In this model, the fetus interprets the mother’s metabolic environment, predicts its likely future environment, and adopts an optimal developmental trajectory. The thrifty phenotype hypothesis models suggest that CVD risk increases with an increase in the disparity between environment \textit{in utero} and environmental conditions in later life.\textsuperscript{7} Accordingly, Gluckman and Hanson’s model has emphasized the disparity between fetal experience and adult lifestyle, and considered fetal life as the most important period that determines ill-health in adulthood. In this model, the “predictive adaptive response”, which reflects the degree of mismatch between pre and postnatal environments, would determine the risk of disease later in life.\textsuperscript{20} Adaptive changes in fetal life may be of benefit for survival in the short-term, however these changes would be maladaptive when the offspring undergoes catch-up growth, or diet-induced obesity, leading to the manifestation of an altered phenotype in later life.\textsuperscript{21}

The developmental period has traditionally been viewed as strictly controlled by genetic programming. However, development, particularly in earlier periods of life, is affected by the surrounding environment.\textsuperscript{22} Epigenetic modifications including DNA methylation, histone covalent modification, and non-coding RNA expression are pathways of the control of gene expression and hence the manifestation of specific phenotypes.\textsuperscript{23} Hence each organism or individual has one genome but has multiple epigenomes.\textsuperscript{22} Moreover, the response of the epigenetic system to the environment is particularly sensitive during the period of developmental plasticity.\textsuperscript{24} The perinatal period is a critical period, with high epigenetic plasticity (Figure 1).\textsuperscript{22} Epigenetic changes vary over the life-course,\textsuperscript{25} and can be the cause or outcome of a disease. DNA methylation patterns in cord blood are related to changes in gene expression, body size and body composition in childhood, suggesting a link between fetal life and later phenotype.\textsuperscript{26} Further, data of the Western Australian Pregnancy Cohort (Raine)
Study has demonstrated an association between DNA methylation with altered head circumference throughout childhood, and with fat distribution pattern in early adulthood.\textsuperscript{27}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Periods of vulnerability to environmental influences. Barouki et al. \textit{Environmental Health} 2012; 11:42}
\end{figure}

\subsection{1.1.2 Animal models and the developmental origins of health and disease}

Animal studies have contributed to the understanding of the concept of DOHaD, by providing proof to the hypothesis.\textsuperscript{28} The concept that interventions in critical developmental periods could exert adverse effect on target organs in the long-term, or that fetal growth restriction is linked to high CVD risk, have been demonstrated in animal models. In newborn female rats treated with testosterone in the first few days of life, regions of the hypothalamus that control reproductive function are remodeled, eventually leading to sterility.\textsuperscript{29} Uterine ligation in rats and guinea-pigs was shown to be associated with elevated blood pressure in the offspring later in life.\textsuperscript{30, 31} Similar findings are also demonstrated in large animal models.\textsuperscript{28} Sheep that were exposed to global nutrition restriction during early stages of gestation had offspring with elevated blood pressure,\textsuperscript{32} although the association is not consistent across studies.\textsuperscript{33} Other studies in pig and sheep also show that maternal nutrition restriction or fetal growth restriction are associated with postnatal insulin sensitivity in postnatal offspring.\textsuperscript{34} Further, in animal models the programmed outcomes may not be apparent until later development in the offspring,\textsuperscript{35} illustrating the mismatch hypothesis. These animal
models across a number of species, with the assessment of a range of biologically-
relevant factors, provide strong support for a causal relationship between fetal
adverse environment and postnatal disease risk.²⁸

Apart from contributing to the understanding of the conceptual models, animal studies
have also addressed the underlying mechanisms for the DOHaD hypothesis.²¹, ²⁸ In
rodent models, a maternal high-fat diet associated with increased levels of obesity,
insulin, and leptin resistance,³⁶ and higher risk of hypertension,³⁷ hepatic steatosis and
non-alcoholic fatty liver disease in the offspring.³⁸, ³⁹ Maternal adiposity but not dietary
fat induced hyperleptinaemia and insulin resistance in the offspring.⁴⁰ Moreover, a
moderate maternal high-fat diet induced insulinaemia and obesity in the offspring,
independent of pre-pregnancy obesity levels.⁴¹ In general, animal models have been
used to investigate the risk of CVD and changes in physiological function that linked to
higher risk of CVD, and the changes in expression of such physiological function, such
as altered renin-angiotensin system activity.²⁸ Overall, animal studies have been, and
continue to be, essential to the understanding of the DOHaD concept by providing
evidence for a causal association between challenges during critical developmental
periods and health risk later in life.²⁸

1.1.3 A life-course perspective of cardiovascular risk

The life-course perspective on health and disease is therefore “the study of long-term
effects on chronic disease risk of physical and social exposure during gestation,
childhood, adolescence, young adulthood and later adult life”, and further, inter-
generational effects on the development of chronic disease.⁴² In this perspective, time
and timing is important in the link between exposure and outcome within an individual
life-course.⁴³ Time lags between exposure and disease manifestations suggest that
early life exposures may involve in starting a disease process long before its
manifestation. Chronic diseases such as CVD progress over long-latent periods,⁴⁴ and
CVD risk factors accumulate gradually across the course of life. On the other hand,
behavioural factors that affect CVD risk, such as smoking, diet pattern, alcohol
consumption or physical activity may have their own natural course for development:
for example, an adult’s dietary habits may importantly be influenced by the dietary
habits adopted in early life. However, at a certain stage of life, a particular exposure can have an important effect on the development of the disease later in the life-course. For example, adolescence may be an important period during which an individual adopts lifestyle habits that could adversely influence later cardiovascular health. The optimal effect in risk reduction is likely to be achieved with timely intervention in early periods of life (Figure 2).

Figure 2 - Risk of chronic disease increases along a trajectory through the life course. Hanson M, and Gluckman P. *Am J Clin Nutr* 2011; 94:1754S-1758S

The DOHaD concept initially was based on the critical period model, which emphasizes the impact of maternal environmental milieu in gestation. However, later-life effect modifiers have also been important in the development of a disease. For example, the association between low birth weight and elevated blood pressure or insulin resistance have been observed in later life sometimes only in obese subjects. A broader life-course perspective has thus recognised that fetal growth is just one out of several developmental periods, which cannot be assessed separately from other life stages. In this respect, the Barry Caerphilly Growth Study, with modelling of growth
trajectories before and after birth, has shown that both pre and post natal factors are important in relating to blood pressure (BP) in early adulthood. Even though research on DOHaD implicates a role of early life influence on disease in later life, the size and relevance of the intrauterine effects on adult disease outcomes are still unclear. From a public health point of view, these effects are small and may be less important than other risk factors.\textsuperscript{17,51}

Health is a product of the life-course, although childhood has been considered as a critical period.\textsuperscript{52} To reduce the burden of CVD in later life, a more practical approach to the life course epidemiology would emphasize prevention measures for both the mother and child not only in pre-pregnancy and during pregnancy, but also in post-natal life.\textsuperscript{22} These measures would include improving nutrition, reducing environmental chemical exposures, and minimising mental stress. The study and testing of critical and sensitive period exposures therefore requires data measurements at multiple time points across the life span, from pre-pregnancy to older ages.\textsuperscript{43} How to intervene between early and later life to reduce chronic disease risk without knowledge of the underlying biological mechanism is still unclear. However, from a health policy point of view, the life course perspective illustrates that each period of an individual’s life is characterized by a new set of developmental challenges, and makes unique contributions to future health.\textsuperscript{53} Therefore, each period requires appropriate health policies to meet the health needs of children. Overall, a life-course model is a multidisciplinary agenda for the understanding of the effects of biological and socio-behavioural exposures in early-life and over the life-course, on the development of adult diseases. These exposures may operate not only through their timing, but also their accumulation over time.\textsuperscript{43}

1.2 Major studies examining the development of cardiovascular risk in children and adolescents

1.2.1 The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study

Atherosclerosis, the pathological processes that underlies ischaemic heart disease, begins in childhood.\textsuperscript{54} These processes involve the deposit of lipid in the intima of large arteries to form early atherosclerotic lesions known as fatty streaks. In early
adulthood, some fatty streaks evolve into fibrous plaques with intra and extra accumulation of lipids. Later in middle-age, fibrous plaques progress to develop advanced lesions that eventually result in clinical ischaemic events. In 1985, the PDAY study was established to examine the natural history of atherosclerosis and the relationship between CVD risk factors and atherosclerosis in youths. The study included 15 clinical centres coordinated by the central pathological laboratory at Louisiana State University Health Sciences Centre. Coronary arteries, aortas, and other tissues from over 3000 subjects aged 15 to 34 who died of accidents, homicides or suicides between 1987 and 1994 were collected.

The PDAY data have shown that CVD risk factors are associated with atherosclerosis since early adolescence, and that the progression of atherosclerosis into advanced lesions is strongly associated with risk factors measured early in life. Low levels of risk factors during adolescence associated with reduced severity of atherosclerosis later in life. Studies of the prevalence of atherosclerotic lesions in the right coronary artery showed an increase trend, with 60% in the 15-19 years group, to >80% in men and 70% in women in the 30-34 years group. Figure 3 shows the extent of atherosclerotic lesions from adolescence to mid adulthood. The PDAY risk scores, developed using logistic regression to produce a weighted summary of the effects of CVD risk factors on advanced atherosclerotic lesions, are associated with all stages of atherosclerosis. Overall, PDAY data have demonstrated that CVD risk factors are associated with both early and advanced stages of atherosclerosis.
1.2.2 The Bogalusa Heart Study

The Bogalusa Heart Study is a long-term population-based investigation of CVD risk factors and lifestyle behaviours related to the development of future CVD, including hypertension and diabetes. The study population includes all children and eligible young adults who live in Ward 4 of the Washington Parish, in which Bogalusa is the major community. The area includes about 22,000 inhabitants, of which about 5,000 are children. The community is biracial, with 65% white and 35% black. The first cross-sectional survey was conducted in 1973-74 on 3524 children aged 5-14 years. Subsequent surveys have been conducted at approximately 5 years intervals.
A major achievement of the Bogalusa study is the investigation of anatomic lesions in relating to CVD risk factors. Autopsy studies were conducted on approximately 80% of the known deaths in the community. The study showed the prevalence of atherosclerotic fatty streaks was 70% for coronary arteries (Figure 4). Additionally, there was an increase in the presence of atherosclerotic lesions in the aorta from young age to middle age. Further, studies using echocardiography have shown functional and structural changes in relation to CVD risk factors. The Bogalusa study has also shown that lifestyle factors such as smoking, alcohol consumption, and unhealthy diet are associated with CVD risk and early stage of CVD. Overall, the study has been an invaluable resource for the understanding of the early natural history of CVD.

1.2.3 The Cardiovascular Risk in Young Finn Study

As coronary heart disease was highly prevalent in Finland in the 1960s and 1970s, an epidemiological project was launched in the late 1970s to investigate cardiovascular health and risk factors in children, adolescents and young adults. The Cardiovascular Risk in Young Finn Study is a collaborative project between five university departments...
The Cardiovascular Risk in Young Finn Study has demonstrated that the biological CVD risk profile is significantly influenced by early lifestyle factors. The study has shown a clustering of adverse lipids, high blood pressure, and high adiposity levels, and the prevalence of clustering is higher in boys compared with girls. Moreover, the study also shows significant clustering of socio-behavioural risk factors such as smoking, alcohol drinking, physical inactivity and unhealthy diet. In general, by linking morbidity and mortality statistics on its longitudinal data, the study has provided strong evidence for the relation between the lifetime burden of CVD risk factors and cardiovascular health.

1.2.4 The Generation R Study

The Generation R Study began in 2002 as a prospective cohort study, with the participation of approximately 10,000 pregnant women in Rotterdam, the Netherlands. The primary objective of the study is to investigate early environmental and genetic causes of growth, development and health from in utero life to young adulthood. Main areas of study include normal and abnormal growth patterns and physical development, including fetal and postnatal brain development; behavioural and cognitive development; diseases in childhood; and healthcare for women in pregnancy and children. Extensive assessments were conducted in mothers and their partners during pregnancy, and subsequently in their children. During pregnancy, assessments were conducted at <18 weeks, 18 to 25 weeks, and ≥ 25 weeks gestation. Information was obtained on several social and environmental aspects of the
participants, including pre and postnatal diet, maternal and child infections, family function, substance use in pregnancy, behaviour, and temperament.\textsuperscript{68}

The Generation R Study has utilized ultrasound imaging to study brain development. Ultrasound studies were conducted in fetal life\textsuperscript{69} and at the first month of life,\textsuperscript{70} to assess cerebral features such as biparietal diameter, cerebellar diameter and ventricular size. Since 2009, brain magnetic resonance imaging has also been conducted in children at 6 to 8 years of age.\textsuperscript{68} The introduction of this sophisticated method of neuroimaging in this population-based pregnancy cohort is of particular importance in the study of genetic and environmental aspects of neurodevelopment.

\textbf{1.2.5 The Western Australian Pregnancy Cohort (Raine) Study}

The Western Australian Pregnancy Cohort (Raine) Study is the source for research in this thesis. The Study recruited 2900 pregnant women from King Edward Memorial Hospital, Perth city’s major tertiary obstetric centre, and other nearby clinics in Perth, Western Australia.\textsuperscript{71} Women were enrolled at or before 18 weeks of pregnancy, over a 30 month period from May 1989 to November 1991. The criteria for enrollment included having a gestational age of 16 – 18 weeks, proficiency in English to understand the implications of participation, an expectation to deliver at KEMH, and an intention to stay in Western Australia. Participants were randomized into either an intensive ultrasound group or a control group. Ultrasound and Doppler examinations were conducted at enrollment (about 18 weeks gestation), and at 24, 28, 34 and 38 weeks gestation. Participants completed questionnaire related to socio-demographic, lifestyle, substance use, medical history, exposure to stress and environmental toxin information.

Over 23 years of continuing data collection, follow-up studies including questionnaire and clinical examinations were conducted at age 1, 2, 3, 4, 8, 10, 14, 17, 20 and 23 years. The number of participants and the reasons for not participating in each review from birth to year 17 are presented in appendix A of this thesis. During this time period, a number of assessments related to cardiovascular risk factors have been employed. Strengths of the Raine Study include the fact that data were collected during the time of pregnancy, and have prospectively been collected over 23 years.
through periodic surveys. Socio-demographic, anthropometric and clinical measurements have been repeated in consecutive follow-ups, which permits the use of longitudinal methods to examine social and health aspects of the mothers and children over time. Data collected on a range of social, behavioural, clinical and biochemical factors has enabled the investigation of health risk whilst adjusting for potential confounders.

The Raine Study data have shown that CVD risk factors start manifesting early in childhood. Burke et al\textsuperscript{72} have shown that overweight/obesity rates increase since 3 years of age, and at 8 years, the prevalence of overweight/obesity in boys and girls was 15\% and 20\%, respectively. Furthermore, at 8 years, systolic and diastolic BP were higher by 6 mmHg and 2 mmHg, respectively; HDL-cholesterol was lower by 8\% (p=0.002), and triglycerides were higher by 27\% (p<0.001). Chivers et al\textsuperscript{73} showed that overweight/obese adolescents at 14 years of age had a different BMI trajectory pattern since birth, compared with those of normal weight. The authors found that BMI increased significantly over time (p<0.001), with girls having a faster rate compared with boys. Investigating adipose trajectory using a semi-parametric group-based method, Huang et al\textsuperscript{74} identified seven distinct adiposity trajectories by year 14 in the Raine Study adolescents. The authors also observed that insulin resistance was greatest in adolescents with increasing adiposity trajectories. Using cluster analysis, Huang et al\textsuperscript{75} showed that 29\% of the adolescents at 14 years fell into a high-risk metabolic cluster group. Compared to those in the low-risk cluster, those in the high-risk cluster group had higher BMI (95\%CI 1.7, 1.8 vs 3.5, 3.9), SBP (95\%CI 110.8, 112.1 vs 116.7, 118.9), triglycerides (95\%CI 0.78, 0.80 vs 1.25, 1.35), C-reactive protein (p<0.001), and lower HDL-cholesterol (1.44, 1.48 vs 1.20, 1.26). Moreover, Ambrosini et al\textsuperscript{76} identified two dietary patterns, “Western” (high in red and processed meat, take-away food, fried and refined foods, confectionary, and full fat dairy products) and “Healthy” (high in legume and fish, fruits, vegetables and whole grain), in the Raine adolescents, and showed that higher scores for the Western pattern were associated with greater odds of being in the high-risk metabolic cluster group.
1.3 Cardiovascular risk factors in adolescence

### 1.3.1 Overweight and obesity

Overweight and obesity are modifiable cardiovascular risk factors associated with adult cardiovascular outcomes including hypertension, type-2 diabetes, and atherosclerotic heart disease.\(^{77, 78}\) In children and adolescents, several studies have demonstrated that childhood and adolescent obesity associated with adverse cardiovascular risk factors and outcomes.\(^{79, 80}\) Worldwide, childhood overweight and obesity prevalence increased from 4.2% to 6.7% during the period 1990-2010, and the prevalence is expected to be approximately 9% in 2020.\(^{81}\) Over the last decade, the prevalence of overweight/obesity in boys and girls in Australia has been about 21% - 25%, and the prevalence of obesity alone has been 5% - 6%.\(^{82}\) These patterns have been fairly consistent across the age range.

Overweight and obesity in children and adolescents are defined based on age- and sex-specific nomograms for body mass index (BMI). Children with BMI values equal to or exceeding the age- and sex-specific 95\(^{th}\) percentiles are defined as obese; those with BMI between the 85\(^{th}\) and 89\(^{th}\) percentiles as overweight.\(^{83}\) The Raine Study data show that the period from birth to 6 years of age is a crucial developmental period for adiposity.\(^{73}\) In this cohort, in those who were obese at 14 years, the critical period was between birth and 2 years, compared with normal children. In children and adolescents, greater adiposity levels may adversely affect cardiovascular risk.\(^{84}\) In the Raine Study, overweight/obese children had higher SBP levels (2, 3, 4 and 6 mmHg, at 1, 3, 5 and 8 years, respectively) compared with normal weight children.\(^{72}\) The atherosclerotic processes, which begin early in life and develop throughout the life course, are influenced by genetic as well as modifiable factors including obesity.\(^{85}\) There has been substantial evidence of childhood obesity in relating to both immediate and long-term cardiovascular health.\(^{86}\) Data from the Bogalusa Heart Study have shown that abnormalities of several cardiovascular risk factors such as higher triglycerides or reduced high-density lipoprotein cholesterol (HDL-C) levels start to manifest at around the 85\(^{th}\) percentile of body weight.\(^{87}\) The impact of childhood BMI on cardiovascular risk profile in adolescents has been shown in the Avon longitudinal
(ALSPAC) study. The ALSPAC data have shown that in girls a 1 standard deviation increase in BMI during 9-12 years of age associated with odd ratios of 1.23 for high systolic blood pressure; 1.19 for raised low-density lipoprotein (LDL-C) levels; 1.43 for high triglycerides; 1.25 for low HDL-C; and 1.45 for high insulin levels, at age 15-16 years. The NHANES data for the period 1999-2008 in the US have shown that 49% of overweight and 61% of obese adolescents had at least one CVD risk factor, findings that indicate a substantial CVD risk factor burden associated with higher adiposity in this age group.

Obesity levels track from childhood to adulthood. The Cardiovascular Risk in Young Finns Study showed that children aged 3 to 9 years, and adolescents aged 12 to 18 years, who were overweight or obese (BMI > 80th percentile) had 3 and 4 times higher risk, respectively, of being obese in adulthood. Several cohort studies across populations have shown the important impact of early adiposity on future cardiovascular outcomes. Early adiposity has been related to changes in the structure and function of the heart. Overweight and obese adolescents have larger left ventricular diameter and mass, and left ventricular hypertrophy was more prevalent in the obese (33.5%) and overweight (12.4%), compared with normal subjects. An echocardiographic study of the Bogalusa Heart data has shown that the cumulative burden of adiposity and systolic BP from childhood predicts left ventricular mass index in young adulthood. In a longitudinal study of 227000 adolescents in Norway, the relative risk of ischaemic heart disease mortality was 2.9 for males and 3.7 for females in the highest BMI categories (i.e. BMI >85th versus BMI 25th to 74th percentiles as reference), with mean follow-up of 34.9 years. In a pooled analysis of 3 British cohorts, persistent overweight from childhood has also been associated with a 12-fold higher risk of having type 2 diabetes.

One issue that is less clear is the relative contribution of obesity at different stages in life to CVD risk. A pooled analysis of three British cohort studies has shown that persistent overweight since childhood to adulthood is associated with significantly higher risk of type 2 diabetes, compared with overweight in adulthood. In this study, the effect of overweight in childhood on type 2 diabetes was not observed in subjects that were not obese later in life. Genetic predisposition underlying persistent obesity may explain later development of metabolic abnormalities.
between overweight in early life and risk of hypertension in adulthood has been equivocal; the above British study demonstrated that overweight in early life was not associated with hypertension risk in adulthood, whereas other studies confirmed the association. Furthermore, some studies have shown that rapid growth during early stages of life may significantly affect risk of hypertension later in life. It has been suggested that there may be multiple mechanistic pathways through which obesity causes hypertension, and therefore it is essential to consider the relative contribution of adiposity and other risk factors, and their development over the life-course, to hypertension risk.

Severe paediatric obesity has increasingly been a major public health issue. Severe obesity is defined as having a BMI ≥120% of the 95th percentile or an absolute BMI ≥35 kg/m2, whichever is lower based on age and sex. Various studies in the US using NHANES data and other cohorts during the years 1999 to 2008 showed that severe obesity affects 4% to 6% children and adolescents. Compared with overweight or obese children and adolescents, those with severe obesity have a more adverse CVD risk factor profile. A study in 2 to 18 year-olds in the Netherlands showed that 67% had at least one CVD risk factor, such as hypertension (56%), elevated blood glucose (14%), type 2 diabetes (0.7%), or low HDL-C (54%). Examining dyslipidaemia, hypertension and hyperinsulinaemia in the Bogalusa Heart Study cohort, Friedman et al. showed that in those children with a BMI ≥95th percentile, 84%, 39% and 18% had at least 1, 2 or 3 of these risk factors, respectively. Severe obesity in children has been associated with arterial stiffness, increased CIMT, endothelial dysfunction, and increased CRP levels. Moreover, severe obesity has been associated with impaired glucose tolerance and prediabetes. In this respect, insulin resistance has been suggested to be the most important factor associated with the development of paediatric severe obesity. Children with severe obesity are highly likely to become severely obese in adulthood. The Bogalusa Heart Study data show that, of the children with severe obesity at 12 years of age, 100% had an adult BMI ≥30 and 88% had BMI ≥35. Overall, given the strong tracking of adiposity, children with severe obesity will be likely to have a poor cardio-metabolic risk profile later in life.
1.3.2 Hypertension

Based on the normal distribution of BP in children and adolescents, the current definition of hypertension in children and adolescents, according to the Fourth Report on Blood Pressure in Children and Adolescents (Fourth Report), is blood pressure values that are above the 95th percentile for age, sex, and height on at least 3 occasions. With the Fourth Report, prehypertension is defined in adolescents as BP values that are between the 90th and 95th percentiles, or > 120/80 mmHg. Unlike the definition of adult hypertension which is based on clinical outcomes, this statistical definition of hypertension for adolescents implies about 4% of the sampled population having sustained hypertension. In the US, the prevalence of prehypertension/hypertension in adolescents was 14% during 1999-2008. Data from children and adolescent studies have suggested an increasing trend of paediatric BP levels as well as the prevalence of prehypertension and hypertension. A report of 5582 children in the National Health and Nutrition Survey showed that BP was (1.4mmHg systolic and 3.3 mmHg diastolic) higher in 2000 compared with 1988-1994. The NHANES data from 1999-2008 showed that the risk of having prehypertension and hypertension increased by 27%. In contrast, other studies observed decreased BP trends in children and adolescents. Methodological issues related to BP measurement or adjustment for confounders during analysis may account for these inconsistencies.

Adiposity is the most important determinant of elevated BP. The observed increase in BP in adolescents likely mirrors the rise in the prevalence of obesity in this age group, which has tripled over the last generation in the developed world. In a prevalence study of hypertension in adolescents, 15.7% had prehypertension and 3.2% had hypertension. Among hypertensive adolescents, 2.6% had stage 1 hypertension and 0.6% had stage-2 hypertension. In this study, obesity significantly correlated with hypertension. Data from the NHANES study for the period 1963-2002 suggest that the rise in BP lags about 10 years behind the rise in obesity prevalence, and the continuous changes in BMI since 1980 may be a crucial factor determining the increased BP prevalence. Furthermore, the Fourth Report mentions weight loss, exercise, and diet as potential measures of lifestyle modification to control obesity hypertension in children and adolescents. The benefit of weight loss on BP in
paediatric populations has been extensively studied. A randomized controlled trial has shown the benefit of diet alone or diet in addition to exercise on BP. However, treatment of obesity is only a part of a complete management plan for the adolescent’s health, given that the adolescent’s obesity is usually a family health problem. Another important issue is the secular increase in sodium intake in childhood populations, and the strong association between high intake of sodium and raised BP particularly in overweight or obese adolescents.

Figure 5 - SBP and DBP tracking correlation coefficients against baseline age.

Chen X and Wang Y. *Circulation.* 2008; 117:3171-3180

Blood pressure tracks from adolescence to adulthood (Figure 5), and adolescents with BP in the higher part of the distribution of BP tend to maintain that rank over time. In an Australian study, 37% of the adolescents remained in the highest SBP quartile between the ages of 9 and 18 years. A systematic analysis on the tracking of blood pressure from childhood to adulthood shows that the tracking correlation was
greater for SBP than DBP, and that sex and baseline age were factors significantly associated with the tracking. Furthermore, in a study of 8500 adolescents with serial single BP taken, annually 7% of the adolescents progressed from prehypertension to hypertension. Although the definition of adolescent hypertension is based on population norms, studies have shown associations between high BP in adolescence and end organ damage. For example, about 30% newly diagnosed hypertensive children already manifested left ventricular hypertrophy. The Bogalusa Heart Study showed that SBP levels in childhood were associated with higher levels of brachial pulse wave velocity, suggesting the importance of BP in children in the evolution of arterial stiffness. The Cardiovascular Risk in Young Finns Study, in a 21 year follow-up study, has shown that SBP levels in adolescents aged 12 to 18 years at baseline, were inversely associated with carotid flow-mediated dilation in adulthood. Moreover, BP in adolescence has been reported to predict future CVD events and mortality. Although SBP is a stronger predictor of death than DBP in middle age and elderly populations, it has been suggested that in younger people elevated DBP is more predominant and may be more important than SBP in determining future outcomes. A Swedish cohort study of 1,207,141 subjects from late adolescence over 37 years demonstrated that the relation between DBP and total mortality was stronger compared with SBP, with regard to the magnitude of prediction of risk as well as the attributable fraction of mortality.

The current high prevalence of childhood and adolescence hypertension is largely due to the paediatric obesity epidemic. Studies in children have shown that BP levels reduce with weight loss. Therefore population-based research aiming to relieve the burden of adolescent elevated BP is crucial. In a study of 11,284 male and 3491 female college students, weight and physical exercise were modifiable risk factors that affected BP. The Fourth Report has reported the important role of reducing weight and salt intake, as well as aerobic exercise, as essential measures to achieve this goal. The first line of management to control obesity hypertension involves lifestyles changes, with reducing caloric intake and increasing regular exercise.
1.3.3 Biochemical risk factors

1.3.3.1 Dyslipidaemias

Abnormal plasma lipids as a result of disorders in lipid metabolism are evident in childhood and adolescence. Approximately 22% of American adolescents aged 12-19 years had borderline-high or high LDL-C and 6% prevalence had low HDL-C (< 35 mg/dL [0.906 mmol/L]) during the period 1999-2008.\textsuperscript{5} The changes in lipid levels and blood pressure by sex from age 11 to 19 years have been described by Moran A et al (Figure 6).\textsuperscript{130} The US NHANES data for the period 1999-2006 showed that the average LDL-C level was 90.2 mg/dL [2.336 mmol/L] among 12-17 years old adolescents, and the average total cholesterol of those from 6-17 years old was 163 mg/dL [4.222 mmol/L].\textsuperscript{131} Earlier surveys of this study showed data of serum triglycerides and LDL-C levels at the 95\textsuperscript{th} percentile were 216 mg/dL [2.441 mmol/L] and 152 mg/dL [3.936 mmol/L], respectively.\textsuperscript{132} In these data, age, sex, and ethnic background were found to be associated with lipid levels. Furthermore, the triglyceride to HDL-C ratio (TG/HDL-C) which has been used as a predictor of small dense LDL-C,\textsuperscript{133} has been correlated with LDL particle size in children and adolescents.\textsuperscript{134} In this respect, a recent study demonstrated that the TG/HDL-C ratio significantly associated with arterial distensibility and pulse wave velocity in healthy adolescents.\textsuperscript{135}
Increased LDL-C and decreased HDL-C levels are present in childhood and adolescence, and track to adulthood. In 1,586 children of the Bogalusa Heart Study that were followed up over a 12 year period, 50% of the children with cholesterol or LDL-C above the 75th percentile at baseline remained in that rank 12 years later. In the Muscatine Study, in 2,446 children and adolescents aged 8 to 18 years, 43% of those with cholesterol levels greater or equal to the 90th percentile measured at baseline remained above the 90th percentile in their adulthood. Moreover, studies have shown that high levels of total cholesterol, LDL-C, or triglycerides, or reduced levels of HDL-C, associated with atherosclerotic lesions in children and adolescents who died

Figure 6 - Changes in lipid levels and blood pressure in boys and girls from age 11 to 19 years. Moran A et al. Circulation. 2008; 117:2361-2368
from accidental death. Epidemiological studies have also shown that LDL-C and obesity in children and adolescents predicted increased carotid intima medial thickness or coronary artery calcium in young adults. Moreover, the Muscatine Hyperlipidemia Family Study showed that lipids levels in children associated with lipid levels of family members, and dyslipidaemia in children can identify families with increased CVD risk. Approximately 50% of offspring with a primary dyslipidaemia had a parent with premature CVD. It has therefore been proposed that early detection of dyslipidaemia in youth requires selective screening of children and adolescents whose parent has premature CVD or dyslilidaemia.

1.3.3.2 Glucose and Insulin resistance

Youth-onset diabetes has been shown to increase mortality in adults, however little is known of the relationship between cardiovascular risk factors, including glucose intolerance, in childhood and lifelong CVD morbidity. A large cohort study of American children without diabetes reported that those in the highest quartile of glucose level had a 73% increase in risk of premature death from endogenous causes during a follow-up period of 23.9 years, compared with children in the lowest quartile. Hyperinsulinaemia and insulin resistance, have been reported in children and adolescents. With regard to the relation between insulin resistance and hypertension, data of the Bogalusa Heart Study have shown a correlation between blood pressure and fasting insulin levels in children. In adolescents, sodium retention was associated with insulin resistance.

Studies have shown an association between insulin resistance and adiposity in children and adolescents, and weight loss has been associated with increased insulin sensitivity. In a study that compared 82 normoglycaemic obese adolescents with 40 lean adolescents, dyslipidaemia was associated with the degree of insulin resistance in those who were obese. In this study, insulin resistance largely explained the variance of triglycerides, LDL-C and HDL-C levels. Furthermore, a European study in 710 obese children aged 6 to 18 years reported that insulin resistance and impaired insulin secretion were associated with hyperglycaemia. Developmental changes during childhood may influence insulin resistance. Data from the Raine Study children
followed up from birth to 14 years of age show that increased insulin resistance is observed in children with increasing adiposity trajectories regardless of birth weight. Homeostasis model assessment of insulin resistance (HOMA-IR) and insulin levels were higher in the “stable high” and “rising to high” adiposity trajectories in both boys (all $p<0.001$) and girls (all $p<0.002$), compared with those in the normal growth trajectory. Additionally, it has been shown that during the transition from childhood to adolescence, the increase in insulin resistance was associated with raised triglycerides and decreased HDL-C levels in males.

Recent data from the US NHANES survey of 1999-2010 suggest that there has been an increase in the prevalence of type 2 diabetes in adolescents. In this study, the prevalence of type 2 diabetes in adolescence was 0.36%. Type 2 diabetic adolescents were reported to be almost invariably overweight or obese, and likely to have other risk factors of CVD such as raised BP and triglycerides levels.

1.3.3.3 Inflammatory biomarkers

Low-grade systemic inflammation in children potentially increases the long-term risk of CVD. A chronic inflammatory state with raised levels of inflammatory markers has been shown to be related to obesity in adolescents. The pathological processes are mediated by the adipocytokines of the immune system. Some cytokines such as tumor necrosis factor alpha (TNFα) and interleukin–1 beta (IL-1β) are pro-inflammatory, whereas others such as IL-10 and cytokine-binding proteins are considered anti-inflammatory. On the other hand, cytokines such as IL-6 can have both pro- and anti-inflammatory actions. Studies in young age groups have shown that markers of low-grade inflammation are associated with individual CVD risk factors or the metabolic syndrome. A study that included 413 adolescents showed a positive correlation between a composite CVD risk factor profile and C-reactive protein (CRP), TNFα, and IL-6 levels. In this study, the risk of having a clustered z-score of the CVD risk factors above 1 standard deviation was increased in those adolescents in the highest quartiles of these cytokines. In another study in adolescents, there was a positive association between IL-6, IL-18, IP-10 and insulin resistance, and a negative association with adiponectin. Furthermore, a study in obese children showed that
the inflammatory and prothrombotic states were similar between pre and post-pubertal stages.\textsuperscript{154}

CRP is the biomarker most commonly used for systemic inflammation assessment. CRP levels in the range of 2-6 mg/L are indicative of low-grade inflammation. Figure 7 demonstrates the relative risk of having a CRP > 10 mg/L in overweight/obese children, compared with those having normal weight.\textsuperscript{153} CRP has been shown to be upregulated across several stages of vascular damage.\textsuperscript{160} Studies in adults demonstrated that CRP predicts future CVD disease.\textsuperscript{160, 161} In a study in adults, those with the highest high-sensitivity CRP (hs-CRP) category had a relative risk of 2.3 to 4.8 for future CVD events, compared with those in the lowest category.\textsuperscript{162} There has been strong evidence of an association between CRP and adiposity.\textsuperscript{163} Smoking and insulin resistance are also important correlates of CRP,\textsuperscript{164} whereas nutritional modifications relate to a decrease in CRP levels.\textsuperscript{165} However, the causative role of CRP in the development of CVD is still debated.\textsuperscript{166}

![Figure 7](image_url)

Figure 7 - Relative risk of having a CRP of >1.0 mg/L in overweight/obese children, compared with healthy-weight children (P < 0.01). Skinner A C et al. Pediatrics 2010; 125:e801-e809
In adolescence, hs-CRP levels are associated with adiposity measures\textsuperscript{167} raised insulin levels and insulin resistance,\textsuperscript{168, 169} as well as smoking and the use of oral contraceptives.\textsuperscript{170} In the Raine Study adolescents, CRP levels were higher in those that were in the high-risk metabolic cluster group, compared with those in the low-risk group.\textsuperscript{75} In studies using non-invasive vascular measures in children and adolescents, CRP associated with increased carotid intima-media thickness and reduced brachial artery flow-mediated dilatation.\textsuperscript{171} In adolescents, however, longitudinal data of hs-CRP in relating to future CVD risk are lacking. CRP levels have been shown to track from childhood to adulthood.\textsuperscript{172} In a large cohort study, CRP measured during childhood and adolescence predicted the metabolic syndrome 21 years later.\textsuperscript{173} Given the scant data of screening tools for future CVD in paediatrics, CRP has been considered as a potential biomarker to identify high-risk adolescents for CVD lifestyle intervention programmes.\textsuperscript{174}

1.3.4 Lifestyle and health behaviours

1.3.4.1 Tobacco smoking

Tobacco smoking is an established major risk factor of CVD including coronary heart disease, stroke and peripheral vascular disease in adults,\textsuperscript{175} and a leading cause of morbidity and mortality.\textsuperscript{176} The atherosclerotic effect of smoking may be direct\textsuperscript{177} or indirect through the mediation of several biological factors.\textsuperscript{178} Smoking associates with an atherosclerotic lipid profile, including higher levels of total cholesterol, triglycerides, and LDL-C, and lower levels of HDL-C.\textsuperscript{178} Moreover, inflammation is an important pathway by which smoking promotes atherosclerosis. In this respect, smoking strongly associates with higher levels of inflammatory markers such as CRP, homocysteine, and fibrinogen.\textsuperscript{179} In adolescence, active tobacco smoking and exposure to secondhand smoke is associated with adverse health problems.\textsuperscript{180} Recent reports in the US have shown that over 20\% of high school students were daily smokers, and the smoking rates were stable over the period 2003 to 2007.\textsuperscript{180} In a prospective observational study of 11,755 male students, active smoking in adolescence and early adulthood increases overall mortality in later life; the association with the mortality reported in this study was due to specific effects of smoking.\textsuperscript{181} Smoking in adolescence promotes early
Atherosclerosis.\textsuperscript{182} Furthermore, active smoking has been associated with decreased glomerular filtration rate in adolescents, an indication of the harmful effects smoking on kidney function early in life.\textsuperscript{183} Overall, there is unequivocal clinical evidence that cigarette smoking is harmful and addictive.

Passive smoking, the inhalation of tobacco smoke by a non-smoker, has been an established environmental risk factor of CVD. Population studies in adults have demonstrated a relative risk of 1.3 for future CVD in those exposed to environmental tobacco smoke, independent of other risk factors.\textsuperscript{184} CVD risk associated with passive smoking is almost as large as that of active smoking.\textsuperscript{185} The mechanisms through which passive smoking affects CVD risk are not clear, however, passive smoking has been shown to associate with lower levels of HDL-C,\textsuperscript{186} higher homocysteine levels\textsuperscript{187} and increased platelet activity.\textsuperscript{188} Moreover, a large population study of 5029 men and women showed that exposure to secondhand smoke, even at low levels, associated with higher levels of a range of inflammatory markers including CRP, fibrinogen, factor VIII, and tissue plasminogen activator.\textsuperscript{189}

It has been estimated that 700 million people are exposed to secondhand tobacco smoking worldwide.\textsuperscript{190} Children and adolescent passive smoking exposure occurs mostly at home, and parental smoking is the main source.\textsuperscript{191} A study of 2767 children and adolescents has shown that non-smokers exposed to parental smoking had higher nicotine concentrations, compared to those not exposed.\textsuperscript{191} It has been suggested that even with low levels of passive smoking, the physiologically developing metabolic systems of the children are still vulnerable to the detrimental effects of environmental smoke.\textsuperscript{185} Parental smoking has been associated with higher systolic BP in pre-school children.\textsuperscript{192} Passive smoking in children and adolescents is also associated with dyslipidaemia, with decreased HDL-C levels as the most consistent finding.\textsuperscript{193, 194} Furthermore, passive smoking in children has been associated with low-grade inflammation, indicated by higher levels of leptin, CRP, fibrinogen, and IL-6, even after adjustment for adiposity.\textsuperscript{195} Passive smoking in youth is detrimental to cardiovascular health in the long-term. In a report of the Cardiovascular Risk in Young Finns Study using brachial flow-mediated dilatation as a marker of atherosclerosis, parental smoking during childhood and adolescence was associated with reduced flow-mediated dilatation 20 years later.\textsuperscript{196}
Maternal smoking in pregnancy is an important adverse fetal exposure that may result in growth restriction of the fetus.\textsuperscript{197} Evidence suggests an increase in vascular resistance of umbilical-placental circulation in mothers who smoke in pregnancy.\textsuperscript{198} In this respect, several studies have shown maternal smoking associated with high systolic \textsuperscript{199, 200} and diastolic\textsuperscript{201} BP in childhood. Jaddoe et al have shown that children whose mother smoked in pregnancy had a 0.12 mmol/L greater annual increase in total cholesterol over a 10 year period, compared to those whose mother did not smoke.\textsuperscript{202} The Generation R Study also showed that both active and passive maternal smoking in late pregnancy associates with higher risk of low birth weight.\textsuperscript{203} Intrauterine exposure to smoking has been shown to adversely affect arterial structure and function in 5-year-old children,\textsuperscript{204} and associated with lower HDL-C levels in 8-year-old children.\textsuperscript{205} Moreover, there has been evidence of an intergenerational association between parental and adolescent smoking.\textsuperscript{206} This association could be due to a genetic influence on tobacco use, or the modeling of parental smoking behavior by offspring.\textsuperscript{207} In this respect, a longitudinal study that employed multigenerational data has shown that parental smoking was significantly associated with the smoking behavior of the children.\textsuperscript{208} Parental smoking at any age would increase the likelihood of their children smoking.\textsuperscript{208}

\subsection*{1.3.4.2 Alcohol consumption}

There has been substantive evidence of the relationship between excessive alcohol use and increased risk of hypertension in adults.\textsuperscript{209} A meta-analysis showed that in those who consumed 3 to 6 drinks per day, reducing alcohol consumption associated with decreased SBP and DBP.\textsuperscript{210} In an Australian study in 491 Caucasian males aged 20 to 45 years, SBP increased progressively with increasing alcohol intake levels ($r=0.18$), and there was no threshold effect.\textsuperscript{211} However, it has been unclear whether consuming light-to-moderate alcohol is beneficial or harmful with regard to hypertension risk. Data from adult studies have documented beneficial,\textsuperscript{212} detrimental,\textsuperscript{213} or equivocal\textsuperscript{209} relationships between light-to-moderate alcohol consumption and risk of elevated BP. These discrepant findings could be due to methodological issues including chance, bias or uncontrolled confounders.
Two large studies\textsuperscript{212, 214} have shown a J-shaped association between light-to-moderate alcohol consumption and hypertension risk in women. Compared to non-drinkers, women who consumed 0.26 to 0.5 drink per day had a 14% lower hypertension risk.\textsuperscript{212} An increase in risk was evident with consuming beyond 2 drinks per day,\textsuperscript{212} and heavier consumption of $\geq 4$ drinks per day significantly increased risk.\textsuperscript{214} There was no benefit of light-to-moderate drinking observed in men, but a strong linear increase in risk beginning with consuming just 5 drinks per week.\textsuperscript{214} A study in young adults aged 18 to 26 years showed a J shaped relationship between alcohol consumption and BP; the lowest BP levels were observed in those who consumed 1 to 3 standard drinks per day.\textsuperscript{215} The timing of alcohol use relative to meals may also be important; those who drink outside of meals have higher risk of hypertension.\textsuperscript{216} Furthermore, binge drinking increases SBP and DBP by 5 mmHg.\textsuperscript{217}

There are scant data on the effects of alcohol consumption on BP in adolescents. The Bogalusa Heart Study data for the period 1981-1991 have shown that alcohol consumption was more prevalent among adolescent boys than girls ($p<0.001$). Of those who drank, 81% to 87% consumed alcohol once or twice a week, but those who drank daily had the highest weekly intake.

\textbf{1.3.4.3 Oral contraceptives use}

Oral contraceptive (OC) pills, a commonly prescribed method for birth control in women, have been available since the 1960s.\textsuperscript{218} However, due to serious adverse effects such as thromboembolism, this first generation of OC were largely replaced in the 1970s by the second-generation OC, which are lower in oestradiol content and a newer generation of progesterol. The third OC generation was introduced in the 1980s, with lower oestrogen content and low-androgenic progestogens. Cardiovascular data on the newer OC generation are scant.\textsuperscript{219} In adult women, OC use associated with an increase of 7 – 8 mmHg SBP;\textsuperscript{220, 221} in contrast, the newer generation of OC such as drospirenone may decrease BP via their antimineralocorticoid diuretic effects.\textsuperscript{222} The use of third-generation OC has associated with increased levels of inflammatory markers such as CRP; high hs-CRP levels in the
range of 3-10 mg/L were reported in 27% of OC users, compared with 8.5% in non-users.\textsuperscript{223}

Endogenous reproductive hormones may have a protective role in CVD, from the observation that cardiovascular risk increases after menopause in women.\textsuperscript{224} However, the Women’s Health Initiative data have shown no overall protection from CVD risk in women without preexisting CVD who used hormone replacement therapy.\textsuperscript{225} In the Nurses’ Health Study, there was no increase in risk of coronary heart disease or non-fatal myocardial infarction in past OC users, compared to that in current users.\textsuperscript{226} However, this study showed a 2.5 relative risk increase in cardiovascular mortality, non-fatal MI, and stroke in current OC users. In women with preexisting CVD risk factors, current use of OC associated with an increase in risk of MI.\textsuperscript{227} In contrast, in an unselected population of 48,321 women aged 30 to 49 years over a period of 11 years there was no increase in risk of MI in former or current OC users.\textsuperscript{228}

In adolescents, epidemiological data on OC use and cardiovascular risk are scant. A cross-sectional study of 120 adolescent girls showed that OC use associated with 5 mmHg higher SBP, and 0.4 mmol/L higher serum total cholesterol.\textsuperscript{229} OC may also be an important marker for systemic inflammation in adolescence. In the Northern Finland Birth Cohort Study, current use of OC in adolescent girls was associated with a 2.83 times higher risk of having elevated CRP levels.\textsuperscript{170}

\textbf{1.3.4.4 Diet and dietary nutrients}

A healthy diet in childhood and adolescence is essential for the primary prevention of CVD throughout the life course.\textsuperscript{230} Dietary behaviour and food choices are established early in life,\textsuperscript{231} and dietary patterns track from childhood to adulthood.\textsuperscript{231, 232} There have been extensive data on the relation between unhealthy diet and poor cardiovascular health. Adiposity is positively associated with macronutrients such as dietary fat, total energy intake,\textsuperscript{230} and sugar sweetened beverages.\textsuperscript{233} Children and adolescents consuming large portion sizes and high energy dense food have higher CVD risk as a result of excess weight gain. Dietary total, animal, and vegetable protein intakes have been adversely related to SBP\textsuperscript{234, 235} and DBP.\textsuperscript{235, 236} In adolescents at high risk for hypertension, BP levels were higher in those having diets low in potassium,
calcium, and magnesium;\textsuperscript{237} these associations were not confounded by sodium intake or adiposity. In the Cardiovascular Risk in Young Finns Study, a dietary pattern that reflects healthy food choices such as high consumption of vegetables, legumes, tea, rye, and dairy products was associated with lower levels of CVD risk factors.\textsuperscript{238} A study of dietary intake in the Raine Study adolescents at 14 years showed that a “Western” or unhealthy dietary pattern was associated with greater odds of being in the high risk cluster of cardio-metabolic factors (p for trend = 0.02).\textsuperscript{76} The intake of polyunsaturated fatty acids, especially omega-3, has been associated with lower BP.\textsuperscript{239} In the Raine Study boys at 13-15 years of age, SBP was inversely associated with intakes of polyunsaturated omega-3 fatty acids, omega-6 fatty acids and long chain omega-3 fatty acids.\textsuperscript{240} Clinical trial data of the Dietary Intervention Study in Children have also shown that diets low in total fat, saturated fat, and cholesterol reduced LDL-C levels.\textsuperscript{241}

There is substantial evidence from studies in adults that sodium intake causally links to blood pressure and thereby to CVD.\textsuperscript{242} The American Heart Association\textsuperscript{243} recommends that daily sodium intake should not be more than 6 g salt. In the INTERSALT study, it was estimated that SBP would be 9 mmHg higher with a 6 g increase per day in salt intake over 30 years.\textsuperscript{244} There was a consistent association between salt intake and the rise of BP with age across 48 centers participating in this study. The benefit of a reduction of salt intake on BP has been shown in several studies including clinical trials and meta-analyses.\textsuperscript{242} Reducing 6 g salt intake per day could reduce SBP by up to 7 mmHg in hypertensives and 4 mmHg in normotensives.\textsuperscript{245} The urinary sodium/creatinine ratio has been used as an index of salt intake and was positively associated with SBP and DBP levels; the differences observed were 7.2 mmHg and 3.0 mmHg, respectively, between the top and bottom quintiles.\textsuperscript{246} Salt intake has been shown to relate to central pulse pressure and its determinants.\textsuperscript{247} In this study, the urinary sodium to potassium ratio was associated with in-office and 24-hour pulse pressure, and central augmentation index.

Despite evidence from epidemiological studies in children and adolescents suggesting an important relation between sodium intake and BP,\textsuperscript{248} there are inconsistent data, with some studies showing no significant association between sodium intake and BP.\textsuperscript{249} Methodological issues, such as small sample size or unreliable salt intake assessment methods, may account for these discrepancies. A study in children with 24-
hour urine collections, a more reliable assessment, has shown a positive linear relation between urinary sodium and SBP.\textsuperscript{250} Moreover, in a large study of 1658 children and adolescents, an increase in 1 g per day in sodium intake was associated with 0.4 mmHg in SBP and 0.6 mmHg in pulse pressure.\textsuperscript{251}

\subsection*{1.3.4.5 Physical activity and fitness}

Physical activity is defined by the US Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents as “any bodily movement produced by contraction of skeletal muscle that increases energy expenditure above a basal level.”\textsuperscript{180} In children and adolescents, a physically active lifestyle is associated with several benefits for health.\textsuperscript{252} Physical activity may have a direct protective effect on cardiovascular health, or a mediating role through its associations with other cardiovascular risk factors.\textsuperscript{253} In line with studies in adults, self-report physical activity data from the Cardiovascular Risk in Young Finns Study\textsuperscript{254} and maximal bicycle ergometry test data from the Muscatine Study\textsuperscript{255} in adolescent populations have shown that those who were physically active had better cardiovascular risk profiles. Regular physical activity improves cardiovascular fitness, and decreases risk of high BP and obesity.\textsuperscript{252, 256} In contrast, increase in time spent in sedentary activities has been associated with lower physical activity levels, as well as higher risk of obesity, hypertension, and dyslipidaemias.\textsuperscript{254, 255} Higher physical activity levels at 9 to 18 years of age predict higher levels of physical activity in adulthood.\textsuperscript{257} Moreover, the Norwegian Nord-Trondelag Health Surveys on 1869 adolescents have shown that those who maintain physical activity from childhood to adulthood had lower waist circumference, BMI, diastolic BP, and increased HDL-C levels.\textsuperscript{258}

Physical activity is related to cardiorespiratory fitness, but physical activity must be sufficiently intense to achieve a certain level of fitness. Studies in adults have shown that lower cardiovascular fitness was associated with higher levels of CVD risk factors, higher prevalence of CVD and all-cause mortality.\textsuperscript{259} Fitness is associated with increased arterial elasticity and decreased carotid intima-media thickness.\textsuperscript{260, 261} A high fitness level decreases the progression of carotid-intima media thickness.\textsuperscript{262} In accordance with adult studies, fitness has been favorably associated with aortic IMT.
and elasticity in adolescents. In this study that followed children from 11 to 17 years of age, the increase in aortic IMT was smaller in adolescents who were fit, compared to those in the lowest fitness level.\textsuperscript{263} Moreover, data of the Amsterdam Growth and Health Longitudinal Study demonstrated that fitness in adolescence predicts decreased carotid IMT in adulthood.\textsuperscript{264}

\textbf{1.3.5 The metabolic consequences of stress in adolescence}

Adolescence is a period of continuous physical and mental development, and also a period of developmental vulnerabilities.\textsuperscript{265} Acute or chronic stress exposure during the adolescence transition may lead to long-term adverse emotional and cardio-metabolic effects. Negative emotions are associated with the activation of the stress systems, of which the hypothalamic-pituitary-adrenal (HPA) axis plays a significant role.\textsuperscript{266} Activation of the HPA axis leads to a cascade of neural events, with the eventual release of cortisol from the adrenal cortex.\textsuperscript{267} HPA axis maintains homeostasis and its activation in response to physiological stress is essential for survival. However, prolonged exposure to stressors may lead to dysregulation of HPA activity\textsuperscript{268} that usually manifests as altered cortisol levels or a flattened pattern of the diurnal cortisol levels throughout the day.

Stress may be more harmful during childhood and adolescence. Little is known about the impact of exposure to stress on the development of the adolescent brain,\textsuperscript{269} however it has been shown in animal studies that substantial changes in stress hormones related to development of the brain in puberty.\textsuperscript{270} An adolescent may be susceptible to physiological perturbations due to changes in neurobehavioural function during adolescent transition.\textsuperscript{265} Chronic dysregulation of the HPA axis expressed as HPA hypo- or hyper-activity during these periods may adversely affect the development of the brain and the endocrine and metabolic systems.\textsuperscript{271} Risk factors in early life may have a substantial impact on health of the individual throughout the life course. In this respect, individual differences in response to stressful situations may be a significant mediator in the relation between early-life factors and coronary heart disease and type 2 diabetes in adults.\textsuperscript{272}
HPA axis dysfunction may play an important role in the pathogenesis of cardio-metabolic disorders. Figure 8 shows the biologic and behavioural pathways through which stress could affect the cardio-metabolic system. In patients with coronary heart disease, the low diurnal decline pattern of cortisol levels has been shown to associate with systemic inflammation, and the reduced cortisol response associated with an increase in CRP levels. Studies in adult populations have shown that higher plasma cortisol levels associated with higher SBP, triglycerides, insulin resistance, fasting glucose, and lower HDL-C levels. Salivary cortisol responses to stress have been associated with a greater degree of coronary calcification; in this study, the cortisol response group associated with a 2.2 fold greater risk of significant coronary artery calcification. Higher cortisol in hair has been associated with a higher prevalence of the metabolic syndrome. A recent study showed that cortisol response to stress also significantly associated with detectable plasma cardiac troponin T levels. The literature on HPA activity and cardio-metabolic risk in children and adolescents is scant. In accordance with data in adults, a few studies in selected obese adolescent populations have shown that higher fasting cortisol was associated with lower insulin sensitivity, and higher BP and LDL-cholesterol levels. A blunted nocturnal cortisol rise pattern has also been associated with higher carotid intima-media thickness in overweight adolescents.
1.3.6 Sex differences in cardiovascular risk

1.3.6.1 Sex differences and the CVD burden in adults

In Australia, coronary heart disease (CHD) mortality rates declined by 46% in men and 51% in women during the period 1986-1996. There has been a male preponderance in CHD and CVD mortality in Australia, with an increased rate in men having BMI>30 and women having BMI>25. In the US, the absolute number of CVD deaths in women has exceeded that of men since 1984, although the rate of CVD mortality rate is higher in men than in women. The mortality rate for CVD in 2007 was 300 per 100,000 in men compared to 212 in women. CVD is the major cause of death in women, although there is a delay in the onset of CVD of 7 to 10 years in women compared with men. The Framingham Study data have shown that the incidence of
CHD increases substantially after menopause in women.\textsuperscript{289} This observation of a female advantage suggests an intrinsic cardioprotective effect in women.\textsuperscript{290} It has been assumed that endogenous oestrogens exposure during the fertile period of a woman’s life delays atherosclerotic CVD.\textsuperscript{290} The incidence of CHD events in women is low before menopause,\textsuperscript{288} and women with early menopause have a lower life expectancy compared to those with normal menopause.\textsuperscript{291} However, oestrogen therapy trials have not demonstrated any cardioprotective effects either in women\textsuperscript{292} or in men.\textsuperscript{293} In a longitudinal epidemiological study based on data from England, Wales and the United States, Vaidya et al\textsuperscript{294} showed that ischaemic heart disease (IHD) mortality rate did not increase around the time of menopause in women, suggesting that IHD in women is a life-course on-going process with no midlife acceleration. The NHANES data from the US have shown that the incidence of myocardial infarction (MI) increases in 35 to 54 year-old women, but decreases in men of the same age range.\textsuperscript{295} The fall in CHD rate in men at age 45, as shown in the study of Vaidya et al,\textsuperscript{294} suggests the concept of “andropause”. Androgens may explain the sex differences in CVD. During middle-age, testosterone levels decline steadily in men.\textsuperscript{296} It has been proposed that the delayed onset of CHD events in women is explained by a reduction in the acceleration of events in middle-age men rather than an acceleration of events in postmenopausal women.\textsuperscript{297} Questions still remain regarding the role of sex hormones on CVD, and whether there is a delay advantage in women or an acceleration advantage in men.\textsuperscript{290} Cardiovascular risk factors, including ethnic and genetic factors, may play a significant role in explaining sex differences in CVD.\textsuperscript{290} The INTERHEART study has shown that modifiable risk factors accounted for over 90\% of myocardial infarctions.\textsuperscript{298} INTERHEART data have also shown that the earlier onset of MI in men could be due to increased dyslipidaemias and higher smoking rates.\textsuperscript{299} However, the harmful effect of the total number of cigarettes smoked per day is larger in women than in men.\textsuperscript{300} Compared with male smokers, in female smokers the first acute MI occurs more prematurely.\textsuperscript{301} Menopause is also an important transitional period with significant worsening in cardiovascular risk profile.\textsuperscript{302} Menopause associates with a decline in oestrogen levels and this may explain, in part, why there is a greater rise in SBP in ageing women than in ageing men.\textsuperscript{303} Other studies have shown that borderline
hypertension associated with more endothelial dysfunction in women than in men. Total cholesterol and LDL-C levels were shown to be increased 10% and 14%, respectively, during menopause, whereas there was no change in HDL-C. However, data from the Framingham study showed that low HDL-C associated with a higher risk of CHD in women compared with men. Kappert et al, in a sex-specific analysis of the ONTARGET and TRANSCEND trial data, have shown that the risk ratio of MI in diabetic women compared with non-diabetic women was significantly higher than that of diabetic men compared with non-diabetic men (hazard ratio, 1.98 in women vs 1.36 in men; interaction p=0.002). Thus diabetes has a severe impact in women in spite of their innate cardiovascular health advantage. In contrast, studies have shown that higher insulin resistance associated with higher hyperadrogenaemia, an observation that may explain the increase in cardiovascular risk in men and diabetic women, but not in non-diabetic women.

Female-specific factors may further impact the sex differences in CVD. In the Women’s Ischemia Syndrome Evaluation (WISE) study, there was an association between a greater clustering of cardiovascular risk factors and an adverse cardiovascular event rate in postmenopausal women. Compared with normoglycaemic pregnant women, those with gestational diabetes had a 7 to 12 fold higher relative risk of future type 2 diabetes. Moreover, a history of hypertensive disease in pregnancy predicts increase risk of future hypertension. For example, women who have experienced pre-eclampsia during pregnancy have a two-fold higher risk of developing CHD later in life, compared to those who were normotensive during pregnancy.

1.3.6.2 The emergence of sex differences in cardiovascular risk factors in early life

Sex differences in cardiovascular risk factors emerge early in life with BP levels higher in boys than in girls during adolescence. The Muscatine study has shown that boys have a 10% higher likelihood of developing high SBP in adulthood, compared with girls. In the Quebec Child and Adolescent Health and Social Survey, the prevalence of high SBP in boys was 3% and 13% higher at 13 years and 16 years, respectively, compared with girls. In another Canadian adolescent cohort followed-up from 12 to 17 years of age, there was little change in SBP in girls, but SBP increased
progressively in boys. During this period, there was an annual increase of 19% in risk of high SBP in boys, but not in girls.

Sex differences have also been demonstrated for other cardiovascular risk factors in children and adolescents. Sexual dimorphism in total body composition has been observed in children. Studies have shown that boys have greater fat free mass and less fat mass, compared with girls.315 Furthermore, fat distribution has been an important cardiovascular risk factor. An android fat pattern is adversely linked to cardio-metabolic risk,316 whereas a gynoid fat pattern is associated with lower risk.317 In this respect, sex differences in fat distribution have been observed in pubertal adolescents,318 and even in pre-pubertal children.319 Moreover, Moran et al have shown in boys an increase in insulin resistance during adolescence in spite of a decrease in percentage of body fat, whereas an opposite pattern was observed in girls.130 Overall, it has been not clear why boys begin to have increased risk in adolescence.

The scope of this thesis

In view of the importance of adolescence as a crucial time for prevention and early recognition of future CVD, this thesis is an investigation of the most important biological and socio-behavioural factors that potentially influence long-term cardiovascular health risk in an Australian birth cohort of adolescents at 17 years of age. The thesis employed data from the Western Australian Pregnancy Cohort (Raine) Study, an on-going prospective population based study, with comprehensive phenotypic, biochemical, and socio-behavioural information that has been collected since 1989 through 10 subsequent surveys.

Adolescence is an important period in which protective and adverse health behaviours are adopted that will influence long-term CVD risk. High BP and lifestyle factors such as active and passive smoking, high sodium intake, and the use of OC in children and adolescent are important determinants of CVD in adulthood. Despite decades of research, the optimal measures to control high BP and exposure to smoking in children
and adolescents have not been established. There have been sex disparities in CVD risk factors in adults, however little is known about the influence of sex on CVD risk factors in adolescents, and how these factors are inter-related. In addition, adolescence is a time during which alterations in the biological stress systems, such as a dysregulated HPA axis, may have long-term effects on the development of cardio-metabolic diseases. To our knowledge, there has been no data on the relationship between basal HPA axis activity and CVD risk factors in a general adolescent population.

This thesis is organised as a series of four scientific papers, of which three have been published. The objectives of the thesis were to investigate (i) the relationship between lifestyle factors and BP at 17 years, with particular reference to sex differences and their interaction with adiposity; (ii) the effects of long-term passive smoking exposure since birth and maternal smoking during pregnancy on HDL-cholesterol (HDL-C) at 17 years; (iii) the influence of active smoking on high-sensitivity C-reactive protein (hs-CRP) at 17 years; and (iv) the link between basal hypothalamus-pituitary-adrenal (HPA) axis activity and cardiovascular risk factors at 17 years.

References


76. Ambrosini GL, Huang RC, Mori TA, Hands BP, O'Sullivan TA, de Klerk NH, Beilin LJ, Oddy WH. Dietary patterns and markers for the metabolic syndrome in Australian adolescents. *Nutrition, Metabolism and Cardiovascular Diseases*. 2010;20:274-283


90. Chinali M, de Simone G, Roman MJ, Lee ET, Best LG, Howard BV, Devereux RB. Impact of obesity on cardiac geometry and function in a population of adolescents - the Strong Heart Study. *Journal of the American College of Cardiology*. 2006;47:2267-2273


104. Kvaavik E, Tell GS, Klepp K. Predictors and tracking of body mass index from adolescence into adulthood: Follow-up of 18 to 20 years in the Oslo Youth Study. *Archives of Pediatrics & Adolescent Medicine*. 2003;157:1212-1218


114. Samuels J. The increasing burden of pediatric hypertension. *Hypertension.* 2012;60:276-277


157. Galcheva SV, Iotova VM, Yotov YT, Bernasconi S, Street ME. Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk


166. Balagopal P, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, McCrindle BW, Mietus-Snyder ML, Steinberger J. Nontraditional risk factors and biomarkers for cardiovascular disease: Mechanistic, research, and clinical considerations for youth: A


242. He FJ, MacGregor GA. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *Journal of Human Hypertension.* 2008;23:363-384


247. Redelinghuys M, Norton GR, Scott L, Maseko MJ, Brooksbank R, Majane OHI, Sareli P, Woodiwiss AJ. Relationship between urinary salt excretion and pulse pressure and
central aortic hemodynamics independent of steady state pressure in the general population. *Hypertension*. 2010;56:584-590


259. Berry JD, Willis B, Gupta S, Barlow CE, Lakoski SG, Khera A, Rohatgi A, de Lemos JA, Haskell W, Lloyd-Jones DM. Lifetime risks for cardiovascular disease mortality by cardiorespiratory fitness levels measured at ages 45, 55, and 65 years in Menthe
Cooper Center longitudinal study. *Journal of the American College of Cardiology*. 2011;57:1604-1610


276. Reynolds RM, Walker BR. Human insulin resistance: The role of glucocorticoids. *Diabetes, Obesity and Metabolism*. 2003;5:5-12


293. The coronary drug project: Findings leading to discontinuation of the 2.5-mg/day estrogen group. *JAMA.* 1973;226:652-657


globulin and the free androgen index are related to cardiovascular risk factors in multiethnic premenopausal and perimenopausal women enrolled in the Study of Women across the Nation (SWAN). *Circulation.* 2005;111:1242-1249


Chapter 2

Oral contraceptive use in girls and alcohol consumption in boys are associated with increased blood pressure in late adolescence

Preamble

Hypertension is an important modifiable risk factor for CVD in adults, and is determined by lifestyle factors such as weight gain, increased salt intake or the use of oral contraceptives. To date, little is known about lifestyle factors, gender, and their inter-relations on blood pressure and hypertension in adolescents.

This chapter is an assessment of blood pressure values and the hypertensive status of the Raine Study adolescents at 17 years of age. It examines the blood pressure and cardiovascular risk factors profile of the cohort, and demonstrates the most important anthropometric and behavioural factors that influence systolic and diastolic blood pressure levels in this age group. By demonstrating a variety of behavioural and phenotypic factors and their differential impact on blood pressure levels, it also addresses a theme central to this thesis, the sex differences in cardiovascular risk factors that already emerge in this young age.

This chapter was published online ahead of print in the European Journal of Preventive Cardiology in July 2012, (printed version, December 2013, vol 20, no 6, 947-955).
Abstract

Aims: Lifestyle behaviours established during adolescence may adversely affect blood pressure (BP) and contribute to gender differences in cardiovascular risk in adulthood. We aimed to assess the association of health behaviours with BP in adolescents in the Western Australian Pregnancy (Raine) Study.

Methods: Cross-sectional analysis on 1248 adolescents aged 17 years, to examine the association between lifestyle factors and BP.

Results: Boys had 8.97 mmHg higher systolic BP compared with girls. The 30% of girls using oral contraceptives (OC) had 3.27 and 1.74 mmHg higher systolic and diastolic BP, respectively, compared with non-users. Alcohol consumption in boys, increasing body mass index (BMI) and sodium-potassium ratio were associated with systolic BP. There was a continuous relationship between BMI and systolic BP in both genders; however, the gradient of this relationship was significantly steeper in boys, compared with girls not taking OC. In boys, systolic BP was 5.7 mmHg greater in alcohol consumers in the upper quartile of BMI and the urinary sodium-potassium ratio compared with teetotallers in the lowest quartile. In girls, systolic BP was 5.5 mmHg higher in those taking OC, in the highest BMI and urinary sodium-potassium ratio quartile as compared to those not taking the OC pill and in the lowest quartile.

Conclusion: In addition to gender-related differences in the effects of adiposity on BP, we found lifestyle-related health behaviours such as high salt intake for both sexes, consumption of alcohol in boys, and OC use in girls were important factors associated with BP measurements in late adolescence. This suggests that gender-specific behavioural modification in adolescence may prevent adult hypertension.

Key words: Oral contraceptives, alcohol consumption, blood pressure, adolescence, gender differences, hypertension, risk factors, lifestyle, salt consumption, body mass index
Introduction

Adolescence is accompanied by the adoption of a range of lifestyle habits that may profoundly influence blood pressure (BP) and adult cardiovascular risk.\(^1\) Obesity, an emerging public health problem in childhood and adolescence worldwide, is associated with multiple cardiovascular risk factors, including raised BP\(^2\) and a higher risk of adult cardiovascular diseases.\(^3\) In adults, lifestyle factors such as dietary patterns, cigarette smoking, alcohol consumption, physical activity, and oral contraceptive (OC) use are known to influence BP; however, it is less clear what their effects are in adolescents, independent of obesity.

In addition, although gender differences in BP levels\(^4,\,5\) and risk of high BP\(^4\) in adolescence have been documented, little is known about the influence of gender on effects of lifestyle factors on BP in this age group. It was previously suggested that OC use could raise BP in adolescent girls and thus increase the risk of hypertension in adult women.\(^6\) Women taking OC have a greater body mass index (BMI),\(^7\) but to our knowledge no one has examined the relationship between OC use and BMI, and whether this affects BP in adolescent girls.

The aim of this study was to examine the relationship of lifestyle factors with BP in a population-based adolescent cohort in Western Australia, with particular reference to possible gender differences and their potential interaction with adiposity.

Methods

Participants

The Western Australian Pregnancy Cohort (Raine) Study,\(^8\) with 2900 pregnant women enrolled from 1989 to 1992, is a longitudinal study of mothers and their children recruited from King Edward Memorial Hospital and nearby clinics in Perth, Western Australia, as described previously.\(^9\) Ninety percent of the eligible women participated
in the study. The 2868 live-births were evaluated and followed up at 1, 2, 3, 5, 8, 10, 14 and 17 years of age.

The Human Ethics Committees of King Edward Memorial Hospital and Princess Margaret Hospital in Perth approved the study protocol. Informed parental and adolescent consent were obtained for participation in the study. The present study was a cross-sectional analysis of data from the 17 year follow-up of the cohort, which was conducted between July 2006 and June 2009.

Assessments

Clinical and biochemical

Resting BP and heart rate were obtained using an oscillometric sphygmomanometer (Dinamap ProCare 100, Soma Technology, USA). After five minutes quiet rest six automatic recordings were taken every 2 minutes with subjects supine and with an appropriate cuff size. The averages of the last 5 readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated. Body weight was measured using a Wedderburn Chair Scale (nearest 100g) and height with a Holtain Stadiometer (nearest 0.1 cm).

Fasting blood samples were analysed in the PathWest laboratory at Royal Perth Hospital for serum lipids. Spot urine samples were analysed for urinary sodium and potassium excretion.

Weight status was determined based on age (17 years) and gender-specific cut-off points from the recommendation for international comparisons of prevalence of overweight and obesity. Overweight was defined as BMI ≥ 24.46 kg/m² and < 29.41 kg/m² for boys, and ≥ 24.70 kg/m² and < 29.69 kg/m² for girls; obese as BMI ≥ 29.41 kg/m² for boys and 29.69 kg/m² for girls. Prehypertension was defined as gender-specific BP that was ≥ 90th percentile for SBP or DBP but below the threshold for hypertension (≥ 95th percentile), and hypertension as gender-specific SBP or DBP ≥ 95th percentile. Hypertensive status was also defined employing adult JNC-7 criteria: prehypertension as SBP 120-139 mmHg or DBP 80-89 mmHg, and hypertension as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg.
Socioeconomic status was assessed from annual family income (Australian dollars) and the SEIFA (Socio-Economic Indexes for Areas) score,\textsuperscript{12} which is based on the adolescent’s geographic postal area (a low score indicates an area of social disadvantage). Family income was used as an interpretable descriptor; however, SEIFA, a more robust measure of SES, was employed in the analysis.

Alcohol consumption information was obtained from an online questionnaire that asked the types (beer, wine or spirits) and amount (can, glass, stubby, nip or standard drink) of alcoholic beverages consumed daily during the past week. Alcohol consumption was defined as the average number of standard drinks consumed per day during the last 7 days where 1 standard drink is 10 g alcohol. Alcohol drinker was defined as consumer of alcohol at any level during the last 7 days.

Adolescents were asked to report the number of cigarettes smoked each day in the last 7 days. Physical activity was assessed from the question “How many hours do you usually exercise in your free time in a week, so much that you get out of breath or sweat?” and a dichotomous variable with cut-point at ≥ 4 hours per week was created.\textsuperscript{13} Oral contraceptive use in girls was defined as answering yes or no to 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?”).

From a 212-item food frequency questionnaire, two dietary patterns (“Western” and “Healthy”) were identified using factor analysis.\textsuperscript{14}

Statistical analysis

SBP and DBP were examined as continuous outcome variables. Candidate factors investigated included age, gender, SEIFA score, BMI, alcohol consumption, smoking, physical activity, dietary patterns, OC use, urinary sodium, potassium, and Na/K ratio. BMI, alcohol consumption and smoking were analysed as continuous variables to avoid the loss of power and the bias of arbitrary choice of categorization of cut-points.
associations between each factor and both SBP and DBP were assessed using robust linear regression, to reduce the influence of potential outliers. To examine the linearity in the relationship between BMI and BP, we used fractional polynomial regression plots. Patterns observed in these plots suggested that for alcohol consumption, a dichotomous arrangement represented the relationship with BP more closely than the continuous variable. In this instance, both the dichotomous and continuous variables were analysed in the initial models. We also investigated the interactions between gender and BMI and other covariates. Initial models were adjusted for sex, and extended models were adjusted for sex and BMI.

Multiple imputation was performed using Royston’s Ice program to estimate the urinary Na/K ratio, missing in approximately one third of the sample. The imputation process, based on iterative multivariable regression, generated 20 new datasets, each containing an estimated value for the missing ratio. These datasets were then analysed separately and estimates combined to obtain the final single solution. Our analysis of complete cases and imputed data did produce the same estimates of the association between Na/K and SBP. Independently of Na/K itself, the reduced sample of complete cases did create bias in the estimated effects of other covariates; hence, imputation maintained the whole sample in the analysis to reduce sample bias.

A 3-level gender variable incorporating oral contraceptive use (girls not using OC, OC using girls, and boys) was created to assess the effect of OC in the final multivariable models. Variables significantly associated with BP in the initial models, as well as the relevant interaction terms, were included in the multivariable regression models. Non-significant variables were removed in a backward process that included examination of the effect on coefficients and p values of all remaining variables, at each step.

Stata MP version 11 (Stata Corp, College Station, Texas) was used for statistical analysis, and significance was set at p <0.05.
Results

At 17 year 1771 adolescents from the original 2868 live-born children participated. There were 13.2% who deferred from participation, 16.6% had withdrawn from the study, 1.2% were deceased and 7.2% were lost to follow up. Of the 1771 participants, 1257 had anthropometric, cardiovascular, and biochemical assessment and detailed health questionnaires. We excluded nine cases with major congenital malformations, resulting in a sample of 1248. Apart from having a higher percentage with higher family income, these participants were comparable to non-participants at the 17-year survey (see supplementary material Table S1). Our study sample was comprised of 88% Caucasians, which is representative of the Western Australian population.

Demographic, anthropometric and socio-behavioural characteristics of the adolescents are shown in Table 1. At around 17 years, boys were heavier and had a larger waist size than girls (p<0.001). Within the previous week, 51% of the adolescents had consumed alcohol at any level. More boys than girls exercised ≥ 4 hours in free time per week (42.6 vs 21.3, p<0.001), while 30% of the girls used OC.

Mean SBP was higher in boys compared with girls (117.9 vs 108.6 mmHg, p<0.0001) (Table 2), whereas mean DBP was lower in boys, as compared with girls (58.2 vs 59.5 mmHg, p=0.0007). Employing gender-specific criteria, we found that 8% of all adolescents were prehypertensive and 7.8% were hypertensive, with similar proportions for boys and girls; but by using hypertensive status defined according to adult (JNC-7) criteria, we found a significantly higher prevalence of prehypertension and hypertension in boys compared with girls (36% vs 8.9%, and 2.2% vs 0.3%, respectively; p<0.001). Overall, 34% of overweight and 38% of obese adolescents had a BP defined as prehypertensive or hypertensive (Table 3).
<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Boys</th>
<th>Whole sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 620 (49.7%)</td>
<td>n = 628 (50.3%)</td>
<td>n = 1,248</td>
</tr>
<tr>
<td><strong>mean (95%CI)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td>17.05</td>
<td>17.02</td>
<td>17.04</td>
</tr>
<tr>
<td></td>
<td>(17.03, 17.07)</td>
<td>(17, 17.04)</td>
<td>(17.02, 17.05)</td>
</tr>
<tr>
<td><strong>Height, m</strong></td>
<td>1.659</td>
<td>1.783</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.653, 1.664)</td>
<td>(1.777, 1.789)</td>
<td></td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>62.5 (61.5, 63.4)</td>
<td>71.0 (69.9, 72)</td>
<td></td>
</tr>
<tr>
<td><strong>Waist circumference, mm</strong></td>
<td>768 (759, 776)</td>
<td>799 (790, 807)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>22.7 (22.4, 23)</td>
<td>22.3 (22.0, 22.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Socio-behavioural</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SEIFA score</strong></td>
<td>1041.4</td>
<td>1045.1</td>
<td>1043.2</td>
</tr>
<tr>
<td></td>
<td>(1035.8, 1046.9)</td>
<td>(1039.6, 1050.5)</td>
<td>(1039.4, 1047.1)</td>
</tr>
<tr>
<td><strong>Alcohol consumption†</strong></td>
<td>0.97 (0.88, 1.08)</td>
<td>1.29 (1.15, 1.46)</td>
<td>1.12 (1.04, 1.22)</td>
</tr>
<tr>
<td><strong>Alcohol drinker‡</strong></td>
<td>50.2 (46.3, 54.2)</td>
<td>51 (47.1, 55)</td>
<td>628 (50.7)</td>
</tr>
<tr>
<td><strong>Smoker§</strong></td>
<td>18.2 (15.2, 21.3)</td>
<td>15.1 (12.3, 17.9)</td>
<td>208 (16.7)</td>
</tr>
<tr>
<td><strong>Oral contraceptives user</strong></td>
<td>30 (26.3, 33.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physically active║</strong></td>
<td>21.3 (17.9, 24.8)</td>
<td>42.6 (38.3, 46.9)</td>
<td>334 (31.5)</td>
</tr>
<tr>
<td><strong>User of at least one recreational drug type¶</strong></td>
<td>13.2 (10.6, 15.9)</td>
<td>15.4 (12.6, 18.3)</td>
<td>179 (14.3)</td>
</tr>
<tr>
<td><strong>Annual family income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ A$ 35,000</td>
<td>13.8 (10.9, 16.8)</td>
<td>12.8 (10.1, 15.6)</td>
<td>145 (13.3)</td>
</tr>
<tr>
<td>A$ 35,001 - ≤ 78,000</td>
<td>33.3 (29.3, 37.4)</td>
<td>33 (29.1, 36.9)</td>
<td>361 (33.2)</td>
</tr>
<tr>
<td>&gt; A$ 78,000</td>
<td>52.8 (48.6, 57.1)</td>
<td>54.2 (50.1, 58.3)</td>
<td>583 (53.5)</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% confidence interval), or for categorical variable as percentage (95% confidence interval) or n (%) as indicated.

BMI: Body Mass Index; SEIFA: Socio-Economic Indexes for Areas

* Index of advantage/disadvantage, Australian Bureau of Statistics Socio-economic Indexes for Areas; † Average number of standard drinks consumed during the last 7 days; ‡ Consumer of alcohol at any level over the last 7 days; § Smoking ≥ 1 cigarettes in a week; ║ Having ≥ 4 hours of exercise in free time per week; ¶ Drug types included amphetamine, marijuana, and party drugs.
### Table 2  Clinical and Biochemical Characteristics, and Comorbidities of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Girls n = 620 (49.7%)</th>
<th>Boys n = 628 (50.3%)</th>
<th>Whole sample n = 1,248</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>mean (95%CI)</strong></td>
<td><strong>mean (95%CI)</strong></td>
<td><strong>mean (95%CI)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>108.6 (107.9, 109.4)</td>
<td>117.9 (117.1, 118.6)</td>
<td>113.3 (112.7, 113.9)</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>59.5 (59, 60)</td>
<td>58.2 (57.7, 58.7)</td>
<td>58.8 (58.5, 59.2)</td>
</tr>
<tr>
<td><strong>HR, bpm</strong></td>
<td>67.2 (66.5, 67.9)</td>
<td>62.9 (62.2, 63.6)</td>
<td>65.0 (64.5, 65.5)</td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.63 (4.59, 4.66)</td>
<td>4.82 (4.78, 4.87)</td>
<td>4.73 (4.70, 4.76)</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.64 (7.21, 8.1)</td>
<td>6.86 (6.49, 7.25)</td>
<td>7.22 (6.94, 7.52)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.30 (4.24, 4.37)</td>
<td>3.93 (3.87, 3.99)</td>
<td>4.11 (4.06, 4.15)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.95 (0.91, 0.98)</td>
<td>0.95 (0.92, 0.99)</td>
<td>0.95 (0.93, 0.97)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.39 (1.36, 1.42)</td>
<td>1.20 (1.18, 1.22)</td>
<td>1.29 (1.28, 1.31)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.43 (2.38, 2.49)</td>
<td>2.24 (2.19, 2.29)</td>
<td>2.33 (2.29, 2.37)</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>132.84 (127.2, 138.47)</td>
<td>122.93 (118.07, 127.78)</td>
<td>127.59 (123.89, 131.29)</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>41.45 (39.23, 43.8)</td>
<td>41.24 (39.37, 43.2)</td>
<td>41.34 (39.89, 42.84)</td>
</tr>
<tr>
<td>Sodium/Potassium ratio</td>
<td>2.85 (2.67, 3.04)</td>
<td>2.69 (2.54, 2.84)</td>
<td>2.76 (2.65, 2.88)</td>
</tr>
<tr>
<td><strong>Hypertensive status</strong></td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Adolescent gender-defined*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prehypertension</td>
<td>7.8 (5.7, 9.9)</td>
<td>8.3 (6.1, 10.5)</td>
<td>100 (8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7.8 (5.7, 9.9)</td>
<td>7.8 (5.7, 10)</td>
<td>97 (7.8)</td>
</tr>
<tr>
<td>Adult criteria†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prehypertension</td>
<td>8.9 (6.7, 11.4)</td>
<td>36 (32.2, 39.9)</td>
<td>280 (22.5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.3 (0.03, 1)</td>
<td>2.2 (1.2, 3.7)</td>
<td>16 (1.3)</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% confidence interval), or for categorical variable as percentage (95% confidence interval) or n (%) as indicated.

SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.

* Gender-specific SBP or DBP ≥ 90th percentile but < 95th percentile (prehypertension); ≥ 95th percentile (hypertension).

† SBP 120-139 mmHg or DBP 80-89 mmHg (prehypertension); ≥ 140 mmHg or DBP ≥ 90 mmHg (hypertension).
In univariate models examining lifestyle and sociodemographic factors (supplementary material Table S2), we found that SBP was significantly associated with male gender, BMI, urinary sodium, the urinary Na/K ratio, and being an alcohol drinker, plus OC use in girls. When adjusted for BMI (supplementary material Table S3), a significant association with SBP remained for male gender, alcohol drinker, OC use in girls, urinary sodium and the Na/K ratio. Dietary patterns were not related to SBP nor DBP. In girls, DBP was positively associated with OC use and negatively with BMI (Table S2). This BMI-DBP negative association in girls became non-significant when four subjects with BMI ≥ 40 were excluded (β=-0.08; p=0.221).

In multivariable models, male gender, alcohol drinking in boys, OC use in girls, BMI, and urinary Na/K ratio were positively associated with SBP (Table 4). SBP in boys
was 8.97 mmHg higher, as compared with girls. Girls using OC measured 3.27 mmHg higher in SBP, as compared with the non-OC users. There was a significant interaction between BMI, gender and OC use in girls. The relationship between BMI and SBP for boys differed when compared to girls not on OC (Figure 1). For an increase in BMI of 1 kg/m$^2$, SBP increased by 0.65 mmHg in boys compared to 0.38 mmHg in the non-OC using girls ($p=0.028$). However, the interaction term indicated there was no difference between boys and girls on OC ($p=0.522$) or OC using and non-OC using girls ($p=0.482$). We found DBP was negatively associated with physical activity and positively with OC use.

Using the final multivariable model, we compared boys and girls in the upper and lower quartiles for those factors that were significantly associated with SBP. In boys who were drinkers and were in the 75th percentiles for BMI and urinary Na/K ratio, their average was 119.9 mmHg SBP, compared to 114.1 mmHg for those who were not drinkers and in the 25th percentiles for both BMI and urinary Na/K ratio. In girls using OC and who were in the 75th percentiles for both BMI and urinary Na/K ratio, average SBP was 114.5 mmHg SBP compared to 105.9 mmHg for those not using OC and in the 25th percentiles for BMI and urinary Na/K ratio.
### Table 4 Multivariable Models for Adolescent Systolic and Diastolic Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender*</td>
<td>8.97</td>
<td>7.43, 10.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OC use</td>
<td>3.27</td>
<td>1, 5.54</td>
<td>0.005</td>
</tr>
<tr>
<td>Urinary Na/K ratio‡</td>
<td>0.27</td>
<td>0.02, 0.51</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>BMI§</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>0.65</td>
<td>0.48, 0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Girls using OC¶</td>
<td>0.52</td>
<td>0.17, 0.87</td>
<td>0.004</td>
</tr>
<tr>
<td>Girls not using OC</td>
<td>0.38</td>
<td>0.21, 0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Alcohol drinker#</strong></td>
<td>2.48</td>
<td>1.09, 3.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Boys</td>
<td>2.48</td>
<td>1.09, 3.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Girls using OC</td>
<td>-0.12</td>
<td>-1.8, 1.56</td>
<td>0.89</td>
</tr>
<tr>
<td>Girls not using OC</td>
<td>0.28</td>
<td>-2.29, 2.85</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Constant</strong></td>
<td>107.46</td>
<td>106.3, 108.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender*</td>
<td>-0.26</td>
<td>-1.15, 0.63</td>
<td>0.571</td>
</tr>
<tr>
<td>OC use</td>
<td>1.74</td>
<td>0.55, 2.93</td>
<td>0.004</td>
</tr>
<tr>
<td>Physical activity†</td>
<td>-1.22</td>
<td>-2.1, -0.34</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Constant</strong></td>
<td>60.22</td>
<td>58.95, 61.49</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*BMI: Body Mass Index; OC: oral contraceptives; Na/K ratio: sodium / potassium ratio.

* Reference group was girls not using OC.
‡ Na/K - mean Na/K.
§ BMI - mean BMI.
|| p=0.028, boys vs non-OC girls.
¶ p=0.482, OC using girls vs non-OC girls.
# Consumer of alcohol at any level over the last 7 days.
† ≥ 4 hours vs < 4 hours of exercise outside study or work per week.
Relationship of BMI and (A) systolic BP (with $p=0.028$, boys vs girls not using OC [Girls - OC]; $p=0.482$, girls using OC [Girls + OC] vs girls not using OC), or (B) diastolic BP in boys versus girls using or not using OC.
Discussion

In this adolescent population we have shown that there was a significant association of a range of lifestyle factors with BP, including some that were gender specific. Higher SBP was significantly associated with use of OC in girls and alcohol consumption in boys. In general, boys had significantly higher SBP than girls. Furthermore, OC use in girls was associated with higher DBP, as compared to non-OC using girls. There were also significant gender differences in the relationship between obesity and BP, as boys showed a steeper gradient for the effect of BMI on SBP than girls not taking OC.

In our study, SBP was 8.97 mmHg higher in boys compared with girls, consistent with previous reports on adolescent SBP.\(^2\) Using gender-specific percentiles to define hypertensive status, not surprisingly there was no difference between the sexes. However, in 17-years-olds it is perhaps more logical to employ adult criteria cut-offs; using the JNC-7 criteria, we found that approximately 24% of the adolescents were prehypertensive or hypertensive. In addition, we found marked gender differences in the prevalence of prehypertension (36% in boys vs 9% in girls) and hypertension (2% in boys vs 0.3% in girls). Gender differences in prehypertension prevalence were previously reported in an adolescent population aged 16-19 years.\(^{16}\)

The proportion of obese girls and boys that were prehypertensive in our study was 15% and 51%, respectively. As a measure of adiposity, BMI is a strong predictor of SBP in children and adolescents \(^{17}\) and was previously shown to have a similar relationship to BP compared with other anthropometric measurements when this same population was studied at age 14.\(^{18}\)

The 30% of our female adolescents who reported taking OC had significantly increased SBP and DBP, as compared with non-OC users. These data agree with results from a study of 17-year-old girls that shows a 4.6 mmHg higher SBP in low-dose OC users, \(^{19}\) which suggests a marked pressor effect of OC in adolescence. Even at low dose, OC use was previously shown to raise BP and prevalence of hypertension in adult women.\(^6\)
We observed a significant and positive association between SBP and drinking alcohol at any consumption level within the last 7 days in boys, but not in girls. Previously, in a cohort of 365 students aged 13-18 years, a weak, albeit significant relationship was found between frequency and quantity of alcohol consumption and DBP, with a greater prevalence of hypertension in male heavy drinkers.\textsuperscript{20} Similarly, others observed higher BP in 18-year-old Australians with alcohol binge drinking behaviours.\textsuperscript{21} Also, a stronger alcohol-SBP association was reported in older, compared with younger men, but not in women.\textsuperscript{22}

The association between sodium consumption and BP has been extensively documented in adults, children and adolescents.\textsuperscript{23} We observed a significant relationship between urinary Na/K ratio and SBP, in both boys and girls. The urinary Na/K ratio is shown to correlate strongly with age-related BP increase in adults.\textsuperscript{24} Our data reinforce evidence in favour of a diet balanced for lower intake of salt-containing foods and higher potassium-rich foods and vegetables.\textsuperscript{25}

With regards to the gender difference in the continuous relationship between BMI and SBP, a novel finding in our study was that in boys, there was a significantly greater increase in SBP with increasing BMI, as compared with girls that were not taking OC. Gender differences in BP most likely relate to different effects of testosterone and oestrogens on BP regulation,\textsuperscript{26} as well as to some of the lifestyle factors demonstrated here.

We confirm that the continuous BP-weight status is associated with hypertension risk, rather than there being a threshold effect. In adults, the existence of linearity or a threshold in the BMI-BP relationship has been equivocal,\textsuperscript{27} with some suggesting a threshold at 21 kg/m\textsuperscript{2} for BMI.\textsuperscript{28} Others have adopted this arbitrary figure for the interpretation of BP data in children.\textsuperscript{29} Our observation of there being a continuous positive linear relationship between BMI and SBP across the entire range of BMI in both boys and girls is important in this context, as it suggested that a BMI threshold in adolescence is not appropriate.

In adolescents of both sexes, moderate to vigorous physical activity has been reported to be inversely related to higher SBP levels.\textsuperscript{30} Our data showed that twice as many boys engaged in more regular physical activity, as compared with girls, a finding
that is consistent with other studies.\textsuperscript{31} We found that self-reported habitual exercise was negatively associated with DBP in both boys and girls. These results are in agreements with other reports\textsuperscript{32,33} about children and adolescents.

Applying our model that best described the association between SBP and lifestyle factors, our findings showed that boys that drank alcohol and were in the 75th percentiles for BMI and the Na/K ratio had 5.7 mmHg higher SBP, as compared to non-drinkers who were in the 25th percentiles for both BMI and urinary Na/K ratio. Similarly, girls taking OC who were in the 75th percentiles for both BMI and Na/K, SBP had 5.5 mmHg higher SBP, as compared to those not using OC and in the 25th percentiles for both BMI and urinary Na/K ratio.

Interpretation of our findings with regard to causality requires caution, in view of the cross-sectional design of the study. However, the strengths of the study include: having a population-based sample; a study large enough to detect gender effects and to analyse for the multiplicative effects of several lifestyle factors; and the collection of comprehensive and standardised phenotypic, lifestyle and socio-behavioural data by trained personnel.

In conclusion, in our study of 17-years-old adolescents, increased BP was associated with OC use in girls and alcohol consumption in boys, as well as increasing BMI, increasing Na/K ratio, and a lower level of physical activity. There were marked gender differences in SBP and its relation to increasing BMI. When applying adult criteria, we found that boys had four times the prevalence of prehypertension compared with girls. These substantial differences in SBP in boys and girls, between those with a healthier versus a less favourable lifestyle pattern, are likely to significantly affect their risk of both ischemic heart disease and stroke in adulthood. Adolescence is a time of life when unhealthy lifestyle behaviours that impact BP and related metabolic disorders tend to become entrenched. Our findings suggest that significant public health benefits may be achieved from implementation of a range of gender-appropriate lifestyle modifications within this age group of adolescents.
Acknowledgements

We thank the Raine Study participants and their families, the Raine Study Team for cohort coordination and data collection, Lynette McCahon for technical assistance and the National Health and Medical Research Council of Australia (NH&MRC) for funding the study.

Sources of Funding

The 17-year follow-up of the Raine Study was supported by NH&MRC Program [grant number 353514] and Project [grant number 403981] Grants. Core management of the Raine Study is funded by The University of Western Australia (UWA); the Raine Medical Research Foundation; the Telethon Institute for Child Health Research; the UWA Faculty of Medicine, Dentistry and Health Sciences; the Women and Infants Research Foundation and Curtin University. Dr Le-Ha is supported by an Endeavour Postgraduate Award from the Australian Government.

Conflict of Interest

None declared.

References


22. van Leer EM, Seidell JC, Kromhout D. Differences in the association between alcohol consumption and blood pressure by age, gender, and smoking. *Epidemiology* 1994;5:576-582.


Table S1  Comparing the 17-Year Sample to Non-Participants at 17-Year Survey

<table>
<thead>
<tr>
<th></th>
<th>Participants</th>
<th>Non-Participants</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1,248</td>
<td>N=1,621</td>
<td></td>
</tr>
<tr>
<td>Family income at 1st year follow-up</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; A$ 24,000</td>
<td>35.4</td>
<td>46.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ A$ 24,000</td>
<td>64.6</td>
<td>53.7</td>
<td></td>
</tr>
<tr>
<td>Gestational age, days</td>
<td>275.3 (14.1)</td>
<td>273.1 (18.1)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mother’s weight, kg*</td>
<td>59.6 (11.8)</td>
<td>60.1 (12.6)</td>
<td>0.31</td>
</tr>
<tr>
<td>Mother’s height, cm*</td>
<td>163.8 (6.7)</td>
<td>163.4 (6.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3327.8 (579.1)</td>
<td>3253.1 (650.6)</td>
<td>0.0014</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>49 (2.6)</td>
<td>48.7 (3.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49.7</td>
<td>49</td>
<td>0.1498</td>
</tr>
<tr>
<td>Male</td>
<td>50.3</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as percentage for categorical variables, and mean (standard deviation) for continuous variables.
* At 18th week of pregnancy.
<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>DBP</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>9.06</td>
<td>8.04, 10.08</td>
<td>&lt;0.001</td>
<td>-1.27</td>
<td>-2.005, -0.539</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.34</td>
<td>-0.72, 3.41</td>
<td>0.201</td>
<td>0.94</td>
<td>-0.54, 2.42</td>
<td>0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Girls</td>
<td>0.38</td>
<td>0.22, 0.53</td>
<td>&lt;0.001</td>
<td>-0.13</td>
<td>-0.25, -0.02</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>0.7</td>
<td>0.53, 0.87</td>
<td>0.005</td>
<td>0.037</td>
<td>-0.09, 0.16</td>
<td>0.555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEIFA score*</td>
<td>-0.007</td>
<td>-0.014, 0.0008</td>
<td>0.08</td>
<td>-0.004</td>
<td>-0.009, 0.0018</td>
<td>0.196</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity†</td>
<td>-0.87</td>
<td>-2.08, 0.34</td>
<td>0.16</td>
<td>-1.29</td>
<td>-2.17, -0.42</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy diet pattern score</td>
<td>-0.23</td>
<td>-0.89, 0.44</td>
<td>0.501</td>
<td>0.099</td>
<td>-0.379, 0.579</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western diet pattern score</td>
<td>0.032</td>
<td>-0.64, 0.71</td>
<td>0.93</td>
<td>0.03</td>
<td>-0.46, 0.52</td>
<td>0.901</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption‡</td>
<td>0.26</td>
<td>-0.07, 0.59</td>
<td>0.12</td>
<td>0.23</td>
<td>-0.009, 0.46</td>
<td>0.059</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol drinker§</td>
<td>1.55</td>
<td>0.53, 2.57</td>
<td>0.003</td>
<td>0.71</td>
<td>-0.026, 1.45</td>
<td>0.059</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking‖</td>
<td>-0.0001</td>
<td>-0.029, 0.029</td>
<td>0.99</td>
<td>-0.002</td>
<td>-0.023, 0.018</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary sodium</td>
<td>0.013</td>
<td>0.003, 0.024</td>
<td>0.008</td>
<td>0.004</td>
<td>-0.003, 0.012</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary potassium</td>
<td>-0.013</td>
<td>-0.036, 0.008</td>
<td>0.23</td>
<td>-0.009</td>
<td>-0.026, 0.007</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary sodium / potassium ratio</td>
<td>0.44</td>
<td>0.18, 0.69</td>
<td>0.001</td>
<td>0.19</td>
<td>-0.007, 0.38</td>
<td>0.059</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive use (girls)</td>
<td>3.11</td>
<td>1.58, 4.64</td>
<td>&lt;0.001</td>
<td>1.78</td>
<td>0.66, 2.91</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table S2** Univariate Models of Lifestyle and Socio-Demographic Factors Affecting Systolic and Diastolic Blood Pressure

*Index of advantage/disadvantage, Australian Bureau of Statistics Socio-Economic Indexes for Areas; † ≥ 4 hours vs < 4 hours of exercise outside study or work per week; ‡ Average number of standard drinks consumed during the last 7 days; § Consumer of alcohol at any level over the last 7 days; ‖ Number of cigarettes smoked in a week. All factors were adjusted for sex, except for OC use. Values for girls and boys were separately presented if the interaction of gender and the factor was significant.

_BMI indicates Body Mass Index; SEIFA, Socio-Economic Indexes for Areas_
Table S3  Sex and BMI Adjusted Models of Lifestyle and Socio-Demographic Factors Affecting Systolic and Diastolic Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>SBP</th>
<th></th>
<th></th>
<th></th>
<th>DBP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>95% CI</td>
<td>p</td>
<td>Regression coefficient</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Male</td>
<td>9.33</td>
<td>8.34, 10.33</td>
<td>&lt;0.001</td>
<td>-1.29</td>
<td>-2.03, -0.56</td>
<td>0.001</td>
</tr>
<tr>
<td>Age</td>
<td>1.35</td>
<td>-0.71, 3.4</td>
<td>0.198</td>
<td>0.81</td>
<td>-0.71, 2.32</td>
<td>0.3</td>
</tr>
<tr>
<td>SEIFA score*</td>
<td>-0.001</td>
<td>-0.009, 0.005</td>
<td>0.64</td>
<td>-0.004</td>
<td>-0.009, 0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Physical activity†</td>
<td>-0.97</td>
<td>-2.14, 0.21</td>
<td>0.11</td>
<td>-1.28</td>
<td>-2.16, -0.41</td>
<td>0.004</td>
</tr>
<tr>
<td>Healthy diet pattern score</td>
<td>-0.315</td>
<td>-0.961, 0.331</td>
<td>0.34</td>
<td>0.105</td>
<td>-0.375, 0.586</td>
<td>0.67</td>
</tr>
<tr>
<td>Western diet pattern score</td>
<td>0.005</td>
<td>-0.652, 0.663</td>
<td>0.99</td>
<td>0.041</td>
<td>-0.448, 0.531</td>
<td>0.87</td>
</tr>
<tr>
<td>Alcohol consumption‡</td>
<td>0.22</td>
<td>-0.09, 0.54</td>
<td>0.17</td>
<td>0.23</td>
<td>-0.003, 0.47</td>
<td>0.053</td>
</tr>
<tr>
<td>Alcohol drinker§</td>
<td>1.53</td>
<td>0.54, 2.52</td>
<td>0.003</td>
<td>0.71</td>
<td>-0.02, 1.45</td>
<td>0.058</td>
</tr>
<tr>
<td>Smoking║</td>
<td>-0.007</td>
<td>-0.036, 0.020</td>
<td>0.6</td>
<td>-0.001</td>
<td>-0.022, 0.019</td>
<td>0.89</td>
</tr>
<tr>
<td>Urinary sodium</td>
<td>0.0104</td>
<td>0.0002, 0.0205</td>
<td>0.045</td>
<td>0.004</td>
<td>-0.003, 0.012</td>
<td>0.24</td>
</tr>
<tr>
<td>Urinary potassium</td>
<td>-0.015</td>
<td>-0.037, 0.006</td>
<td>0.18</td>
<td>-0.009</td>
<td>-0.026, 0.007</td>
<td>0.29</td>
</tr>
<tr>
<td>Urinary sodium / potassium ratio</td>
<td>0.37</td>
<td>0.12, 0.63</td>
<td>0.004</td>
<td>0.19</td>
<td>-0.003, 0.39</td>
<td>0.055</td>
</tr>
<tr>
<td>Oral contraceptive use (girls)</td>
<td>3.5</td>
<td>2.007, 4.99</td>
<td>&lt;0.001</td>
<td>1.71</td>
<td>0.58, 2.84</td>
<td>0.003</td>
</tr>
</tbody>
</table>

SEIFA, Socio-Economic Indexes for Areas
* Index of advantage/disadvantage, Australian Bureau of Statistics Socio-Economic Indexes for Areas; † ≥ 4 hours vs < 4 hours of exercise outside study or work per week; ‡ Average number of standard drinks consumed during the last 7 days; § Consumer of alcohol at any level over the last 7 days;║ Number of cigarettes smoked in a week. All factors were adjusted for Sex and BMI, except for OC use adjusted for BMI.
Chapter 3

Gender Difference in the Relationship between Passive Smoking Exposure and HDL-Cholesterol Levels in Late Adolescence

Preamble

Smoking exposure, both active and passive, is highly associated with atherosclerotic CVD, and has been an important component of cardiovascular health promotion programmes. In adolescents, little is known about the sex differences in the relationship between passive smoking and CVD risk factors.

In Chapter Three the long-term detrimental impact of passive smoking exposure in the household from birth, and the impact of maternal smoking during pregnancy, on HDL-cholesterol levels of the adolescents at 17 years of age were examined.

This chapter was published in the Journal of Clinical Endocrinology and Metabolism in May 2013, vol 98, issue 5, 2126-2135.
Abstract

Background: High-density lipoprotein-cholesterol (HDL-C) levels are influenced by gender and by genetic and environmental factors. We aimed to assess the impact of passive smoking exposure since birth on HDL-C levels of non-smoking adolescents at age 17 years and to determine whether there was a gender difference in the relationship between smoking exposure and HDL-C.

Methods: A total of 804 non-smoking adolescents with biochemical, anthropometric and lifestyle data from a cohort of 1754 adolescents (mean age, 17 ± 0.25 y) of the Western Australian Pregnancy Cohort (Raine) Study had data of maternal smoking during pregnancy and smoking exposure in the household over 17 years. HDL-C was analysed using multivariable linear regression, with adjustment for early-life, adiposity and current lifestyle confounders.

Results: HDL-C levels were significantly lower in girls exposed to passive smoking compared to those not exposed (regression coefficient b = -0.09 [95% confidence interval, -0.15, -0.03]); this was not observed in boys (b = 0.02 [95% confidence interval, -0.04, 0.08]), with a significant sex interaction p = 0.009. The effects of passive smoking in girls persisted after adjusting for oral contraceptive use.

Conclusions: This study has shown a gender difference in the relationship between passive smoking exposure since birth and HDL-C in late adolescence. Exposure to passive smoking in girls could have adverse consequences on their risk of cardiovascular disease in adulthood. These findings reinforce the need for future public health measures to reduce children’s exposure to passive smoking.
Introduction

Passive tobacco smoke exposure is an important modifiable risk factor of cardiovascular disease (CVD). In non-smoking adults, passive smoking is associated with 50% higher CVD events than in those not exposed.\textsuperscript{1} Arterial changes of preclinical arteriosclerosis have been shown in children exposed to tobacco smoke,\textsuperscript{2} and parental smoking exposure in childhood has been associated with brachial artery flow-mediated dilatation in adulthood.\textsuperscript{3} In adults, low levels of high density lipoprotein cholesterol (HDL-C) are associated with cardiovascular events and are a major indicator of elevated CVD risk.\textsuperscript{4} Studies of samples of boys and girls that did not differentiate by sex, demonstrated that children exposed to passive smoking had an unfavourable lipid profile,\textsuperscript{5} including lower HDL-C levels.\textsuperscript{6} Furthermore, maternal smoking in pregnancy has recently been reported to be associated with HDL-C in 8-year-old children.\textsuperscript{7} However, because maternal prenatal smoking is almost always associated with postnatal smoking, it is methodologically difficult to address the role of prenatal smoking \textit{per se}.

Because CVD is the leading cause of death in women in the western world, gender has been an important issue in risk assessment for CVD.\textsuperscript{8,9} Smoking may be one factor influencing this gender difference. Despite higher rates of smoking in men compared with women, smoking confers a 25% greater coronary heart disease risk in women compared with men.\textsuperscript{10} In adults, environmental factors such as smoking and alcohol consumption can affect plasma HDL-C concentrations, and this association varies by gender.\textsuperscript{11,12} The question arises as to whether passive smoking influences HDL-C levels in children and whether there is a significant gender difference in this relationship.

Using data from a longitudinal birth cohort, we aimed to determine whether passive smoking exposure since birth and throughout childhood is associated with lower HDL-C levels in non-smoking 17-year-old adolescents and whether there is a gender difference in this association. We also investigated whether maternal smoking during pregnancy, apart from passive smoking exposure in the household, was associated with lower HDL-C in the adolescents.
Methods

The study population comprised 1754 adolescents and their families who participated in the 17-year follow-up of the Western Australian Pregnancy Cohort (Raine) Study. Details of the cohort at birth were previously published. In brief, 2868 children born to the 2900 women were enrolled at 18 weeks gestation between 1989 and 1992 at King Edward Memorial Hospital and nearby clinics in Perth, Western Australia. Ongoing longitudinal follow-up investigations were carried out at 1, 2, 3, 5, 8, 10, 14, and 17 years of age. The study and all follow-ups were approved by the Human Ethics Committee at King Edward Memorial Hospital and Princess Margaret Hospital for Children in Perth. The adolescents and their primary care giver provided written informed consent for participation. With children of socially disadvantaged families less likely to remain in the study beyond the third year, participants at 17 years were more representative of the Western Australian population. These adolescents were more likely to be from a higher income family, of later gestational age, and have higher birth weight.

Figure 1 provides a CONSORT diagram of the study samples. At age 17 years, 1754 adolescents underwent the following assessments: 1) height, weight, body mass index (BMI) and skinfold thickness; 2) measures of arterial stiffness and blood pressure; 3) fasting blood for biochemistry; and 4) completed questionnaires regarding nutrition, physical activity, drug and alcohol behaviour. A total of 1057 adolescents had biochemical, anthropometric, and lifestyle data available for analysis.
Figure 1  CONSORT diagram of adolescents attending the Raine Study 17-year follow-up
Smoking exposure

Maternal smoking exposure status was assessed by questionnaire at the first clinical visit at 18 weeks gestation. From the question “Do you smoke cigarettes now?”, mothers were asked to quantify their smoking as: 0 cigarette per day, 1-5/day, 6-10/d, 11-15/d, 16-20/d, or 21 or more cigarettes per day. Maternal smoking in pregnancy and passive smoking was defined as consuming 1 or more cigarettes daily. During follow-up visits from years 1 to 17, information on passive smoking exposure in the household was obtained by questionnaire to the mother or caregiver at each of the surveys. Mothers/caregivers were asked “How many cigarettes do you smoke a day now?”, “Does anyone living in your house smoke?”, and “How much do they smoke?” The number of cigarettes smoked was quantified into categories as above. By taking mid-point values for each range (0.5, 3, 8, 13, 18, 20, respectively), the number of cigarettes smoked daily was calculated. From smoking information obtained across 8 survey time points in 17 years, a total quantity of cigarettes smoked daily in the household was calculated as the sum of smoking by the mother/caregiver and others living in the house. The average quantity of passive smoking exposure across 17 years was assessed as the total quantity of cigarettes smoked daily in the household divided by 8 survey points and then categorised using the original ranges. Using a cut point of ≥ 1 cigarettes smoked daily defined the dichotomous indicator of passive smoking exposure.

The smoking status of the adolescents at 17 years was obtained via an online questionnaire. The adolescents were asked: “Have you ever smoked cigarettes in the past 12 months?” and “Have you smoked cigarettes in the past 4 weeks?”, and the number of cigarettes consumed in the last 7 days were recorded.

Anthropometry measures and blood analyses

As described previously, venous blood for the determination of serum insulin, glucose, total cholesterol, triglycerides, HDL-C, low density lipoprotein cholesterol (LDL-C), and high-sensitivity C-reactive protein was obtained after an overnight fast. Analyses were performed in the PathWest Laboratory at Royal Perth Hospital.
Homeostasis assessment for insulin resistance, an estimate of insulin resistance,\textsuperscript{16} was computed by dividing the product of insulin (mU/L) and glucose (mmol/L) by 22.5. Body weight was measured with a Wedderburn Chair Scale (nearest 100g), and height, with a Holtain Stadiometer (nearest 0.1 cm). Resting supine blood pressure were obtained after resting 5 minutes, using an oscillometric sphygmomanometer (Dinamap ProCare 100, Soma Technology, Bloomfield, Connecticut) with an appropriate cuff size. The average of the last 5 of 6 readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively, was calculated.

**Demographic and socio-behavioural features of the adolescents at 17 years**

Annual family income was categorised as Australian dollars ≤ 35,000, 35,001 to ≤ 78,000, and > 78,000 (calendar years 2005-2007). The adolescents were asked, via online questionnaire, the alcoholic beverage types (beer, wine or spirits) and amount (can, glass, stubby, nip or standard drink) consumed during the past week. Adolescent alcohol drinker was defined as consumer of alcohol at any level in a drinking day during the last 7 days. Oral contraceptive (OC) use was obtained 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?”). Physical fitness was determined using an objective estimate of aerobic fitness with the PWC 170 test, which assesses physical working capacity at a heart rate of 170 beats per minute\textsuperscript{17} and has been validated in our study population.\textsuperscript{18} Dietary patterns (“Healthy” and “Western”) were identified from a 212-item food frequency questionnaire using factor analysis with varimax rotation\textsuperscript{19}; separate scores for each pattern were generated.

**Early life factors**

Birth weight (in grams) was obtained from the baby’s medical records. Gestational age (in weeks) was calculated based on the date of the last menstrual period, unless there was discordance of more than seven days with ultrasound measurements <18-weeks; in those cases the estimate was based on ultrasound biometry at 18-weeks gestation. Breast feeding (duration < 4 months vs ≥ 4 months) information was obtained from the
questions to the mother at year 1: “Did you breastfeed your baby?” and “Are you still breastfeeding?”.

**Statistical analysis**

HDL-C was analysed as a continuous outcome variable. Candidate factors examined comprised age, sex, BMI, family income, alcohol consumption, dietary patterns, PWC170 aerobic fitness score, OC use, and early-life factors including birth weight, gestational age, and breastfeeding duration. To avoid the loss of power, BMI, dietary pattern scores, and PWC170 scores were analysed as continuous variables. The relationship between quantities of alcohol consumed and HDL-C levels was investigated using fractional polynomials. This indicated an absence of additional effects on HDL-C for increasing consumption of 1 or more standard drinks, hence a dichotomous yes/no variable was chosen for the analysis. Robust linear regression was employed in all analyses, to minimise the influence of potential outliers. Univariate models were initially adjusted for sex, and extended models additionally adjusted for BMI.

Interactions between maternal smoking in pregnancy and passive smoking exposure in the household as well as the 3-way interaction between these and gender were of particular interest. The small numbers (n=4) in the category of prenatal smoking with no subsequent passive smoking hindered the formal assessment of interactions. Hence, this group was excluded, and a composite smoking variable was generated with 3 mutually exclusive categories: 1) no maternal smoking in pregnancy and no passive smoking in the household during 17 years; 2) no maternal smoking in pregnancy and passive smoking; and 3) maternal smoking in pregnancy and passive smoking.

Multivariable regression models for HDL-C were established with the above composite smoking variable. Confounding factors included sex, BMI, family income, dietary patterns score, PWC170 score, alcohol drinker, birth weight, gestational age and breastfeeding duration. Differences in gender effects were assessed via sex interaction terms. Non-significant factors were removed in a backward process,
examining the effects on coefficients and p values of all remaining factors, at each step. Further adjustment for OC use was carried out in the girls. The variance inflation factor was investigated to ensure that associations between the covariates were not adversely affecting the standard errors. Given the known inverse relationship between HDL and triglycerides, the final model obtained for HDL-C was repeated substituting triglycerides as the outcome to examine for any relationship between passive smoking and triglycerides.

In a separate analysis in 1035 adolescents with complete information on active smoking at 17 years (22 participants did not provide complete information on their current smoking status), the relationship between current active smoking and HDL-C was examined in multivariable stepwise models, adjusting for the potential confounders as mentioned above.

Analysis was performed using Stata version 12 (Stata Corp, College Station, Texas). Results are interpreted with reference to significance at p<0.05.

Results

The whole sample

Of the 1754 adolescents that participated at the 17-year survey, 1057 had biochemical, anthropometric and lifestyle data available. There were no differences in age, gender, birth weight, gestational age, family income, maternal smoking in pregnancy, and maternal alcohol consumption, between the 1057 adolescents in the study and those that did not participate. Demographic, anthropometric and socio-behavioural features are shown in Table 1. In the whole sample, 51% of the adolescents were drinkers, 21% smokers, and 31.5% of the girls used OC. Sixty nine percent of the adolescents were breastfed ≥ 4 months.

At week 18 of pregnancy, 19.8% of the mothers and 32.6% of the fathers smoked, and the overall smoking rate in the household was 40.2%. From birth to 17
years, 48% of the sample had been exposed to passive smoking in the household. In regression models adjusting for sex (Table 2), higher HDL-C levels were associated with lower BMI (p<0.001) and longer duration of breastfeeding (p=0.007). Additional adjustment for BMI showed that breastfeeding duration remained significantly (p=0.02) associated with HDL-C levels. Alcohol consumption was associated with increased HDL-C levels in boys (p=0.04), but not in girls (sex interaction p=0.012).

**Non-smoking adolescents**

To exclude the effects of current active smoking on HDL-C, analysis of maternal prenatal smoking and passive smoking effect was performed on the 800 non-smoking adolescents (mean age, 17 ± 0.25 y), after excluding the 4 with prenatal smoking but no subsequent passive smoking (Table 1). With the composite passive smoking exposure variable, the numbers of boys and girls were similar within the categories.
Table 1  Demographic and Socio-behavioural Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>Whole sample (n = 1,057)</th>
<th>Non-smoking adolescent sample (n = 800)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No passive smoking &amp; no maternal smoking in pregnancy (n = 441)</td>
</tr>
<tr>
<td></td>
<td>Girls (n = 511; 48.3%)</td>
<td>Boys (n = 546; 51.7%)</td>
</tr>
<tr>
<td>Age, y</td>
<td>17.06 (17.04, 17.08)</td>
<td>17.02 (17, 17.04)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>62.8 (61.8, 63.8)</td>
<td>71.3 (70.1, 72.4)</td>
</tr>
<tr>
<td>Waist circumference, mm</td>
<td>769 (760, 779)</td>
<td>800 (791, 808)</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>22.8 (22.4, 23.1)</td>
<td>22.4 (22.1, 22.8)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>108.6 (107.8, 109.4)</td>
<td>118 (117.2, 118.8)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>59.4 (58.8, 60)</td>
<td>58.1 (57.6, 58.7)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3290.3 (3239.4, 3341.3)</td>
<td>3364.7 (3315.9, 3413.6)</td>
</tr>
<tr>
<td>Gestational age, w</td>
<td>39.3 (39.1, 39.5)</td>
<td>39.3 (39.1, 39.5)</td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.63</td>
<td>4.82</td>
</tr>
<tr>
<td>----------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>(4.59, 4.66)</td>
<td>(4.78, 4.87)</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.65</td>
<td>6.87</td>
</tr>
<tr>
<td></td>
<td>(7.22, 8.11)</td>
<td>(6.5, 7.26)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.30</td>
<td>3.93</td>
</tr>
<tr>
<td></td>
<td>(4.24, 4.37)</td>
<td>(3.87, 3.99)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>(0.91, 0.98)</td>
<td>(0.92, 0.99)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.39</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(1.36, 1.42)</td>
<td>(1.18, 1.23)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.44</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>(2.38, 2.49)</td>
<td>(2.19, 2.3)</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>0.86</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>(0.77, 0.96)</td>
<td>(0.46, 0.57)</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>1.57</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>(1.48, 1.67)</td>
<td>(1.39, 1.56)</td>
</tr>
<tr>
<td>Socio-behavioural</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWC170 scoreb</td>
<td>1.59</td>
<td>2.17</td>
</tr>
<tr>
<td></td>
<td>(1.55, 1.62)</td>
<td>(2.12, 2.22)</td>
</tr>
<tr>
<td>Alcohol drinker, %c</td>
<td>49.2</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>(44.8, 53.5)</td>
<td>(47.8, 56.2)</td>
</tr>
<tr>
<td>Smoker at year 17, %d</td>
<td>23.7</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td>(19.9, 27.4)</td>
<td>(15.2, 21.9)</td>
</tr>
<tr>
<td>OC user, %</td>
<td>31.5</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>(27.4, 35.6)</td>
<td>(19.2, 31.8)</td>
</tr>
<tr>
<td>Physically active, %e</td>
<td>20.9</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>(17.2, 24.7)</td>
<td>(38.5, 47.7)</td>
</tr>
<tr>
<td>Annual family income, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ A$35,000</td>
<td>14.3</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>(11, 17.6)</td>
<td>(9.7, 15.6)</td>
</tr>
<tr>
<td>A$35,001 – ≤ A$78,000</td>
<td>34.1</td>
<td>33.4</td>
</tr>
<tr>
<td></td>
<td>(29.7, 38.6)</td>
<td>(29.2, 37.6)</td>
</tr>
<tr>
<td>Breathing (%)</td>
<td>51.5 (46.7, 56.1)</td>
<td>53.8 (49.4, 58.3)</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Breast feeding%</td>
<td>5.9 (3.8, 8.1)</td>
<td>7.1 (4.9, 9.3)</td>
</tr>
<tr>
<td>≤ 4 months</td>
<td>24.4 (20.6, 28.3)</td>
<td>24.4 (20.7, 28.2)</td>
</tr>
<tr>
<td>&gt; 4 months</td>
<td>69.5 (65.4, 73.6)</td>
<td>68.4 (64.4, 72.4)</td>
</tr>
</tbody>
</table>

Abbreviations:

SBP, systolic blood pressure; DBP, diastolic blood pressure; hsCRP: high-sensitivity C-reactive protein;

HOMA-IR, homeostasis model of assessment for insulin resistance. Data are expressed as mean or percentage (95% confidence interval)

a After excluding 4 adolescents whose mother smoked in pregnancy and with no subsequent exposure to passive smoking;
b Score of the Physical Working Capacity 170 test, adjusted for weight;
c Consumer of alcohol at any level over the last 7 days; d Smoking ≥ 1 cigarettes in a week;
e Having ≥ 4 hours of exercise in free time per week
<table>
<thead>
<tr>
<th></th>
<th>Models adjusted for Sex</th>
<th>Models adjusted for Sex and BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDL-C</td>
<td>HDL-C</td>
</tr>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.015</td>
<td>-0.019, -0.011</td>
</tr>
<tr>
<td>Healthy diet pattern score</td>
<td>0.004</td>
<td>-0.018, 0.026</td>
</tr>
<tr>
<td>Western diet pattern score</td>
<td>0.003</td>
<td>-0.022, 0.028</td>
</tr>
<tr>
<td>Aerobic fitness pwc170 score</td>
<td>-0.0001</td>
<td>-0.0006, 0.0004</td>
</tr>
<tr>
<td>Oral contraceptive use (girls)</td>
<td>0.025</td>
<td>-0.03, 0.08</td>
</tr>
<tr>
<td>Drinker&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>-0.03</td>
<td>-0.078, 0.015</td>
</tr>
<tr>
<td>Boys</td>
<td>0.03</td>
<td>-0.01, 0.08</td>
</tr>
<tr>
<td>Annual family income in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australian dollars&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A$ 35,001 - ≤ 78,000</td>
<td>0.03</td>
<td>-0.02, 0.08</td>
</tr>
<tr>
<td>&gt; A$ 78,000</td>
<td>0.04</td>
<td>-0.01, 0.09</td>
</tr>
<tr>
<td>Breast feeding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4 mo</td>
<td>0.04</td>
<td>-0.028, 0.118</td>
</tr>
<tr>
<td>≥ 4 mo</td>
<td>0.09</td>
<td>0.025, 0.161</td>
</tr>
</tbody>
</table>

*Abbreviation: CI, confidence interval; <sup>a</sup> Sex interaction p=0.045, and p=0.012 (adjusted for BMI); <sup>b</sup> Reference: ≤ A$ 35,000.*
Adolescents whose mother smoked during pregnancy and subsequently exposed to passive smoking had significantly higher levels of daily cigarette quantity consumed in the household over 17 years, compared to those exposed to passive smoking whose mother did not smoke in pregnancy \( p < 0.001 \) (Supplemental Table 1, published on The Endocrine Society’s Journals Online website at http://jcem.endojournals.org). There were higher proportions of the adolescents in the maternal smoking in pregnancy group that were exposed to passive smoking, compared with the no maternal smoking group in the 2 most recent surveys (58.1% vs 40.4% at year 17 \( p < 0.001 \) and 66.2% vs 46.8% at year 14 \( p < 0.001 \)).

In stepwise multivariable analysis (Table 3), passive smoking exposure since birth in the adolescents with no maternal smoking in pregnancy was negatively associated with HDL-C levels in girls \( (b = -0.094; p = 0.003) \), but not in boys \( \text{sex interaction} p = 0.009 \), after adjusting for the effects BMI. However maternal smoking in pregnancy with subsequent passive smoking in the household was not associated with HDL-C in either girls \( (b = -0.018; p = 0.624) \) or boys \( (b = 0.001; p = 0.971) \). The inclusion of triglycerides as a covariate in the final HDL-C model demonstrated the expected inverse relationship with HDL-C as well as the independence of the relationship between HDL-C and passive smoking from triglycerides. In a model with triglycerides as the outcome and identical covariates as the final HDL-C model, no association was detected with passive smoking. Consuming alcohol had no effect on the passive smoking-HDL-C relationship (Supplemental Table 2). The inverse association between HDL-C and passive smoking in the absence of maternal smoking in pregnancy remained significant in multivariable analysis in girls, after further adjustment for OC use (Table 4).
Table 3  Multiple effects of sex, BMI, passive smoking, and maternal smoking in pregnancy on HDL-C levels in non-smoking adolescents (n=800)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Passive smoking exposure(^b)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In girls not exposed to maternal smoking in pregnancy</td>
<td>-0.094</td>
<td>-0.155, -0.033</td>
<td>0.003</td>
</tr>
<tr>
<td>In girls exposed to maternal smoking in pregnancy</td>
<td>-0.018</td>
<td>-0.09, 0.054</td>
<td>0.624</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>-0.229</td>
<td>-0.278, -0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sex interaction with exposure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive smoking and no maternal smoking in pregnancy</td>
<td>0.112(^c)</td>
<td>0.028, 0.196</td>
<td>0.009</td>
</tr>
<tr>
<td>Passive smoking and maternal smoking in pregnancy</td>
<td>0.019(^d)</td>
<td>-0.081, 0.12</td>
<td>0.706</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>-0.015</td>
<td>-0.02, -0.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Constant</strong></td>
<td>1.421</td>
<td>1.384, 1.457</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Multivariable model adjusted for BMI, birth weight, gender, family income, alcohol consumption, dietary patterns, physical fitness, gestational age, and breast feeding duration; \(^a\) After excluding 4 adolescents whose mother smoked in pregnancy and with no subsequent exposure to passive smoking; \(^b\) reference: No passive smoking and no maternal smoking in pregnancy; \(^c\) equates to coefficient of 0.018 (95%CI -0.04, 0.076) for boys; \(^d\) equates to coefficient of 0.001 (95%CI -0.069, 0.071) for boys.
Table 4  Multivariable models for the effects of passive smoking and maternal smoking in pregnancy on HDL-C in non-smoking adolescent girls (n=374)

<table>
<thead>
<tr>
<th></th>
<th>Model A</th>
<th>Model B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>Passive smoking and no maternal smoking in pregnancy</td>
<td>-0.085</td>
<td>-0.152, -0.018</td>
</tr>
<tr>
<td>Passive smoking and maternal smoking in pregnancy</td>
<td>-0.01</td>
<td>-0.089, 0.068</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.02</td>
<td>-0.027, -0.013</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>Constant</td>
<td>1.891</td>
<td>1.735, 2.046</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

Model A, adjusted for BMI, birth weight, gender, family income, alcohol consumption, dietary patterns, physical fitness, gestational age, and breast feeding duration; Model B, adjusted further for oral contraceptive use.

Reference: No passive smoking and no maternal smoking in pregnancy. Italicised values represent the constant in the statistical model.

To assess an acute effect of passive smoking, in separate multivariable regression models we examined the association between passive smoking exposure at year 17 and HDL-C. There was no association between passive smoking exposure at year 17 and HDL-C in those adolescents with or without maternal smoking in pregnancy (p=0.678 and p=0.554, respectively).
**Active smoking in the adolescents**

The effect of active smoking on HDL-C at 17 years was examined in 1035 adolescents, of whom 21% were active smokers. There was a negative association between active smoking and HDL-C levels in girls (b=-0.077; p=0.005), but not in boys (sex interaction p=0.012) (Supplemental Table 3). In models examining girls only (n=503), the effect on HDL-C with additional adjustment for OC use remained significant (b=-0.078; p=0.011) (Supplemental Table 4).

**Discussion**

To our knowledge, this is the first study that shows a gender difference in the relationship between passive smoking exposure and HDL-C levels in late adolescence, with smoking data prospectively collected during pregnancy and over 17 years in a longitudinal population-based birth cohort. Passive smoking during the period since birth was associated with reduced HDL-C levels at 17 years of age in girls but not boys. In contrast, there was no evidence of an effect of smoking by the mother in pregnancy on HDL-C levels of their offspring.

The present findings can be viewed in the context of reports regarding gender differences in adult cardiovascular disease risk. HDL-C has been considered to be a more important CVD risk factor in women than in men. In adults, female smokers but not male smokers had lower HDL-C and large HDL-C particle concentrations compared with non-smokers. Although in children and adolescents, passive smoking has been shown to be associated with lower HDL-C levels, the estimates have mostly been from pooled samples of boys and girls. In a study of pre-pubertal children exposed to passive smoking, non-significant lower HDL$_2$ cholesterol levels were reported in boys compared with girls, whereas HDL$_3$ cholesterol levels were lower in girls. Complex interactions between passive smoking, HDL-C levels, adiposity, gender and race were shown when these children were re-examined at puberty. However, the issue of gender difference does not appear to have previously been addressed in the late adolescent age group. In our study, non-
smoking girls exposed to passive smoking since birth had HDL-C levels on average 0.094 mmol/L lower than those not exposed to passive smoking. Furthermore, our finding of no association between HDL-C levels and passive smoking exposure at year 17 excludes an effect of recent exposure to passive smoking on HDL-C. In a separate analysis of the effect of active smoking in the adolescents on HDL-C, we found the same gender difference pattern with similar effect size, again in girls but not boys. These findings are of clinical significance because it has been shown in the Framingham Heart Study and other population clinical trials that an increase of 0.026 mmol/L in HDL-C was associated with a significant CVD risk reduction of 2% in men and 3% in women.\(^{25}\) Furthermore, in adults it has been suggested that the impact of passive smoking on cardiovascular risk is almost as great as that of active smoking.\(^{26}\) In adult men and women, a low level of passive smoking exposure may also adversely affect endothelial function.\(^ {27}\)

Gender is an important modulator in the complex interaction between genetic, biological and environmental factors, and disease. The role of hormonal status in the gender difference in the genotype-environment interaction on HDL-C has been proposed.\(^ {28}\) In our study, however, adjustment for OC use did not alter the effect size of passive or active smoking on HDL-C in girls. With 98% of the boys and 97% of the girls in our study being post-pubertal (Tanner stages 4 or 5), puberty \textit{per se} could not account for these findings. In genetic studies of HDL-C metabolism, \textit{TagI}B polymorphism at the cholesteryl ester transfer protein gene locus associated with HDL-C levels in women but not men, with similar association reported for female smokers but not male smokers.\(^ {28}\) In adults, smoking increases LDL-C and total cholesterol in men, whereas in women HDL-C levels are reduced and triglycerides are elevated.\(^ {21}\) In a Canadian study, the interactive effect of smoking, alcohol, and adiposity measures on HDL-C levels was shown to be gender-dependent; furthermore, the apolipoprotein E genotype-specific impact of alcohol consumption and smoking was noted only in women.\(^ {29}\) In our study, boys that drank alcohol had higher HDL-C levels compared with girls that consumed alcohol. However, alcohol consumption did not affect the relationship between passive smoking and HDL-C in either girls or boys, thus excluding the confounding effect of alcohol in the gender difference. These findings are in line
with a previous report in adults that smoking does not affect the relationship between alcohol consumption and HDL-C.\textsuperscript{30}

As prenatal smoking is almost always associated with post-pregnancy smoking, it is methodologically difficult to address the role of maternal smoking in pregnancy \textit{per se} in relating to later health outcomes in the offspring.\textsuperscript{31, 32} Maternal prenatal smoking was shown to be negatively associated with HDL-C levels in the offspring at 8 years of age,\textsuperscript{7} with regression models that treated post-natal passive smoking as a covariate. Other studies have examined the impact of maternal smoking in pregnancy on children’s health outcomes\textsuperscript{33-35}, but have not addressed the separate effects of prenatal and post-pregnancy smoking exposure and none has addressed this issue with HDL-C levels. Given that passive smoking is on the causal path to HDL-C, ideally the effect of prenatal smoking \textit{per se} can be determined in those whose mothers smoked in pregnancy but with no subsequent passive smoking. In our study, there were only 4 adolescents in this category, making it difficult to conduct meaningful analyses to estimate the effect. However, in the adolescents exposed to long-term passive smoking, we examined and compared the effects on HDL-C between those with prenatal smoking and those without.

Our data showed that girls exposed to passive smoking but not maternal smoking during pregnancy had significantly lower HDL-C levels at 17 years. Unexpectedly, there was no evidence of an association with HDL-C in those girls whose mother smoked in pregnancy \textit{and} subsequently were exposed to passive smoking. We found higher proportions of adolescents of the maternal smoking group that were exposed to passive smoking in the two most recent surveys, compared to the no maternal smoking group; this demonstrated that lower levels of recent smoking exposure in the maternal smoking group do not appear to be an explanation for the non-significant result. The absence of an effect in this group might be due to confounders that we could not identify and control. In addition, the construction of the passive smoking variable used in this study was somewhat constrained by the recording of categorical ranges of quantity rather than actual quantity of cigarettes smoked. Furthermore, the resulting dichotomous variable does not reflect variations in quantity or distinguish between long-term and short-term exposure or the time
between exposure and the measurement of HDL-C. These factors may be important in the relationship with HDL-C.

Strengths of our study include detailed and comprehensive anthropometric, phenotypic, clinical and socio-behavioural data that were prospectively collected during pregnancy and across 17 years, and a sufficient study sample to enable the examination of gender effects in smoking exposure categories in multivariable models. A limitation of the study was that smoking behaviour was assessed from self-reporting, which could underestimate the smoking effects. However, maternal smoking was assessed at 18th week of pregnancy, and passive smoking information prospectively collected at each of the survey follow-ups, excluding potential recall bias. Furthermore, self-reported smoking has been shown to have high sensitivity and specificity in a large meta-analysis.36

In conclusion, our data from a longitudinal population-based birth cohort shows a gender difference in the relationship between passive smoking exposure since birth and HDL-C levels in late adolescence, with girls, but not boys, showing significantly lower HDL-C levels associated with passive smoking. In view of the relation between reduced HDL-C levels and CVD events in adults, exposure to passive smoking in girls could increase their risk of cardiovascular disease in adulthood. The gender difference in the epidemiology of smoking exposure and HDL-C in this young age group may highlight important gender-specific cardio-metabolic pathophysiological aspects. Assuming causality in these relationships, there are strong public health implications concerning the need to avoid exposure of children, particularly girls, to passive smoking in the household.

Acknowledgements

We acknowledge the Raine Study participants and their families, the Raine Study Team for cohort co-ordination and data collection, Lynette McCahon for technical assistance and the National Health and Medical Research Council of Australia (NH&MRC) for funding the study.
Funding Sources

NH&MRC has been contributing to funding the study over the last 20 years. The 17-year follow-up of the Raine Study was supported by NH&MRC Program [grant number 353514] and Project [grant number 403981] Grants. Core management of the Raine Study is funded by the University of Western Australia (UWA); the Raine Medical Research Foundation; the Telethon Institute for Child Health Research; the UWA Faculty of Medicine, Dentistry and Health Sciences; the Women and Infants Research Foundation and Curtin University. C Le-Ha is supported by an Endeavour Postgraduate Award from the Australian Government, and a Raine Study PhD Scholarship.

References


Supplemental materials for Chapter 3

**Supplemental Table 1  Average daily cigarettes consumed in the household in passive smoking (with versus without maternal prenatal smoking) categories**

<table>
<thead>
<tr>
<th></th>
<th>Daily quantity of cigarettes consumed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-5</td>
<td>6-10</td>
</tr>
<tr>
<td>Passive smoking and no maternal smoking in pregnancy</td>
<td>127 (88.2)</td>
<td>52 (68.4)</td>
</tr>
<tr>
<td>Passive smoking and maternal smoking in pregnancy</td>
<td>17 (11.8)</td>
<td>24 (31.6)</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>76</td>
</tr>
</tbody>
</table>

*Data presented as n (%)*

Likelihood-ratio chi2 = 103.39; p < 0.001
Supplemental Table 2  Multiple effects of sex, BMI, passive smoking and alcohol consumption on HDL-C in non-smoking adolescents (n=800)

<table>
<thead>
<tr>
<th>Exposure categories in girls*</th>
<th>Regression coefficient</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No passive smoking and consuming alcohol</td>
<td>-0.04</td>
<td>-0.114, 0.033</td>
<td>0.285</td>
</tr>
<tr>
<td>Passive smoking and not consuming alcohol</td>
<td>-0.078</td>
<td>-0.148, -0.009</td>
<td>0.027</td>
</tr>
<tr>
<td>Passive smoking and consuming alcohol</td>
<td>-0.092</td>
<td>-0.166, -0.017</td>
<td>0.015</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.268</td>
<td>-0.332, -0.204</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Sex interaction with exposure

| No passive smoking and consuming alcohol | 0.091† | -0.008, 0.19 | 0.072 |
| Passive smoking and not consuming alcohol | 0.093‡ | -0.004, 0.191 | 0.053 |
| Passive smoking and consuming alcohol | 0.144§ | 0.042, 0.245 | 0.005 |
| BMI                          | -0.015 | -0.02, -0.011 | <0.001 |
| Constant                     | 1.802  | 1.693, 1.91   | <0.001 |

Multivariable model adjusted for BMI, gender, family income, dietary patterns, and physical fitness; main predictor was a 4-level composite variable incorporating passive smoking and alcohol consumption information.

* reference: No passive smoking and no consuming alcohol

† equates to coefficient of 0.05 (95%CI -0.015, 0.116) for boys

‡ equates to coefficient of 0.014 (95%CI -0.054, 0.083) for boys

§ equates to coefficient of 0.052 (95%CI -0.017, 0.121) for boys
Supplemental Table 3  Multiple effects of sex, BMI, and active smoking on HDL-C levels in all adolescents (n=1035)

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active smoking</td>
<td>-0.077</td>
<td>-0.131, -0.023</td>
<td>0.005</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.193</td>
<td>-0.229, -0.156</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex interaction with smoking</td>
<td>0.1</td>
<td>0.021, 0.179</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.015</td>
<td>-0.018, -0.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>1.73</td>
<td>1.643, 1.822</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Multivariable model adjusted for BMI, sex, family income, alcohol consumption, dietary patterns, and physical fitness
Supplemental Table 4

Multivariable models for the effects of active smoking on HDL-C in girls (n=503)

<table>
<thead>
<tr>
<th></th>
<th>Model A</th>
<th>Model B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient</td>
<td>95%CI</td>
</tr>
<tr>
<td>Active smoking</td>
<td>-0.074</td>
<td>-0.133, -0.015</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.018</td>
<td>-0.023, -0.012</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>1.8</td>
<td>1.67, 1.934</td>
</tr>
</tbody>
</table>

Model A, adjusted for BMI, sex, family income, alcohol consumption, dietary patterns, and physical fitness

Model B, adjusted further for oral contraceptive use
Chapter 4

Gender and the Active Smoking and High-Sensitivity C-Reactive Protein Relation in Late Adolescence

Preamble

In the previous chapter, the more detrimental effects of long-term passive smoking exposure in the household on HDL-cholesterol on girls at year 17, compared with boys were demonstrated.

Chapter Four is an investigation of the effects of active smoking on high-sensitivity C-reactive protein (hs-CRP), an important biomarker of CVD risk, and potential sex differences in this relationship, given that hs-CRP levels are affected by the use of OCs, and that 30% of the Raine Study adolescent girls used OCs. The interrelationships among smoking, OC use, and CRP is the theme of this chapter.

This chapter was published online ahead of print in the *Journal of Lipid Research* in February 2014, (printed version, April 2014, vol 55, 758-764).
Abstract

C-reactive protein (CRP), smoking, and oral contraceptive (OC) use are associated with cardiovascular disease risk in adults. This study examines the effect of smoking on high-sensitivity CRP (hs-CRP) levels, and the interactive effects of sex and OC use on this relationship, in an adolescent cohort. A total of 1050 adolescents (mean age 17±0.25 years) from the Western Australian Pregnancy Cohort (Raine) Study had anthropometric, lifestyle and metabolic measures recorded. The association between smoking status and log-transformed hs-CRP was analysed using multivariable Tobit linear regression models, with adjustment for adiposity, lifestyle, and early-life confounders. A three-level variable (girls not using OCs, girls using OCs, and boys) was employed to assess the interactive effects of sex, OC use, and smoking. Smoking associated with higher hs-CRP levels in girls not using OCs (b=0.571; p=0.001), but not in girls using OCs (b = -0.117; p=0.598) or in boys (b=0.183; p=0.2). OC use in non-smoking girls was the strongest factor associated with higher hs-CRP levels (b=1.189; p<0.001). This study has demonstrated a more robust effect of smoking on hs-CRP levels in girls not using OCs, compared with boys. The findings may explain why cardiovascular disease risk conferred by smoking is higher in women than in men.

Supplemental keywords:

Adolescent smoking; Cardiovascular risk factors; Oral contraceptives; Sex differences
Introduction

Atherosclerosis, the primary underlying pathological process that eventually leads to ischemic heart disease, begins early in life, and is influenced by potentially modifiable behavioural and lifestyle factors over the life course. Inflammation is a major component of atherosclerosis and an important factor in the early phase of arteriosclerosis.\(^1\) C-reactive protein (CRP), an acute phase reactant and a biomarker of systemic inflammation, is associated with adverse cardiovascular outcomes.\(^2\) There is also strong evidence that CRP is an independent predictor of increased cardiovascular risk,\(^3\) supporting a role for CRP in CVD risk prediction.\(^4, 5\) Smoking exposure, adiposity, and the use of oral contraceptives (OCs) have been shown to be related to higher CRP levels in studies in adults.\(^6-9\) Raised CRP levels later in life reflect lifetime exposure to smoking as well as adiposity levels.\(^10\) Further, sex differences in the association between CRP levels and other risk factors have been described. For example, leptin was shown to be associated with higher CRP levels in women but not in men,\(^11\) while adiponectin was shown to be inversely associated with CRP in females.\(^12\) Elevated CRP levels in smokers may contribute to the adverse effect of smoking on CVD risk, hence both smoking and CRP have been incorporated in the Reynolds Risk Score for women in CVD risk assessment,\(^13\) as recommended by the American Heart Association.\(^14\) Moreover, sex differences have been reported in the relationship between smoking and subclinical inflammation in adults.\(^15\)

Studies in adults have documented that smoking may reduce the efficacy of OCs,\(^16\) and may adversely affect menstrual cycle control in OC users.\(^17\) Oestrogen levels were shown to be lower in smokers, compared to non-smokers.\(^18\) The cause of the anti-oestrogenic effects of smoking may be an increase in the catabolism of oestrogen.\(^19\) To our knowledge, the potential interaction between smoking and OC use in relating to CRP levels in adolescence has not been studied.

To date, there is scant and inconsistent literature on the relationship between smoking exposure and CRP in adolescence.\(^20, 21\) In view of the importance of understanding how cigarette smoking increases the long-term risk of cardiovascular disease, this population-based study aimed to examine the effect of active smoking on
high-sensitivity CRP (hs-CRP) levels, and the potential interactive effects of sex and OC use on this relationship in 17-year-old adolescents from the Western Australian Pregnancy Cohort (Raine) Study. Our primary hypothesis was that in this adolescent population, smoking increases CRP levels, and other factors such as sex and OC use modify this relationship.

Methods

Study population

This study is based on the 17-year-old follow-up of the Western Australian Pregnancy Cohort (Raine) Study, a longitudinal population-based cohort study. Details of the cohort at birth have been published previously.22 In brief, 2868 live births from 2900 women recruited from King Edward Memorial Hospital and nearby clinics in Perth, Western Australia, were enrolled at 18 weeks of pregnancy between 1989 and 1991. Data collection was in accordance with the Guidelines for Ethical Conduct in Human Research of the Australian National Health and Medical Research Council, and was approved by the Human Ethics Committees of King Edward Memorial Hospital and Princess Margaret Hospital for Children in Perth. Written informed consent was obtained from the mother/primary care giver and the adolescent.

In the 17 year survey, there were 1748 active adolescents from the original cohort. Of these, 1248 adolescents consented to participate and completed questionnaires for nutrition and lifestyle information including physical activity, smoking, drug, and alcohol consumption. Participants were predominantly Caucasian (93%). The adolescents underwent assessments for height, weight, body mass index, and skinfold thickness; arterial stiffness and blood pressure; and had fasting blood for biochemistry measured. Adolescents participating in the 17 year survey were more representative of the Western Australian population than the original cohort, which
had included a slightly higher proportion of socially disadvantaged families (Supplemental Table I) admitted to the public hospital.

**Demographic and socio-behavioural features**

The active smoking status and other socio-behavioural features of the adolescents were assessed via a computer-based questionnaire. The adolescent was asked: “Have you ever smoked cigarettes in the past 12 months?” and “Have you smoked cigarettes in the past 4 weeks?”, and the number of cigarettes consumed each day in the last 7 days were recorded. Information of the use of OCs was obtained from the question “In the last 6 months, have you taken any prescription medication(s), e.g., the Pills?” (if yes, “which medication(s), and are you still taking it?”). Participants were also asked about the amount (can, glass, stubby, nip, or standard drink) and type of alcoholic beverage (beer, wine or spirits) consumed in the past week. An alcohol drinker was defined as consuming alcohol at any level in a drinking day during the last 7 days. The PWC$_{170}$ aerobic fitness test, that predicts physical working capacity at a heart rate of 170 beats per minute and has been validated in our study population, was employed to measure physical fitness. From a 212-item food frequency questionnaire, “Healthy” and “Western” dietary patterns were identified using factor analysis with varimax rotation, and scores for each pattern were generated. Annual family income was categorised as Australian dollars ≤ 35000, 35001 to ≤ 78000, and > 78000.

**Blood biochemistry analysis and anthropometry measures**

After an overnight fast, venous blood samples were obtained for the determination of glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, leptin, adiponectin, and hs-CRP, as reported previously. Height was measured using a Holtain Stadiometer (nearest 0.1 cm), and body weight using a Wedderburn Chair Scale (nearest 100 g). After resting 5 minutes, supine blood pressure was recorded using an oscillometric sphygmomanometer (Dinamap Pro Care 100; Soma Technology, Bloomfield, Connecticut) with an
appropriate cuff size. The average of the last 5 of 6 readings of systolic and diastolic blood pressure, respectively, was recorded.

*Early-life factors*

Birth weight (in grams) was obtained from the medical records of the baby. Gestational age (in weeks) was computed 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.

*Statistical analysis*

A smoking yes/no variable was used to indicate current cigarette smoking, because fractional polynomials showed no additional effects on hs-CRP levels beyond smoking more than 1 cigarette (Supplemental Figure I). Similarly, fractional polynomial analysis showed no effects on hs-CRP of additional consumption beyond 1 standard drink; hence the effect of alcohol consumption was analysed using a dichotomous yes/no variable. Given that OC use is an important factor in determining CRP levels in previous reports in adults, we created a three-level sex variable (girls using OCs, girls not using OCs, and boys) to examine the effects of sex and OC use, as well as their interactions, on the relationship between smoking and hs-CRP.

Tobit regression models, which take into consideration the censored nature of data distribution, were employed in all analyses, due to censored data as a result of the lower limit of the CRP assay. Hs-CRP values were log-transformed and hs-CRP values ≥ 10 mg/L, which are presumed to be the result of acute inflammation, were excluded in all analyses, based on recommendations by the American Heart Association. Univariate Tobit regression models assessing the relationship between cardiovascular risk factors and hs-CRP were initially adjusted for sex, and additionally adjusted for BMI.
Employing the whole adolescent sample, multivariable Tobit regression models for the association between adolescent active smoking and hs-CRP were built, considering the potential confounding effects of BMI, alcohol consumption, dietary pattern scores, PWC \textsubscript{170} aerobic fitness score, family income, birth weight, and gestational age. In a backward stepwise process, non-significant factors were removed considering the effects on coefficients and p values at each step. Likelihood ratio tests were performed after the removal of each variable to confirm that its exclusion had no impact on the model. Multivariable models examined potential interactions between the covariates and the three-level sex variable, which assessed the potential sex and OC use effect modification. A p value of <0.05 was used to maintain variables in the model. Conventionally, BMI is used to measure adiposity effects on CRP in regression models, but given the known relationship between adipokines and CRP, in separate multivariable models, leptin and adiponectin, respectively, were added to the above final model to assess the independent effects of these cytokines on hs-CRP levels.

Stata version 12 (StataCorp, College Station, Texas) was used for analysis. Results are interpreted at p<0.05 significance level.

Results

Of the 1248 adolescents that participated in the 17-year survey, 1050 had completed anthropometric, phenotypic and socio-behavioural data. Tables 1 and 2 describe the phenotypic and socio-behavioural features, and biochemistry, respectively, of the adolescents studied. Overall, 31.5% of the girls used OCs, 51% of all adolescents were drinkers, 17% were smokers, and 12.5 % had hs-CRP levels in the range of 3 to 10 mg/L. Compared with the girls not using OCs, a larger percentage of smokers (23% vs 18%) as well as a greater number of cigarettes smoked (14 vs 7) in the last 7 days were observed in the girls who used OCs. Hs-CRP levels were significantly higher in girls than boys, and particularly in girls using OCs (all p<0.001). There was no difference in the
patterns of the relationship between smoking and BMI across the 3 adolescent groups (interaction p=0.561, OC using girls vs non OC girls; p=0.911, boys vs non OC girls).

In univariate models of the associations between individual socio-behavioural factors and hs-CRP (Table 3), being male was associated with lower hs-CRP levels (p<0.001) compared to being female, whereas the use of OCs in girls and smoking in all adolescents were associated with higher levels of log hs-CRP (all p<0.001).

Multivariable analysis showed a significant interaction between the three-level sex variable and smoking, indicating there was a significant difference between using or not using OCs in the association of smoking with hs-CRP (interaction p=0.014, girls using OCs and smoking vs girls using OCs and not smoking; p=0.078, smoking boys vs non-smoking boys). Table 4 shows the effects of smoking on hs-CRP in the final multivariable model. In girls not using OCs, smoking associated with a significant increase in log hs-CRP levels (b=0.571; p=0.001). In contrast, in girls using OCs, there was no significant effect of smoking on log hs-CRP (b=−0.117; p=0.598). In boys, there was a positive, albeit non-significant effect of smoking that is not as large as that seen in girls not using OCs. In these models, BMI was significantly independently associated with higher log hs-CRP levels, with similar patterns of association in the 3 groups (as indicated by the non-significant interaction p=0.845, OC using girls vs non OC girls; and p=0.347, boys vs non OC girls). Further, OC use in non-smoking girls was the strongest factor independently associated with higher log hs-CRP levels (b=1.189; p<0.001). Figure 1 demonstrates that there was a significant difference in the linear prediction of log hs-CRP levels between smokers and non-smokers in girls not using OCs; this was not clearly demonstrated in girls using OCs or in boys.
Figure 1  Predicted log hs-CRP levels by smoking status in the three adolescent groups (girls not using OCs [No OC], girls using OCs [OC], and boys).

Further adjustment for leptin in multivariable models showed that leptin was associated with higher log hs-CRP levels ($b=0.009; p=0.001$), independent of BMI as a measure of adiposity (Supplemental Table II). Adjustment for leptin did not alter the coefficients of the smoking variables in the models. However, there was no independent association between adiponectin and log hs-CRP, with adjustment for BMI. In these models, there was no sex difference in the patterns of leptin or adiponectin in the relationship between smoking and hs-CRP.
Table 1  Anthropometric, phenotypic and behavioural characteristics of the study samples

<table>
<thead>
<tr>
<th></th>
<th>All adolescents (n=1050)</th>
<th>Non-smoking adolescents (n=870)</th>
<th>Smoking adolescents (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls not using OCs (n=345)</td>
<td>Girls using OCs (n=159)</td>
<td>Boys (n=546)</td>
</tr>
<tr>
<td>Age, y</td>
<td>17.04 (17.01, 17.06)</td>
<td>17.07 (17.03, 17.1)</td>
<td>17.02 (17, 17.04)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>107.7 (106.9, 108.6)</td>
<td>110.9 (109.5, 112.2)</td>
<td>117.8 (117.1, 118.6)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>58.8 (58.2, 59.4)</td>
<td>60.7 (59.7, 61.6)</td>
<td>58.2 (57.7, 58.7)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.34 (22.87, 23.81)</td>
<td>22.61 (22.08, 23.14)</td>
<td>22.68 (22.35, 23.01)</td>
</tr>
<tr>
<td>Waist circumference, mm</td>
<td>77.89 (76.72, 79.06)</td>
<td>76.73 (75.28, 78.17)</td>
<td>80.52 (79.65, 81.39)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3281.8 (3223.9, 3339.8)</td>
<td>3286.9 (3212.7, 3361.2)</td>
<td>3376.1 (3331.3, 3420.8)</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.2 (38.9, 39.4)</td>
<td>39.5 (39.2, 39.7)</td>
<td>39.3 (39.2, 39.5)</td>
</tr>
<tr>
<td>Healthy diet pattern score †</td>
<td>0.483 (0.401, 0.484)</td>
<td>0.478 (0.347, 0.392)</td>
<td>0.482 (0.394, 0.397)</td>
</tr>
<tr>
<td></td>
<td>0.582)</td>
<td>0.675)</td>
<td>0.583)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Western diet pattern score †</td>
<td>0.329 (0.24, 0.45)</td>
<td>0.395 (0.282, 0.553)</td>
<td>0.499 (0.421, 0.592)</td>
</tr>
<tr>
<td>PWC 170 score a</td>
<td>1.613 (1.571, 1.655)</td>
<td>1.558 (1.504, 1.613)</td>
<td>2.18 (2.133, 2.226)</td>
</tr>
<tr>
<td>Drinker, % b</td>
<td>47.1 (41.7, 52.3)</td>
<td>54.7 (46.8, 62.5)</td>
<td>52 (47.8, 56.2)</td>
</tr>
<tr>
<td>Smoker, % c</td>
<td>17.6 (13.6, 21.7)</td>
<td>23.2 (16.6, 29.9)</td>
<td>15 (12, 18)</td>
</tr>
<tr>
<td>Number of cigarettes smoked d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual family income, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ A$ 35,000</td>
<td>14.3 (10.3, 18.3)</td>
<td>14.7 (8.6, 20.7)</td>
<td>12.7 (9.7, 15.6)</td>
</tr>
<tr>
<td>A$ 35,001 - ≤ 78,000</td>
<td>31 (25.7, 36.2)</td>
<td>41.1 (32.7, 49.5)</td>
<td>33.4 (29.2, 37.6)</td>
</tr>
<tr>
<td>&gt; A$ 78,000</td>
<td>54.6 (49, 60.3)</td>
<td>44.1 (35.6, 52.5)</td>
<td>53.8 (49.4, 58.3)</td>
</tr>
</tbody>
</table>

Data are expressed as arithmetic or geometric (†) mean, or percentage (95% confidence interval).

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; A$, Australian dollars

a Score of the PWC 170 test (watts), adjusted for weight.
b Consumer of alcohol at any level over the last 7 days.
c Smoking ≥ 1 cigarette in a week.
d in the last 7 days.
## Table 2  Serum biochemistry features of the study samples

<table>
<thead>
<tr>
<th></th>
<th>All adolescents (n=1050)</th>
<th>Non-smoking adolescents (n=870)</th>
<th>Smoking adolescents (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Girls not using OCs (n=345)</td>
<td>Girls using OCs (n=159)</td>
<td>Boys (n=546)</td>
</tr>
<tr>
<td>Hs-CRP, mg/L †</td>
<td>0.593 (0.523, 0.671)</td>
<td>1.447 (1.216, 1.721)</td>
<td>0.481 (0.439, 0.528)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.388 (1.355, 1.421)</td>
<td>1.41 (1.362, 1.457)</td>
<td>1.2 (1.186, 1.228)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.344 (2.283, 2.405)</td>
<td>2.619 (2.509, 2.730)</td>
<td>2.245 (2.19, 2.299)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L †</td>
<td>0.868 (0.832, 0.907)</td>
<td>1.142 (1.079, 1.208)</td>
<td>0.95 (0.916, 0.985)</td>
</tr>
<tr>
<td></td>
<td>Arithmetic Mean (95% CI)</td>
<td>Geometric Mean (95% CI)</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin, mU/L†</strong></td>
<td>7.496 (6.969, 8.062)</td>
<td>7.952 (7.216, 8.763)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.868 (6.498, 7.258)</td>
<td>7.485 (6.933, 8.080)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.134 (7.262, 9.112)</td>
<td>6.76 (6.372, 7.173)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.549 (6.092, 9.355)</td>
<td>7.388 (6.092, 8.959)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.511 (6.443, 8.757)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HOMA-IR†</strong></td>
<td>1.545 (1.432, 1.666)</td>
<td>1.632 (1.474, 1.806)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.471 (1.388, 1.559)</td>
<td>1.539 (1.421, 1.666)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.665 (1.478, 1.875)</td>
<td>1.445 (1.357, 1.538)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.574 (1.256, 1.973)</td>
<td>1.527 (1.25, 1.866)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.631 (1.386, 1.918)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Leptin, µg/L†</strong></td>
<td>24.51 (22.61, 26.57)</td>
<td>23.93 (21.57, 26.54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.58 (3.29, 3.89)</td>
<td>23.89 (21.88, 26.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.47 (3.18, 3.79)</td>
<td>23.8 (21.22, 26.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.62 (22.48, 33.94)</td>
<td>24.35 (19.06, 31.09)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.25 (3.3, 5.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adiponectin, µg/L†</strong></td>
<td>10.08 (9.54, 10.65)</td>
<td>9.68 (9.06, 10.35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.13 (6.83, 7.45)</td>
<td>10.27 (9.66, 10.92)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.95 (9.26, 10.69)</td>
<td>7.08 (6.74, 7.44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.23 (8.16, 10.45)</td>
<td>8.86 (7.52, 10.44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.43 (6.79, 8.14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as arithmetic or geometric (†) mean (95% confidence interval).

HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; HOMA-IR, homeostasis model of assessment for insulin resistance.
### Table 3  Univariate associations between cardiovascular risk factors and hs-CRP

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Regression coefficient</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>-0.581</td>
<td>-0.75, -0.412</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.135</td>
<td>0.117, 0.153</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight</td>
<td>8.70e-07</td>
<td>-0.0001, 0.0001</td>
<td>0.991</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.021</td>
<td>-0.020, 0.063</td>
<td>0.322</td>
</tr>
<tr>
<td>Smoking(^a)</td>
<td>0.466</td>
<td>0.239, 0.692</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol drinking(^b)</td>
<td>0.285</td>
<td>0.113, 0.456</td>
<td>0.001</td>
</tr>
<tr>
<td>PWC (_{170}) score(^c)</td>
<td>-0.657</td>
<td>-0.805, -0.508</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Healthy diet pattern</td>
<td>-0.032</td>
<td>-0.142, 0.077</td>
<td>0.567</td>
</tr>
<tr>
<td>Western diet pattern</td>
<td>-0.116</td>
<td>-0.238, 0.005</td>
<td>0.061</td>
</tr>
<tr>
<td>OC use</td>
<td>1.126</td>
<td>0.897, 1.355</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Annual family income in Australian dollars(^d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$35 001 to ≤$78 000</td>
<td>-0.157</td>
<td>-0.451, 0.136</td>
<td>0.292</td>
</tr>
<tr>
<td>&gt;$78 000</td>
<td>-0.288</td>
<td>-0.567, -0.01</td>
<td>0.042</td>
</tr>
</tbody>
</table>

\(^a\) Smoking ≥ 1 cigarette in a week.

\(^b\) Consuming alcohol at any level over the last 7 days.

\(^c\) adjusted for weight

\(^d\) Reference: ≤ $35 000.
Table 4  Multivariable model for the effects of active smoking on hs-CRP in the whole adolescent sample (n=1022)

<table>
<thead>
<tr>
<th></th>
<th>Regression coefficient</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls not using OCs and smoking</td>
<td>0.571</td>
<td>0.244, 0.898</td>
<td>0.001</td>
</tr>
<tr>
<td>Girls using OCs and smoking</td>
<td>-0.117</td>
<td>-0.556, 0.32</td>
<td>0.598</td>
</tr>
<tr>
<td>Girls using OCs and not smoking</td>
<td>1.189</td>
<td>0.937, 1.441</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Boys not smoking</td>
<td>-0.118</td>
<td>-0.296, 0.058</td>
<td>0.19</td>
</tr>
<tr>
<td>Boys smoking</td>
<td>0.183</td>
<td>-0.097, 0.465</td>
<td>0.2</td>
</tr>
<tr>
<td>BMI</td>
<td>0.133</td>
<td>0.116, 0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>-3.851</td>
<td>-4.267, -3.436</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Final multivariable model represents sub-categories in all variables that maintain their significance at p<0.05. Non-significant terms in the model are components of the significant interaction between smoking and sex/OC use, hence are retained.
Discussion

In a large well-phenotyped population of 17-year-olds, we have shown that smoking was significantly associated with higher hs-CRP levels in girls not using OCs, but not in girls using OCs or in boys. BMI was independently associated with higher hs-CRP levels in all adolescents, and OC use in non-smoking girls was the strongest factor associated with higher hs-CRP levels. To our knowledge, this is the most comprehensive study to date evaluating these associations in this age group, and the first to demonstrate that the effects of smoking on hs-CRP is more robust in girls than in boys.

OC use increases hs-CRP levels in adult women. Studies in adolescents have shown a positive association between active smoking and hs-CRP, however, they did not examine the potential interactions between sex and OC use on the smoking effects. In the present study, with over 30% of the girls using OCs, we found that OC use in non-smoking girls was a strong determinant of higher hs-CRP levels. Further, we observed a clear-cut effect of smoking on hs-CRP in the girls not using OCs. However, there was no effect of smoking on hs-CRP in the girls who smoked and used OCs, even though the latter group smoked a larger number of cigarettes and the smoking rates in this group were higher. Changes in endocrine patterns with smoking and the potential anti-estrogenic effect of smoking may help explain our findings of a modifying role of OCs on hs-CRP levels in the present study. Smoking has been reported to enhance oestradiol metabolism in the liver, and changes in adrenal metabolism by smoking that leads to enhanced adrenal activity may contribute to an anti-estrogenic effect of smoking on hs-CRP.

In relation to risk discrimination and effect magnitude, CRP and lipids have the same capability to identify who is at risk for future cardiovascular events. Recent data from the JUPITER clinical trial provides substantial evidence that CRP levels predict CVD events independently from other cardiovascular risk factors, a finding that strongly supports a role for CRP in CVD risk assessment. Moreover, a large meta-analysis demonstrated that the measurement of CRP in people of intermediate CVD risk could help prevent one major cardiovascular event over 10 years for every 400 people screened; further in that study, CRP had greater predictive value in CVD risk.
assessment in current smokers than in non-smokers. The Reynolds Risk Score, of which both smoking and CRP are major components, has been validated and shown to significantly improve CVD risk discrimination particularly in women, compared to the conventional Framingham-based CVD models which do not incorporate CRP. Given that even with smoking cessation CRP levels remain increased over 19 years, and that CRP levels in childhood predict CRP in adulthood, our novel finding of a predominant effect of smoking on hs-CRP levels in girls not using OCs is important in this context. It has been reported that smoking exposure is more harmful to health in women than in men: e.g. female smokers were more vulnerable to colon cancer than male smokers, and smoking in women was associated with 25% greater coronary heart disease risk compared with smoking in men. In line with these reports and the present study, we have previously shown in this adolescent population that long-term passive smoking exposure was associated with lower levels of HDL-cholesterol in 17-year-old girls but not boys.

We observed higher levels of hs-CRP in girls using OCs and also in girls not using OCs, compared with boys, a finding which is consistent with most previous studies in adults and adolescents which showed higher levels of CRP in females compared with males. Some studies suggest that leptin levels may explain the sex difference in CRP levels. In our study, sex differences in hs-CRP levels were accounted for by leptin, even after adjusting for BMI. However, we observed no impact of leptin on the sex-smoking interaction effects on hs-CRP. Further, pubertal development may affect CRP levels differently between boys and girls, which could be due to the sex differences in adiponectin levels during puberty. Nonetheless, in our study sample, of which 97% of the girls and 98% of the boys were post-pubertal (Tanner stages 4 or 5), we found no association between adiponectin and hs-CRP levels independently of BMI.

A strength of our study is that we employed data from a large population-based pregnancy cohort, with comprehensive anthropometric, phenotypic, and socio-behavioural information prospectively collected from 16-20 weeks gestation and over 17 years. Analyses examined the relationship between smoking and hs-CRP with adjustment for a variety of potential confounders including sex and OC use. Limitations
of the study include the relatively small number of participants in some subgroups, which may have limited the detection of interaction effects. Smoking exposure was assessed from self-reporting and therefore could underestimate the smoking effect. However, to minimise potential reporting bias, the adolescents provided confidential smoking and other behavioural information via a computer-based structured questionnaire. Moreover, a large meta-analysis has shown high sensitivity and specificity in self-reporting for smoking exposure. OC use was documented but we did not ascertain the type of OCs used, therefore cannot be certain that the findings appertain to all varieties of these agents.

Conclusion

In a large cohort of adolescents at 17 years of age, we have shown a more robust effect of smoking in girls that were not using OC on hs-CRP levels, compared with girls taking OCs or boys. Our findings support the important role of CRP in CVD risk prediction, in particular CVD risk assessment in women. Given that both smoking behaviour and CRP levels track from childhood to adulthood, the findings help explain why CVD risk conferred by smoking is higher in women than in men.

Acknowledgements

The authors are grateful to the Raine Study participants and their families, the Raine Study Team for cohort co-ordination and data collection, and Ms Lynette McCahon for technical assistance.

Funding Sources

The 17-year follow-up of the Raine Study was supported by the National Health and Medical Research Council of Australia Program [grant number 353514] and Project [grant number 403981] Grants. Core management of the Raine Study is funded by the University of Western
Australia (UWA); the Raine Medical Research Foundation; the Telethon Institute for Child Health Research; the UWA Faculty of Medicine, Dentistry and Health Sciences; the Women and Infants Research Foundation and Curtin University. C Le-Ha was supported by an Endeavour Postgraduate Award from the Australian Government, and a Raine Study PhD Scholarship.

References


7. Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation,


29. Ridker PM, Kastelein JJP, Genest J, Koenig W. C-reactive protein and cholesterol are equally strong predictors of cardiovascular risk and both are important for quality clinical care. *Eur Heart J*. 2013; doi10.1093/eurheartj/eht022


### Supplemental Table I  
Comparing the 17-year sample to non-participants at 17-year survey

<table>
<thead>
<tr>
<th></th>
<th>Participants (N = 1,248)</th>
<th>Non-Participants (N = 1,621)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family income at 1st year follow-up</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; A$ 24,000</td>
<td>35.4</td>
<td>46.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ A$ 24,000</td>
<td>64.6</td>
<td>53.7</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age, days</strong></td>
<td>275.3 (14.1)</td>
<td>273.1 (18.1)</td>
<td>0.0003</td>
</tr>
<tr>
<td><strong>Mother’s weight, kg</strong></td>
<td>59.6 (11.8)</td>
<td>60.1 (12.6)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Mother’s height, cm</strong></td>
<td>163.8 (6.7)</td>
<td>163.4 (6.5)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Birth weight, g</strong></td>
<td>3327.8 (579.1)</td>
<td>3253.1 (650.6)</td>
<td>0.0014</td>
</tr>
<tr>
<td><strong>Birth length, cm</strong></td>
<td>49 (2.6)</td>
<td>48.7 (3.2)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Placenta weight, g</strong></td>
<td>610.5 (148.1)</td>
<td>609.3 (152.7)</td>
<td>0.843</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49.7</td>
<td>49</td>
<td>0.1498</td>
</tr>
<tr>
<td>Male</td>
<td>50.3</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td><strong>Previous medical history of mother</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>42.2</td>
<td>57.7</td>
<td>0.549</td>
</tr>
</tbody>
</table>

*Data are shown as percentage for categorical variables, and mean (standard deviation) for continuous variables.  * At 18th week of pregnancy.*
Supplemental Table II

Multivariable model for the effect of smoking on hs-CRP – adjusting further for leptin (n=1022)

<table>
<thead>
<tr>
<th></th>
<th>b- coefficient</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls not using OCs and smoking</td>
<td>0.547</td>
<td>0.221, 0.872</td>
<td>0.001</td>
</tr>
<tr>
<td>Girls using OCs and smoking</td>
<td>-0.113</td>
<td>-0.548, 0.322</td>
<td>0.61</td>
</tr>
<tr>
<td>Girls using OCs and not smoking</td>
<td>1.19</td>
<td>0.94, 1.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Boys not smoking</td>
<td>0.111</td>
<td>-0.113, 0.336</td>
<td>0.33</td>
</tr>
<tr>
<td>Boys smoking</td>
<td>0.185</td>
<td>-0.093, 0.464</td>
<td>0.192</td>
</tr>
<tr>
<td>BMI</td>
<td>0.106</td>
<td>0.083, 0.129</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.009</td>
<td>0.003, 0.015</td>
<td>0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>-3.526</td>
<td>-3.982, -3.071</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: OCs, oral contraceptives; BMI, body mass index.
Supplemental Figure I

Fractional polynomial plot showing the relationship between the number of cigarettes consumed in the last 7 days and log hs-CRP levels.
Chapter 5

Basal Hypothalamic-pituitary-adrenal Axis Activity Associates with Increased Levels of Cardiovascular Risk Factors in an Adolescent Population

Preamble

Previous chapters have shown the relationships between socio-behavioural factors and the CVD risk profile in adolescents. The transitional period from late childhood through adolescence represents important physical, social and psychological changes. As discussed in Chapter One, adolescence is a period of emotional development and brain plasticity, during which alterations in the biological stress systems may have a long-term impact on the cardio-metabolic system.

The study reported in Chapter Five addresses the associations between the hypothalamic-pituitary-adrenal axis activity, a component of the biological stress systems, and cardiovascular risk factors in the Raine Study adolescents. It highlights the potential impact of chronic stress, as reflected in the dysregulated HPA axis activity observed, on future CVD risk.
Abstract

Background: One route by which psychosocial stress affects the cardiovascular system is through activation of the hypothalamic-pituitary-adrenal (HPA) axis. This study aimed to assess the relation between basal HPA axis activity and cardiovascular risk factors in a general population of adolescents.

Methods: A total of 1052 adolescents participating in the age 17 review of the Western Australian Pregnancy Cohort (Raine) Study had complete phenotypic and socio-demographic data. The associations between HPA axis measures (including plasma ACTH, total cortisol, calculated free cortisol, corticosteroid binding globulin (CBG), and salivary cortisol) and a range of conventional cardiovascular risk factors were examined using multivariable linear regression models, with adjustment for adiposity, and early-life and socio-behavioural factors.

Results: There was no association between BMI and total cortisol or CBG. In all adolescents, total cortisol positively associated with systolic blood pressure (SBP) (p=0.01), total cholesterol (p<0.001), HDL-cholesterol (p<0.001), and high-sensitivity C-reactive protein (hs-CRP) (p=0.047). Salivary cortisol positively associated with HDL-cholesterol (p=0.03) and negatively associated with LDL-cholesterol (p=0.015); plasma free cortisol positively associated with triglycerides (p=0.003); and CBG positively associated with total cholesterol and HDL-cholesterol (both p<0.001), LDL-cholesterol (p=0.017), and hs-CRP (p=0.001). In girls but not boys, total cortisol and CBG positively associated with triglycerides (p<0.001 and p=0.025, respectively). Plasma ACTH was not associated with any cardiovascular risk factor.

Conclusion: In a general adolescent population, high basal HPA axis activity associates with increased SBP, total cholesterol, and hs-CRP levels. Understanding the mechanisms by which basal HPA axis activity regulates cardiovascular function in adolescence would be the first step to assess the impact of psychosocial stress on long-term cardiovascular risk.
Introduction

Psychosocial stress, mediated by heightened hypothalamic-pituitary-adrenal (HPA) axis activity, is a risk factor for cardiovascular disease (CVD). Chronic stress exposure alters HPA axis activity, which in turn adversely affects the cardiovascular system by promoting atherosclerosis, resulting in a higher risk of CVD. Regular functions of the HPA axis affect the cardio-metabolic system. In adults, HPA axis dysfunction has been associated with elevated blood pressure, insulin resistance, dyslipidaemia, and higher levels of central adiposity. Inflammatory processes, which underlie the pathogenesis of atherosclerosis, have been linked to HPA axis activation. Population data in adults has demonstrated that elevated plasma cortisol increases the prevalence of ischaemic heart disease; high long-term cortisol levels in hair are related to a history of CVD; and high urinary cortisol levels strongly predict cardiovascular mortality.

The scant paediatric literature on HPA activity and cardio-metabolic risk includes studies conducted in obese children and adolescents, and has shown associations between HPA axis measures and cardiovascular risk factors as well as the metabolic syndrome. Moreover, in obese Latino children, salivary cortisol has been associated with increased carotid artery intima-media thickness.

During adolescence, HPA axis maturation reflects the on-going maturation of the adolescent forebrain. 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. Animal data have shown that adolescents are likely more vulnerable to the impact of exposure to stressors than adults. The question arises as to whether dysfunctional HPA activity adversely affects the cardio-metabolic risk profile in a general adolescent group.

Reynolds et al have previously reported in the Raine Study adolescents gender specific sensitivity of the basal HPA axis. Plasma levels of ACTH were lower, but total cortisol and corticosteroid binding globulin (CBG) were higher in females compared with males. Further, the authors have shown that the relative higher levels of salivary cortisol measured at awakening were significantly reduced by the use of oral contraceptives (OC).
The present study aimed to investigate, in a large general population of 17-years-old adolescents, the associations between the HPA axis measures of plasma ACTH, corticosteroid binding globulin (CBG), total cortisol, calculated free cortisol, and salivary cortisol, with a range of conventional cardiovascular risk factors under resting conditions. Our *a priori* hypothesis was that altered basal HPA functions at 17 years of age would be associated with CVD risk factors including blood pressure, lipids, and the inflammatory marker C-reactive protein.

**Methods**

*Study population*

Participants were adolescents from the 17-year review of the Western Australian Pregnancy Cohort (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; they were serially enrolled at 18 week’s gestation between 1989 and 1991. Details of the cohort at birth were previously described. 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, and drug use. Participants underwent assessments for height, weight, body mass index and skinfold thickness; cardiorespiratory fitness; blood pressure and arterial stiffness; and had fasting blood collected for biochemical analyses. At the time of the 17-year review, Raine Study adolescents were representative of the current Western Australian population. The 17-year participants, as well as those 17-year-olds 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 status.\textsuperscript{19,20}

**Assessments**

**Hormonal**

The details of the protocol for hormone measures and analysis have been described elsewhere.\textsuperscript{16} 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, and date and time was documented. 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 \textsuperscript{125} I radioimmunoassay (GammaCoat cortisol RIA – DiaSorin, MN USA and CBG RIA 100 – BioSource Europe S.A., Belgium). Plasma ACTH was measured by \textsuperscript{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: $U = v (Z^2 + 0.0122T) - Z$, where $Z = 0.0167 + 0.0182 (G - T)$. In this formula, $U$, $G$ and $T$ represent free cortisol, CBG and total cortisol, respectively.\textsuperscript{21}

**Clinical, biochemical, and anthropometric**

Fasting plasma 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 recorded, after 5 minutes of quiet rest, using an oscillometric sphygomanometer (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 100g). Pubertal development was estimated with self-reported Tanner stage.

Early-life factors

Birth weight (g) was obtained from the baby’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.

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 alcohol at any level in a drinking day during the last 7 days. Oral contraceptive (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. 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.
Statistical analysis

HPA axis parameters comprised plasma ACTH, total cortisol, calculated free cortisol, CBG and salivary cortisol. In preliminary analyses, using fractional polynomial and linear regression models, these HPA measures were corrected for the timing factors known to influence their expression, including time of blood sample collection, date of blood sample collection, season, and time of sunrise. Mixed models with both a random slope and intercept were employed for the repeated measures of salivary cortisol, to account for within-subject correlation between repeated measures. Sampling time of year was categorized into one of the four seasons in the southern hemisphere. Time of sunrise was calculated based on computation procedures by Geoscience Australia (http://www.ga.gov.au/geodesy/astro/sunrise.jsp). Regression residuals were added to the observed mean to produce temporally and seasonally corrected HPA axis measures.

Cardiovascular risk factor outcomes included SBP, DBP, glucose, insulin, total cholesterol, triglycerides, LDL-C, HDL-C, hs-CRP, and BMI. For all cardiovascular risk factors except hs-CRP, robust linear regression models were employed to examine the association with the corrected HPA axis measure. Due to censored data as a result of the lower limit of the CRP assay, all analyses of hs-CRP were conducted using tobit regression models, which take into account the censored nature of data distribution. Hs-CRP values ≥ 10 mg/L which are presumed to be due to acute inflammation, were excluded as recommended by the American Heart Association.

In a preliminary analysis with separate models for girls and boys, pubertal stage did not significantly affect the relationship of any of the HPA parameters with cardiovascular risk factors. Subsequent analyses were conducted using males and females combined. A three-level sex variable (girls using OC, girls not using OC, and boys) was used to evaluate the effects of sex, OC use (in girls), and their interaction, on the relationship between HPA axis activity and cardiovascular risk factors. Multivariable regression models were used to examine the associations between each of the HPA axis parameters with each CVD risk factor. In these models, potential confounding effects of birth weight, gestational age, BMI, smoking, alcohol consumption, dietary patterns,
physical fitness, and family income were considered. In a backward stepwise process, non-significant confounder covariates were removed considering the effects on coefficients and p-values at each step. The multivariable process also examined the potential interactions between the covariates and the three-level sex variable.

All analyses were conducted using Stata version 12 (StataCorp, College Station, Texas). Results are interpreted with reference to significance at P<0.05.

Results

Five pregnant girls at the time of sampling were excluded. A total of 1052 adolescents that participated in the 17-year cohort had complete phenotypic, biochemical and socio-behavioural data for analysis (Table 1). Of the participants, 51% consumed alcohol, 17% were smokers, and 32% of the girls used OC. In fractional polynomial analysis, plasma total cortisol, calculated free cortisol, CBG and ACTH were significantly associated with time of blood collection; and plasma total cortisol and calculated free cortisol were associated with season and time of sunrise (p<0.001 and p=0.004 for time of sunrise, respectively). Figure 1 shows the relationship of plasma total cortisol with time of blood sample collection and time of sunrise. HPA axis components, corrected for temporal and seasonal factors, are shown in table 2. Smoking was not associated with any of the HPA parameters. There were no significant associations between BMI and plasma total cortisol (b= -0.0009; p=0.075) or CBG (b= -0.0067; p=0.128).
Table 1  Clinical and socio-demographic characteristics of the 17-years-old participants

<table>
<thead>
<tr>
<th></th>
<th>All adolescents (n=1052)</th>
<th>Girls (n=506)</th>
<th>Boys (n=546)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>17.04 (17.02, 17.05)</td>
<td>17.06 (17.04, 17.08)</td>
<td>17.02 (17, 17.04)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.6 (22.4, 22.8)</td>
<td>22.7 (22.4, 23.1)</td>
<td>22.4 (22.1, 22.8)</td>
</tr>
<tr>
<td>Waist circumference, mm</td>
<td>785 (778, 791)</td>
<td>769 (760, 779)</td>
<td>800 (791, 808)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>113.5 (112.9, 114.1)</td>
<td>108.6 (107.8, 109.4)</td>
<td>118.1 (117.3, 118.8)</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>58.7 (58.4, 59.1)</td>
<td>59.4 (58.9, 59.9)</td>
<td>58.1 (57.6, 58.7)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.1 (4.05, 4.15)</td>
<td>4.29 (4.22, 4.35)</td>
<td>3.93 (3.87, 3.99)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.94 (0.92, 0.97)</td>
<td>0.94 (0.90, 0.97)</td>
<td>0.95 (0.91, 0.98)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.29 (1.28, 1.31)</td>
<td>1.39 (1.36, 1.42)</td>
<td>1.21 (1.18, 1.23)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.33 (2.29, 2.37)</td>
<td>2.43 (2.37, 2.48)</td>
<td>2.24 (2.19, 2.3)</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.73 (4.7, 4.76)</td>
<td>4.63 (4.6, 4.66)</td>
<td>4.82 (4.78, 4.87)</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>7.23 (6.94, 7.52)</td>
<td>7.65 (7.22, 8.11)</td>
<td>6.86 (6.49, 7.25)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.52 (1.45, 1.58)</td>
<td>1.57 (1.48, 1.67)</td>
<td>1.47 (1.38, 1.55)</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>0.656 (0.607, 0.709)</td>
<td>0.849 (0.757, 0.952)</td>
<td>0.516 (0.467, 0.571)</td>
</tr>
<tr>
<td>Smoker † %</td>
<td>17 (15, 19)</td>
<td>19 (16, 22)</td>
<td>15 (12, 18)</td>
</tr>
<tr>
<td>Drinker ‡ %</td>
<td>51 (48, 54)</td>
<td>50 (45, 54)</td>
<td>52 (48, 56)</td>
</tr>
<tr>
<td>Oral contraceptive user %</td>
<td>32 (28, 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWC 170 score §</td>
<td>1.81 (1.77, 1.84)</td>
<td>1.54 (1.5, 1.58)</td>
<td>2.09 (2.04, 2.14)</td>
</tr>
<tr>
<td>Family income %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ A$ 35,000</td>
<td>13 (11, 15)</td>
<td>14 (11, 17)</td>
<td>13 (10, 15)</td>
</tr>
<tr>
<td>A$ 35,001 - ≤ 78,000</td>
<td>34 (31, 37)</td>
<td>34 (30, 39)</td>
<td>33 (29, 37)</td>
</tr>
<tr>
<td>&gt; A$ 78,000</td>
<td>53 (49, 56)</td>
<td>52 (47, 56)</td>
<td>54 (49, 58)</td>
</tr>
</tbody>
</table>

Data are expressed as mean or percentage (95% confidence interval). Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, Homeostasis model of assessment for insulin resistance; PWC 170, physical working capacity at a heart rate of 170 beats per minute.

† Smoking ≥ 1 cigarettes in a week; ‡ Consumer of alcohol at any level over the last 7 days; § Score of the Physical Working Capacity 170 test, adjusted for weight.
Fractional polynomial plots showing the significant associations of time of blood sample collection (A; p<0.001) and time of sunrise (B; p<0.001), with plasma total cortisol.
Table 2  HPA axis measures,† by sex and the use of oral contraceptives in girls

<table>
<thead>
<tr>
<th></th>
<th>Girls not using OC (n=340)</th>
<th>Girls using OC (n=159)</th>
<th>All girls (n=499)</th>
<th>Boys (n=546)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma ACTH, pg/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.67 (42.71, 46.62)</td>
<td>43.45 (39.67, 47.22)</td>
<td>44.22 (42.44, 46.00)</td>
<td>52.15 (49.94, 54.37)</td>
</tr>
<tr>
<td><strong>Total plasma cortisol, nM</strong></td>
<td>590.73 (571.01, 610.46)</td>
<td>895.08 (849.49, 940.67)</td>
<td>686.81 (663.56, 710.05)</td>
<td>551.97 (539.31, 564.62)</td>
</tr>
<tr>
<td><strong>Plasma CBG, μg/ml</strong></td>
<td>49.07 (47.35, 50.78)</td>
<td>98.91 (92.93, 104.88)</td>
<td>64.69 (61.68, 67.70)</td>
<td>45.45 (44.52, 46.39)</td>
</tr>
<tr>
<td><strong>Calculated plasma free cortisol§, nM</strong></td>
<td>41.96 (39.42, 44.49)</td>
<td>31.26 (28.39, 34.13)</td>
<td>38.66 (36.68, 40.63)</td>
<td>40.44 (38.68, 42.21)</td>
</tr>
<tr>
<td><strong>Salivary free cortisol, nM</strong></td>
<td>27.50 (26.81, 28.18)</td>
<td>26.44 (25.50, 27.38)</td>
<td>27.15 (26.60, 27.70)</td>
<td>25.89 (25.44, 26.33)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (95% confidence interval)

† corrected for time of blood sample collection, date, season, and time of sunrise

§ using Coolen’s equation

Abbreviations: OC, oral contraceptives; CBG, corticosteroid binding globulin
Multivariable models showed significant associations between the HPA axis components and SBP, the lipids, and hs-CRP, after adjusting for sex, BMI, OC use, and predefined early-life and socio-behavioural covariates (Table 3). Full details of these models are presented in Supplemental tables I, II, and III. In boys and girls combined, plasma total cortisol positively associated with SBP, total cholesterol, HDL-C, and hs-CRP. Salivary cortisol positively associated with HDL-C and negatively with LDL-C; plasma calculated free cortisol positively associated with triglycerides; and CBG positively associated with HDL-cholesterol, LDL-C, total cholesterol, and hs-CRP. In these models, for every one standard deviation (SD) increase of total cortisol, SBP increased by 0.85 mmHg, and total cholesterol increased by 0.02 mmol/L. Further, one SD increase in CBG was associated with 0.12 mmol/L higher in total cholesterol, and 0.06 mmol/L higher in LDL-C. HPA measures were not associated with insulin levels or HOMA-IR. There was no significant interaction of the use of OC in girls in all these associations, indicating that these relationships were similar in girls regardless of OC use status. Plasma ACTH was not associated with any cardiovascular risk factor.

In girls, total cortisol and CBG were positively associated with triglycerides (b=0.0005; p<0.001 and b=0.0028; p=0.025, respectively) independent of OC use and other covariates, however this effect was not observed in boys (b= 0.0001; p= 0.292 and b= -0.0018; p= 0.211, respectively).
Table 3  Significant associations between HPA axis parameters and cardiovascular risk factors at 17 years

<table>
<thead>
<tr>
<th></th>
<th>b coefficient</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cortisol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP †</td>
<td>0.0039</td>
<td>0.0009, 0.0068</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.0002</td>
<td>0.0001, 0.0003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.0004</td>
<td>0.0002, 0.0007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hs-CRP †‡</td>
<td>0.0004</td>
<td>0.00005, 0.0008</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Salivary cortisol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C ‡</td>
<td>0.0040</td>
<td>0.0003, 0.0077</td>
<td>0.033</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.0105</td>
<td>-0.0189, -0.0020</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>Plasma free cortisol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.0016</td>
<td>0.0005, 0.0026</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>CBG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.0021</td>
<td>0.0013, 0.0030</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.0045</td>
<td>0.0022, 0.0068</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.0025</td>
<td>0.0005, 0.0045</td>
<td>0.017</td>
</tr>
<tr>
<td>Hs-CRP ‡</td>
<td>0.0066</td>
<td>0.0027, 0.0105</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: SBP, systolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; CBG, corticosteroid binding globulin.

Data from final multivariable models retain variables significantly associated with the outcomes, after the stepwise elimination process adjusting for the potential confounding effects of birth weight, gestational age, BMI, sex, OC use, smoking, alcohol consumption, dietary patterns, physical fitness, and family income.

Significant additional factors in final model: † drinker; ‡ smoker
Discussion

To our knowledge, this is the first study that shows significant associations between basal HPA axis activity and a range of conventional cardiovascular risk factors in a large general population of adolescents at 17 years of age. Specifically, high basal HPA activity was associated with higher levels of SBP, total cholesterol, and hs-CRP, after adjustment for sex, OC use, adiposity and several early-life and socio-behavioural factors.

The HPA axis, a component of the stress response systems, has an important role in long-lasting adaptations to stress. Dysregulation of the HPA axis, in the form of either hypo- or hyper-functioning, may be one of the mechanisms through which negative emotion and chronic stress affect the development of CVD. In this respect, heightened HPA activity has been proposed as a risk factor for coronary heart disease. In adults there is an association between altered cortisol and higher risk of atherosclerosis, carotid and coronary calcification, and ischemic heart disease. The few available paediatric studies, conducted in selected groups of obese children, have similarly shown that cortisol measures were associated with elevated blood pressure, high LDL-C, and increased carotid-intima media thickness. Further, a study in adolescents has shown that HPA axis dysregulation was related to abnormal brain structures such as smaller hippocampal volume and increased frontal lobe atrophy, and these associations were mediated through metabolic abnormalities. Our findings that in a general adolescent population, measures of the HPA axis activity under non-stressed conditions associate with CVD risk factors and in particular, blood pressure, lipids, and an acute marker of inflammation, are relevant in this context.

Previous reports have demonstrated a significant relationship, often an inverse association, between adiposity and cortisol measures. However, our data did not show an association of BMI with any HPA axis parameter. Dysregulation of the HPA axis can promote the development of CVD through the deposition of visceral fat, but another pathway is its direct effect on endothelial function, particularly through elevated blood pressure. In this respect, we observed that HPA measures significantly associated with increased SBP and higher levels of hs-CRP and various lipid
parameters. The overall impact of the altered basal HPA activity in adolescents may be clinically important, especially assuming persistence into adulthood. To put this in perspective, the predicted increase in SBP from a one standard deviation increase in plasma total cortisol represented an effect-size of almost half that of BMI on SBP in the same model (data not shown). The clinical impact of BMI on SBP is well established, as we have previously shown in this cohort that BMI significantly associated with higher SBP in both boys and girls.\textsuperscript{19}

To our knowledge, these are the first population data examining the relationship of CBG with cardiovascular risk factors in adolescents. Clinical studies in adults have shown that plasma CBG was negatively related to adiposity levels and insulin resistance.\textsuperscript{32} CBG is conventionally viewed as a transporter for and reservoir of cortisol in plasma. Under basal conditions, approximately 80 - 90% of CBG is bound to cortisol. Nonetheless, CBG may have a more important role in the neurobehavioural response to stress, beyond simply being a cortisol carrier.\textsuperscript{33} CBG is widely expressed across the central nervous system including the human hypothalamus,\textsuperscript{34} and is selectively present in the anterior pituitary in animal models.\textsuperscript{35} These neuroanatomical observations suggest an important role of CBG in the regulation of stress responses in the central nervous system.\textsuperscript{36} Sex hormones regulate HPA function, and animal data have suggested that changes in the brain over adolescence may play a role in this process.\textsuperscript{37}

At the 17-year review of the Raine Study, approximately 30% of the girls used OCs. We have previously shown in this cohort that OC use in girls significantly associated with higher SBP\textsuperscript{19} and hs-CRP levels.\textsuperscript{38} In accordance with Reynolds et al,\textsuperscript{16} we also observed in the present study that OC use in girls associated with higher plasma CBG and total cortisol levels (corrected for timing factors; data not shown); however, there was no evidence of an interaction of OC use in the associations between CBG or other HPA measures with any cardiovascular risk factor.

Our data show significant positive associations of plasma total cortisol, calculated free cortisol, CBG, and salivary cortisol, with a variety of cardiovascular risk factors, suggesting that increased activation of the basal HPA axis activity adversely affects cardiovascular risk in this age group. Cortisol, the key end-product of HPA activation, can be viewed as a primary intermediary by which chronic stressors may adversely influence cardiovascular risk. Both hypo- and hyperactivity of the HPA axis can
facilitate the pathogenesis of CVD.\textsuperscript{31} However, literature on the relationship of chronic stress with HPA activity has been contradictory; several studies show increased activation\textsuperscript{39} whereas others show a decrease.\textsuperscript{40} In this context, the question arises as to how the positive associations demonstrated in the present study should be interpreted, in view of a potential adverse effect of chronic psychosocial stress on CVD risk through the mediation of HPA activity. In a meta-analysis, Miller et al\textsuperscript{41} suggested that chronic stress can either increase or decrease HPA activity, depending on the timing and nature of the stress, and the person experiencing it. The authors reported that chronic social stressors experienced during adolescence can invariably activate the HPA axis.\textsuperscript{41} Overall, the interrelation among chronic stress, HPA activity and cardiovascular risk is complex.

The strengths of this study include the use of data from a large, well-phenotyped population-based adolescent cohort at 17 years of age. With comprehensive anthropometric, clinical, and socio-behavioural information prospectively collected during pregnancy and over 17 years, we were able to adjust regression models for a number of anthropometric, early-life, and lifestyle factors. We employed a complementary set of HPA axis measures, including ACTH, cortisol measures and CBG, that enabled an integrated assessment of HPA function downstream of the pituitary. Limitations of our study include the study design which is cross-sectional, and thus we cannot infer causality in the relation between HPA activity and cardiovascular risk. Apart from salivary cortisol samples which were consecutively collected over 3 days, other HPA axis components were measured on a single occasion in the morning. However, it has been reported that cortisol measured at awakening was correlated with total daily cortisol exposure,\textsuperscript{42} and reliably predicts carotid intima-media thickness,\textsuperscript{13} a surrogate marker of atherosclerosis. Plasma total cortisol, as used in our study, has been shown to be stable over adolescence,\textsuperscript{43} therefore this cortisol measure may be feasible and reliable as an HPA activity marker to be used in large-scale epidemiological studies.

In conclusion, we have shown in a non-select general adolescent population at 17 years of age that basal HPA axis activity closely associates with increased SBP, cholesterol and hs-CRP levels. Given that adolescence is a developmental period with unique and dramatic changes in neurobehavioural function, understanding the
mechanisms by which basal HPA axis activity regulates cardiovascular function in adolescence would be the first step to assess the impact of psychosocial stress on long-term cardiovascular risk.

Acknowledgements

We are thankful to the Raine Study adolescents and their families, the Raine Study Team for cohort co-ordination and data collection, and Ms Lynette McCahon for technical assistance. We acknowledge the support of the National Health and Medical Research Council of Australia (Program grant 353514 and Project grants 403981 and 458623), and the Canadian Institutes of Health Research (Grant MOP 82893). Core management of the Raine Study is funded by the University of Western Australia (UWA); the Raine Medical Research Foundation; the Telethon Institute for Child Health Research; the UWA Faculty of Medicine, Dentistry and Health Sciences; the Women and Infants Research Foundation and Curtin University. Dr Le-Ha is supported by an Endeavour Postgraduate Award from the Australian Government, and a Raine Study PhD Scholarship.
References


Supplemental materials for Chapter 5

**Supplemental Table I**  Final multivariable models of the associations between plasma total cortisol and cardiovascular risk factors

<table>
<thead>
<tr>
<th></th>
<th>b coefficient</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cortisol</td>
<td>0.0039</td>
<td>0.01</td>
<td>0.0009, 0.0068</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>2.4293</td>
<td>0.013</td>
<td>0.5217, 4.3369</td>
</tr>
<tr>
<td>Boys</td>
<td>10.5885</td>
<td>&lt;0.001</td>
<td>9.3711, 11.8058</td>
</tr>
<tr>
<td>BMI</td>
<td>0.5671</td>
<td>&lt;0.001</td>
<td>0.4392, 0.6951</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>1.4335</td>
<td>0.01</td>
<td>0.3496, 2.5174</td>
</tr>
<tr>
<td>constant</td>
<td>91.3311</td>
<td>&lt;0.001</td>
<td>87.6264, 95.0358</td>
</tr>
<tr>
<td><strong>HDL-Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cortisol</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>0.0001, 0.0003</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>-0.0352</td>
<td>0.224</td>
<td>-0.0921, 0.0217</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.1632</td>
<td>&lt;0.001</td>
<td>-0.1994, -0.1270</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0151</td>
<td>&lt;0.001</td>
<td>-0.0189, -0.0114</td>
</tr>
<tr>
<td>constant</td>
<td>1.6165</td>
<td>&lt;0.001</td>
<td>1.5073, 1.7258</td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cortisol</td>
<td>0.0004</td>
<td>&lt;0.001</td>
<td>0.0002, 0.0007</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.3179</td>
<td>&lt;0.001</td>
<td>0.1683, 0.4676</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.2195</td>
<td>&lt;0.001</td>
<td>-0.3148, -0.1243</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0200</td>
<td>&lt;0.001</td>
<td>0.0102, 0.0299</td>
</tr>
<tr>
<td>constant</td>
<td>3.4093</td>
<td>&lt;0.001</td>
<td>3.1219, 3.6968</td>
</tr>
<tr>
<td></td>
<td>Estimate 1</td>
<td>Estimate 2</td>
<td>Estimate 3</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Total cortisol</td>
<td>0.0004</td>
<td>0.047</td>
<td>0.00005, 0.0008</td>
</tr>
<tr>
<td>BMI</td>
<td>0.1370</td>
<td>&lt;0.001</td>
<td>0.1196, 0.1545</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.4569</td>
<td>0.011</td>
<td>0.1051, 0.8086</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>1.0424</td>
<td>&lt;0.001</td>
<td>0.7533, 1.3314</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.1044</td>
<td>0.255</td>
<td>-0.2841, 0.0753</td>
</tr>
<tr>
<td>Smoking interaction with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC use</td>
<td>-0.6186</td>
<td>0.032</td>
<td>-1.1841, -0.0532</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.3387</td>
<td>0.135</td>
<td>-0.7833, 0.1058</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>0.1545</td>
<td>0.049</td>
<td>0.0005, 0.3086</td>
</tr>
<tr>
<td>constant</td>
<td>-4.2334</td>
<td>&lt;0.001</td>
<td>-4.7399, -3.7268</td>
</tr>
</tbody>
</table>

Final multivariable models retain variables significantly associated with the outcomes, after the stepwise elimination process adjusting for the potential confounding effects of birth weight, gestational age, BMI, sex, OC use, smoking, alcohol consumption, dietary patterns, physical fitness, and family income.

Abbreviations: CI, confidence interval; OC, oral contraceptives; SBP, systolic blood pressure; hs-CRP, high sensitivity C-reactive protein.

† reference: girls not using OC.
### Supplemental Table II  Final multivariable models of the associations of salivary cortisol and free calculated plasma cortisol with cardiovascular risk factors

<table>
<thead>
<tr>
<th></th>
<th>b coefficient</th>
<th>p value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salivary cortisol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary cortisol</td>
<td>0.0040</td>
<td>0.033</td>
<td>0.0003, 0.0077</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.0552</td>
<td>0.065</td>
<td>-0.0034, 0.1137</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.1571</td>
<td>&lt;0.001</td>
<td>-0.2002, -0.1140</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0165</td>
<td>&lt;0.001</td>
<td>-0.0211, -0.0119</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.0837</td>
<td>0.002</td>
<td>-0.1371, -0.0302</td>
</tr>
<tr>
<td>constant</td>
<td>1.6387</td>
<td>&lt;0.001</td>
<td>1.4936, 1.7838</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary cortisol</td>
<td>-0.0105</td>
<td>0.015</td>
<td>-0.0189, -0.0020</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.3646</td>
<td>&lt;0.001</td>
<td>0.2330, 0.4962</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.0792</td>
<td>0.110</td>
<td>-0.1764, 0.0180</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0262</td>
<td>&lt;0.001</td>
<td>0.0159, 0.0365</td>
</tr>
<tr>
<td>constant</td>
<td>1.9679</td>
<td>&lt;0.001</td>
<td>1.6402, 2.2956</td>
</tr>
<tr>
<td>Plasma free cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma free cortisol</td>
<td>0.0016</td>
<td>0.003</td>
<td>0.0005, 0.0026</td>
</tr>
<tr>
<td>sex†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.3059</td>
<td>&lt;0.001</td>
<td>0.2351, 0.3767</td>
</tr>
<tr>
<td>Boys</td>
<td>0.0714</td>
<td>0.005</td>
<td>0.0216, 0.1212</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0216</td>
<td>&lt;0.001</td>
<td>0.0165, 0.0268</td>
</tr>
<tr>
<td>constant</td>
<td>0.3110</td>
<td>&lt;0.001</td>
<td>0.1765, 0.4456</td>
</tr>
</tbody>
</table>

*Final multivariable models retain variables significantly associated with the outcomes, after the stepwise elimination process adjusting for the potential confounding effects of birth weight, gestational age, BMI, sex, OC use, smoking, alcohol consumption, dietary patterns, physical fitness, and family income.*

*Abbreviations: CI, confidence interval; OC, oral contraceptives. † reference: girls not using OC.*
### Supplemental Table III  Final multivariable models of the associations between corticosteroid binding globulin and cardiovascular risk factors

<table>
<thead>
<tr>
<th></th>
<th>b coefficient</th>
<th>p value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL-Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBG</td>
<td>0.0021</td>
<td>&lt;0.001</td>
<td>0.0013, 0.0030</td>
</tr>
<tr>
<td><strong>sex†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>-0.0797</td>
<td>0.018</td>
<td>-0.1458, -0.0135</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.1632</td>
<td>&lt;0.001</td>
<td>-0.1995, -0.1269</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0149</td>
<td>&lt;0.001</td>
<td>-0.0187, -0.0111</td>
</tr>
<tr>
<td><strong>constant</strong></td>
<td>1.6049</td>
<td>&lt;0.001</td>
<td>1.5000, 1.7098</td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBG</td>
<td>0.0045</td>
<td>&lt;0.001</td>
<td>0.0022, 0.0068</td>
</tr>
<tr>
<td><strong>sex†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.2492</td>
<td>0.005</td>
<td>0.0744, 0.4240</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.2213</td>
<td>&lt;0.001</td>
<td>-0.3172, -0.1255</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0205</td>
<td>&lt;0.001</td>
<td>0.0105, 0.0305</td>
</tr>
<tr>
<td><strong>constant</strong></td>
<td>3.4373</td>
<td>&lt;0.001</td>
<td>3.1602, 3.7144</td>
</tr>
<tr>
<td><strong>LDL-Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBG</td>
<td>0.0025</td>
<td>0.017</td>
<td>0.0005, 0.0045</td>
</tr>
<tr>
<td><strong>sex†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC girls</td>
<td>0.2028</td>
<td>0.010</td>
<td>0.0486, 0.3571</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.0784</td>
<td>0.069</td>
<td>-0.1631, 0.0063</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0236</td>
<td>&lt;0.001</td>
<td>0.0147, 0.0324</td>
</tr>
<tr>
<td><strong>constant</strong></td>
<td>1.6482</td>
<td>&lt;0.001</td>
<td>1.4037, 1.8928</td>
</tr>
<tr>
<td><strong>Hs-CRP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBG</td>
<td>0.0066</td>
<td>0.001</td>
<td>0.0027, 0.0105</td>
</tr>
<tr>
<td>BMI</td>
<td>0.1404</td>
<td>&lt;0.001</td>
<td>0.1232, 0.1577</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.4909</td>
<td>0.005</td>
<td>0.1485, 0.8333</td>
</tr>
</tbody>
</table>
sex†

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OC girls</td>
<td>0.8249</td>
<td>&lt;0.001</td>
<td>0.4959,</td>
<td>1.1539</td>
</tr>
<tr>
<td>Boys</td>
<td>-0.0886</td>
<td>0.331</td>
<td>-0.2672,</td>
<td>0.0900</td>
</tr>
</tbody>
</table>

Smoking interaction with

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OC use</td>
<td>-0.6066</td>
<td>0.034</td>
<td>-1.1677,</td>
<td>-0.0455</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.2751</td>
<td>0.226</td>
<td>-0.7209,</td>
<td>0.1707</td>
</tr>
</tbody>
</table>

constant  | -4.3323  | <0.001 | -4.8112,   | -3.8534    |

Final multivariable models retain variables significantly associated with the outcomes, after the stepwise elimination process adjusting for the potential confounding effects of birth weight, gestational age, BMI, sex, OC use, smoking, alcohol consumption, dietary patterns, physical fitness, and family income.

Abbreviations: CI, confidence interval; OC, oral contraceptives; hs-CRP, high sensitivity C-reactive protein; CBG, corticosteroid binding globulin.

† reference: girls not using OC.
Chapter 6

An overview of the main findings and future directions

Although CVD does not clinically manifest until adulthood, risk factors and behaviours that promote the development of atherosclerotic CVD begin early in childhood. Adolescence is a critical stage of life that marks developmental transition from childhood to adulthood and during which there are important biological, psychological and behavioural changes. Early adolescence represents a period of rapid physical and physiological development. However, it is in late adolescence that the individual begins making choices concerning his or her future adult life and ideals. Behavioural patterns developed in adolescence are likely to last for life and will be difficult to change. This is also a period in which adolescents often adopt adverse health behaviours, such as smoking, excess alcohol intake, or consuming an unhealthy diet, that potentially influence cardiovascular health risk in adulthood. The prevalence of poor cardiovascular health behaviours and lifestyle factors in adolescents is high, and the low prevalence of ideal cardiovascular health behaviour in adolescents is predicted to contribute to their worsening future prevalence of obesity, hypertension, and dyslipidaemias. CVD risk factors are conventionally defined as measurable features in the pathological causal path that predict a cardiovascular outcome such as myocardial infarction. Biomarkers, on the other hand, are biological indicators that are involved in the developmental process of CVD but may not necessarily be causal. In the Western Australian Pregnancy Cohort (Raine) Study adolescents at 17 years of age, I have described the biological and behavioural factors that affect their cardiovascular risk profile.

Obesity, high salt intake, and the use of OCs have been important determinants of hypertension in adults. I have shown in Study 1 that male sex and BMI are important factors associated with hypertension status in the adolescents of the Raine Study.
Being male and having higher BMI prospectively predict hypertension in diabetic adolescents in the Treatment Options for Type 2 Diabetes in Adolescent and Youth (TODAY) clinical trial. Recent data from the US NHANES on childhood BP trends for the period 1988-2008 showed that BMI and sodium intake are major factors that were independently associated with elevated BP in children and adolescents. A meta-analysis has shown that a modest sodium intake causes immediate BP reduction in children and adolescents. I have demonstrated in Study 1 that boys who consumed alcohol and were in the upper quartile for both BMI and the urinary sodium to potassium ratio had 5.7 mmHg higher systolic BP, compared to teetotalers in the lowest quartile. Similarly, systolic BP was 5.5 mmHg higher in girls who used OCs and were in the highest BMI and urinary sodium to potassium ratio quartile, compared to those not using OCs and in the lowest quartile.

Studies in adults have been equivocal with regard to the existence of a linear or threshold in the association between BMI and BP. In Study 1, I have observed a continuous linear BMI-BP relationship. Moreover, I have described sex differences in this relationship: there was a greater increase in BP levels with increasing BMI in boys, compared with girls not using OCs in this age group. Sex differences in the development of BP over time have been described previously. However, my data have shown that sex differences in lifestyle and behavioural factors play an important role in explaining the emergence of sex differences in the prevalence of BP in this age group. Given that BP levels in childhood and adolescence predict hypertensive status in adulthood, my findings suggest that future public health policies focusing on the adolescent age will not only be on weight and salt intake reduction, but also on the modification of lifestyle related factors such as alcohol consumption and the use of OCs in girls.

Active smoking and exposure to passive smoking have been well established as CVD risk factors. In children and adolescents, smoking exposure has been associated with an adverse CVD risk factor profile that includes higher levels of the inflammatory marker CRP or lower levels of HDL-C. In studies 2 and 3, I have demonstrated sex differences in the relationship between smoking exposure and these CVD risk factors in late adolescence. Girls at 17 years of age who had been exposed to passive smoking in the household since birth had significantly lower levels of HDL-C, compared with boys.
Similarly, I have shown that active smoking in girls not using OCs associated with higher levels of hs-CRP. These sex differences may highlight important gender-specific cardio-metabolic pathophysiological aspects in the relation between smoking and CVD risk prevention. It has been documented that women may be more vulnerable to the harmful effects of smoking, for example in adult studies showing that the risk of colon cancer was higher in female smokers compared with male smokers, and female smokers had 25% higher coronary heart disease risk compared with male smokers. Future public health programmes and policies should focus on strategies to control active smoking and passive smoking exposure in adolescence. Equally as important, programmes should include a female perspective in tobacco smoking control.

Psychosocial stress has been shown to be a risk factor for atherosclerotic heart disease. Alterations in the stress system activity, either in the form of hypo- or hyper-functioning of the HPA axis are linked to changes in the cardio-metabolic system. In adults, heightened cortisol reactivity has been linked to an increase in BP, a pro-inflammatory state, or a higher extent of coronary calcification. Heightened HPA axis activity has therefore been proposed as a mechanism for this increase in CVD risk. As early as in this adolescent age, I showed in Study 4 that basal HPA axis hyperactivity associates with a range of conventional cardiovascular risk factors, specifically with higher systolic BP, cholesterol, and hs-CRP levels. Adolescence is a period of on-going brain development and maturation of the HPA axis. Alterations in this biological stress system in this critical developmental period may have permanent effects on cardio-metabolic risk in the life-course. Future research should focus on psychosocial stress and its link to a dysfunctional HPA axis, as a component in prediction models of cardio-metabolic risk.

The Raine Study has provided an excellent opportunity to investigate cardiovascular risk factors from a developmental perspective. The emergence of CVD is multifactorial, and is affected by dynamic inter-relations between protective and promoting factors including biological, behavioural, psychological, and social influences. Using a contemporary cohort of adolescents with comprehensive phenotypic, clinical and socio-behavioural data that were prospectively collected during pregnancy and over 17 years, I was able to adjust for a range of covariates, as well as to examine the interactive relationship of these factors, in my statistical analyses of CVD risk factors.
Furthermore, the large sample size enabled precise estimation of the findings. The low p values and narrow confidence intervals reported in my papers suggested that the associations observed were unlikely to occur by chance.

Methodological limitations of the studies include sample attrition, which is more common in the socially disadvantaged families in the Raine Study. Behavioural and emotional problems are prevalent in socially and economically disadvantaged groups. In our studies, behavioural information such as smoking or alcohol intake was obtained by self-reports. To minimise bias, the adolescents provided information of their habits via a computer-based questionnaire. Compared with previous surveys of the Raine Study, the 17-year review collected a more comprehensive range of biomedical and socio-behavioural data, which enabled an extensive examination of various aspects of the cardiovascular risk factor profile of the adolescents through statistical cross-sectional modelling. Longitudinal modelling, although a stronger method, was not feasible for my analyses, given that data on the use of OC were available only for the 17-year survey.

Overall, my findings in this thesis highlight the role of gender and its relationship with the cardiovascular risk factor profile in late adolescence. If the associations observed are causal, the findings suggest that unhealthy lifestyle, adverse health-related behaviours, and psychosocial stress, and their interrelations, in this age group are important antecedents of CVD later in life.

References


Appendix A

The Western Australian Pregnancy Cohort (Raine) Study – Participants from birth to the 17-year review

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>deferred</th>
<th>lost</th>
<th>withdrawn</th>
<th>deceased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>n=2,868</td>
<td>n=204</td>
<td>n=174</td>
<td>n=21</td>
<td>n=28</td>
</tr>
<tr>
<td>Year 1</td>
<td>n=2,441</td>
<td>n=381</td>
<td>n=418</td>
<td>n=51</td>
<td>n=30</td>
</tr>
<tr>
<td>Year 2</td>
<td>n=1,988</td>
<td>n=321</td>
<td>n=158</td>
<td>n=79</td>
<td>n=30</td>
</tr>
<tr>
<td>Year 3</td>
<td>n=2,280</td>
<td>n=339</td>
<td>n=135</td>
<td>n=127</td>
<td>n=30</td>
</tr>
<tr>
<td>Year 5</td>
<td>n=2,237</td>
<td>n=376</td>
<td>n=124</td>
<td>n=198</td>
<td>n=30</td>
</tr>
<tr>
<td>Year 8</td>
<td>n=2,140</td>
<td>n=281</td>
<td>n=162</td>
<td>n=348</td>
<td>n=30</td>
</tr>
<tr>
<td>Year 10</td>
<td>n=2,047</td>
<td>n=357</td>
<td>n=207</td>
<td>n=412</td>
<td>n=32</td>
</tr>
<tr>
<td>Year 14</td>
<td>n=1,860</td>
<td>n=379</td>
<td>n=206</td>
<td>n=447</td>
<td>n=35</td>
</tr>
<tr>
<td>Year 17</td>
<td>n=1,771</td>
<td>n=379</td>
<td>n=206</td>
<td>n=447</td>
<td>n=35</td>
</tr>
</tbody>
</table>

Deferred: remaining in the cohort but declined to participate in the current review;

Lost: lost to follow up;

Withdrawn: withdrawn from cohort, no further contact;

Deceased: child is deceased.
Appendix B

Published papers originating from this thesis

NOTE: Papers removed due to copyright issues.