Relationship between social adversity in two year olds and C-reactive protein in eighteen year olds in the Birth-to-Twenty cohort

A research report presented

By

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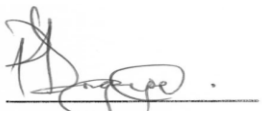
DECLARATION

I, Phuti Dascious Ngwepe hereby declare that this research report is my own unaided work. It is being submitted for the degree of Masters of Science in the field of Epidemiology and Biostatistics at the University of Witwatersrand, Johannesburg. It has not been submitted entirely or partially for any degree or examination at this or any other University.

Signature: 

Date: 15/06/2017

DEDICATION

DEDICATED TO

THE **ALMIGHTY GOD** WHO HAS MADE IT POSSIBLE FOR ME TO ACCOMPLISH THIS WORK

AND

TO

MY LOVING FAMILY

FOR THE UNLIMITED SUPPORT

AND

ENCOURAGEMENT I RECEIVED

ESPECIALLY MY MOTHER FOR ALL THE THINGS SHE HAS DONE FOR ME WHILE I WAS PREPARING THIS WORK

ABSTRACT

Introduction: Worldwide, cardiovascular diseases are the number one cause of death with a three-quarter of cardiovascular disease deaths occurring in low-middle income countries. Childhood social adversity as a proxy of psychosocial stress has been found to be associated with later adult risk of cardiovascular diseases, with studies investigating the mechanisms linking early exposure to social adversity and later risk of cardiovascular diseases. CRP has been a biomarker that is found to be associated with the risk of cardiovascular diseases in adults, however, the association between CRP levels in adolescence and social adversity in children (prenatal and postnatal periods) is not well documented. Assessing the association between childhood social adversity and CRP levels in late adolescent period will encourage further studies to explore whether high levels of CRP tracks from adolescence to adulthood and ultimately increase the risk of cardiovascular diseases in the South African context.

Aim: This study aims to determine the association between social adversity from the prenatal period to two years of age and the level of CRP in the same cohort at the age of 18 (from 1990 to 2008)

Methods: The study was a secondary data analysis of the Birth to Twenty longitudinal study which recruited 3273 singleton children. Four measures (prenatal and postnatal (0-2 years)) of social adversity in children (which are maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household socioeconomic status) were used. The high-sensitivity C-reactive protein was grouped into tertiles (1^{st} tertile: hs-CRP$\leq$0.48 mg/l, 2^{nd} tertile: 0.48<hs-CRP$\leq$1.16, 3^{rd} tertile: hs-CRP>1.16) and multinomial logistic regressions were therefore used to assess the association between childhood social adversities and tertiles of high sensitivity C-reactive protein.

Results: The primary Birth to Twenty longitudinal study had more than 35% loss to follow-up at 18 years. No statistically significant difference (p>0.05) was found on demographic variables of those included in the analysis compared to those not included (due to current study criteria and loss to follow-up). A unit increase in maternal marital stress score during pregnancy was associated with an increase by 2.23 (p=0.03) in the relative risk of the youth being in the 2^{nd} high sensitivity

C-reactive protein tertile in comparison to being in the 1st high sensitivity C-reactive protein tertile. For a unit increase in maternal family stress score, the relative risk of the youth being in the 3rd high sensitivity C-reactive protein is 1.61 (p=0.04) times greater in comparison to being in the 1st high sensitivity C-reactive protein tertile. No statistically significant associations were observed among the other categories of social adversity (p>0.05) and high sensitivity C-reactive proteins. Low social support to mothers during pregnancy was associated with elevated high-sensitivity C-reactive protein in adolescents.

Conclusion: A positive association was observed between a prenatal measure of social adversity and elevated high-sensitivity C-reactive protein; In particular, increased levels of family and relationship-related prenatal stress during pregnancy is a predictor of elevated high-sensitivity C-reactive protein in children. This study contributes to the empirical evidence from studies done in animals suggesting that early development of adult health complication starts during the intrauterine period. The findings of this study will further guide intervention research to target conditions during intrauterine period in preventing adult health complications.

Keywords: High sensitivity C-reactive protein, cardiovascular diseases, intrauterine, tertile, adolescents, pro-inflammation, pre-and postnatal social adversity.

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Last but not least I want to thank God for all the blessings and favour upon me.

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ABBREVIATIONS/ACRONYMS

BT20 : Birth-To-Twenty cohort study

CDC : Centre for Disease Control and Prevention

CRP : C-Reactive Protein

CTQ : Childhood Trauma Questionnaire

CVDs : Cardiovascular Diseases

DPHRU : Developmental Pathways for Health Research Unit

HREC : Health Research Ethics Committee

Hs-CRP : High sensitivity C-Reactive Protein

IL-6 : Interleukin 6

MSc : Master of Science

SES : Socio-Economic Status

WHO : World Health Organization

TERMINOLOGY

Atherosclerosis: a specific form of arteriosclerosis (a type of cardiovascular diseases) in which an artery wall thickens as a result of the formation of multiple atheromatous (accumulation of degenerative material/plaques) within the arteries and accumulation of white blood cells.

Cytokine : generic term for non-antibody proteins released by diverse type of cells of the organism on contact with a specific antigen, which acts as intercellular mediators of inflammation in the generation of an immune response.

Homeostasis : physiological process allowing the regulation of the internal physiological conditions of the organism, usually by a system of feedback controls to maintain vital functions (e.g. body temperature) into normal range regardless of the outside changing conditions

Interleukin-6 : a multifunctional pro-inflammatory cytokine that plays a role in host defense (e.g in the case of an invasion of a foreign body) due to its wide range of immune and hematopoietic (formation of blood cellular components) activities and its potent ability to induce an acute phase response.

C-Reactive Protein : An acute-phase protein that serves as an early marker of inflammation. It is primarily synthesized by the liver and its concentration can be detected in the blood.

Tumor Necrosis Factor Alpha: a cell signalling protein (cytokine) which is involved in systematic inflammation, it is chiefly produced by activated macrophages and it serves as the primary regulator of immune cells and makes up the acute phase reaction.

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CHAPTER ONE: INTRODUCTION

1.1 Background

In developed countries, cardiovascular diseases (CVDs), which affect the blood vessels and the heart (Mendis et al., 2011) are the number one cause of death which resulted in an estimated 17.5 million deaths in 2012 (WHO, 2012). In most African countries, CVDs are the second most common cause of death after infectious diseases, accounting for almost 11% of total deaths in Africa (WHO, 2015). In South Africa, CVDs are the leading cause of non-communicable disease mortality and morbidity (Maredza et al., 2011). Several risk factors such as hypertension, high blood cholesterol, lack of exercise, obesity, smoking, age, diet and psychosocial stress are associated with CVDs (Walton et al., 2002).

Psychosocial stress has been associated with health conditions and consistently with CVDs (Dong et al., 2004; Galobardes et al., 2006). Furthermore, studies have shown that early life exposure to social adversity, a source of child psychosocial stress, is associated with incidents of CVDs in adulthood (Felitti et al., 1998; Galobardes et al., 2006). For instance, a study conducted in New Zealand found that the exposure to social adversity during childhood, measured by social isolation (children who are isolated in the family and at school) using the Rutter's child scale when the children were aged 5, 6, 7 and 11 years, is associated with CVDs risk in adulthood at age 26 (Caspi et al., 2006). As a result, few studies have been researching the potential mechanisms which link the exposure to social adversity during childhood and the increased risk of CVDs in adulthood (Slopen et al., 2013; Miller et al., 2009a). Interestingly, a recent study implicated the innate immune system as a mechanism which links childhood experiences and adulthood disease (Baumeister et al., 2016)

In the last two decades, mounting evidence shows that immune dysregulations, particularly of the inflammatory pathways, could play a role in the pathophysiological pathways linking social adversity during childhood to CVDs risk in adulthood (McDade et al., 2006, Miller et al. 2011). It is assumed that social adversity during childhood, via its alteration of the regulation of the

hypothalamic–pituitary-adrenal (HPA) axis, would impact inflammation processes, as measured by the secretion of biomarkers such as interleukins and the C-reactive protein (CRP), and would ultimately result in higher risks of CVD in adulthood. Indeed, most patients who experienced CVD incidents (such as heart attack, stroke, and atherosclerosis) were found with a higher concentration of circulating biomarkers of inflammation such as CRP and interleukin-6 and 4 (Pearson et al., 2003). This inflammatory status has been defined as a chronic low-grade inflammation state (Pepys and Hirschfield, 2003) and is differentiated from acute inflammatory responses resulting from infections or injuries (Jaye and Waites, 1997). Although no causal mechanistic relationship has been established between the increase in circulating inflammatory biomarkers and CVDs as yet, CRP is now recommended to identify the risk of CVDs in adult patients in clinical settings by the Centres for Disease Control and Prevention and American Heart Association (Pearson et al., 2003).

Associations between childhood social adversity and chronic low-grade inflammation in adulthood, measured by CRP, interleukins, fibrinogen, Tumour necrosis factor, and interferons have been found in several studies (Aiello and Kaplan, 2009; Danesh et al., 1998). A recent systematic review have highlighted the need to conduct more studies which explores the relationship between childhood social adversity and inflammatory changes in young adults. These study will aid in investigating whether immunologic alterations are apparent as early as during adolescence period and whether pro-inflammatory phenotype in late adolescence predict higher risk of CVDs in adulthood. Furthermore more studies can aid in identifying which inflammatory biomarkers during adolescence are best indicators of risks of CVDs (Slopen et al., 2012) .

1.2 Literature review

Introduction

This sub-chapter focuses on the current published literature on the association between social adversity and high-sensitivity CRP. The sub-chapter introduces the derivation of social adversity from stress and further looks at the immunological/biological impacts of social adversity. Published literature on the association between childhood social adversity and adult CRP are then analogized demonstrating the discrepancies in the literature and the need to conduct the current study.

1.2.1 *Definition of stress*

Stress is a state of mental or emotional strain resulting from adverse or demanding stressors such as environmental conditions or physical manifestations (such as physical trauma, and fatigue). Stress is a body's method of reacting to a challenge or foreign stimuli (Black, 2003). However, there are various types of stress including but not limited to psychosocial stress and physiological stress. Psychosocial stress can be defined as a state of threatened homeostasis provoked by the combined effect of psychological factors and the surrounding social environmental factors. Psychological factors include mental fear, anxiety, depression or mental disorders whereas social factors include but not limited to personal relationships, community problems, and/or financial distress (Chrousos & Gold, 1992; Peterson et al., 1991).

1.2.2 Definition of childhood social adversity

Social adversity in childhood is defined as experiences of social conditions or stressors that threaten or are perceived to threaten the physiological equilibrium in childhood (Loman and Gunnar, 2010). Social adversity in childhood refers to challenges to childhood development from the prenatal to postnatal development which are created by stressors outside the family (such as poverty, stranger assaults, community safety and support during/after pregnancy) and within the family (such as relationship problems, neglect, emotional and physical abuse, or early emotional trauma) encountered by the mother during pregnancy or by both mother and child after birth. Childhood social adversity can be assessed by measuring stressors encountered during and after pregnancy (Slopen et al., 2012). These stressors are associated with psychological disturbances in childhood (such as Anxiety disorders, or Mood disorders). (Slopen et al., 2012)

Nonetheless, social adversity in childhood has been defined differently in different studies. For instance, McDade and colleagues (2013) defined and measured social adversity through the instability of caregiving relationship measured by the absence of the mother in the child's life for an extended period and through the socioeconomic resources (household income and household assets) (McDade et al., 2013). In the systematic review by Slopen and colleagues (2012) social adversity in childhood is defined as any type of stressful experience (such as family or relationship conflicts) for the child and material hardship measured by family SES, maternal personal stress,

stressful life events (such as physical or mental trauma) (Slopen et al., 2012). Due to difficulties in measuring childhood stress, social adversity in childhood has been widely used as a proxy (Garner and Shonkof, 2012). The current study measures both aspects of social adversity by assessing the prenatal maternal factors as well as the postnatal maternal and childhood factors.

1.2.3 *Biological impacts of social stressors*

During stress, there is an induction of dysregulations in immune and inflammation processes which involve the secretion of a variety of cytokines through a cascade of biochemical reactions (Black, 2003; Danesh et al., 2004).

Stressful experiences in early life may be one mechanism through which the social environment may be biologically embodied and lead to a cascade of physiological process increasing the risk of CVD in adulthood. In detail (**Figure 1.1**), acute and chronic stressors induce biological responses altering the regulation of the hypothalamic pituitary adrenal (HPA) axis and leading to the stimulation of the sympathetic nervous system (SNS). These processes may result in excessive glucocorticoid production, blunted HPA function and excessive inflammation as measured by CRP, interleukin-6, and fibrinogen levels in the blood (McEwen, 1998).

It is suggested (**Figure 1.1**) that childhood social adversities induce the secretion of the pro-inflammatory cytokine IL-6, among others cytokines, which in turn induce the release of CRP in the blood, produced by the kuppfer cells (macrophages) of the liver, and thus enhance inflammation (Danese et al., 2007; Libby et al, 2002; Dowd et al., 2010). Hypothetically, a stressed child is assumed to develop chronic low-grade inflammation and immune response characterised by the release of IL-6 which will trigger the liver to release CRP in the bloodstream. The progression of inflammation will result in endothelial cell damage and plaque formation in blood vessels, thereby leading to development and progression of CVDs (Slopen et al., 2012).

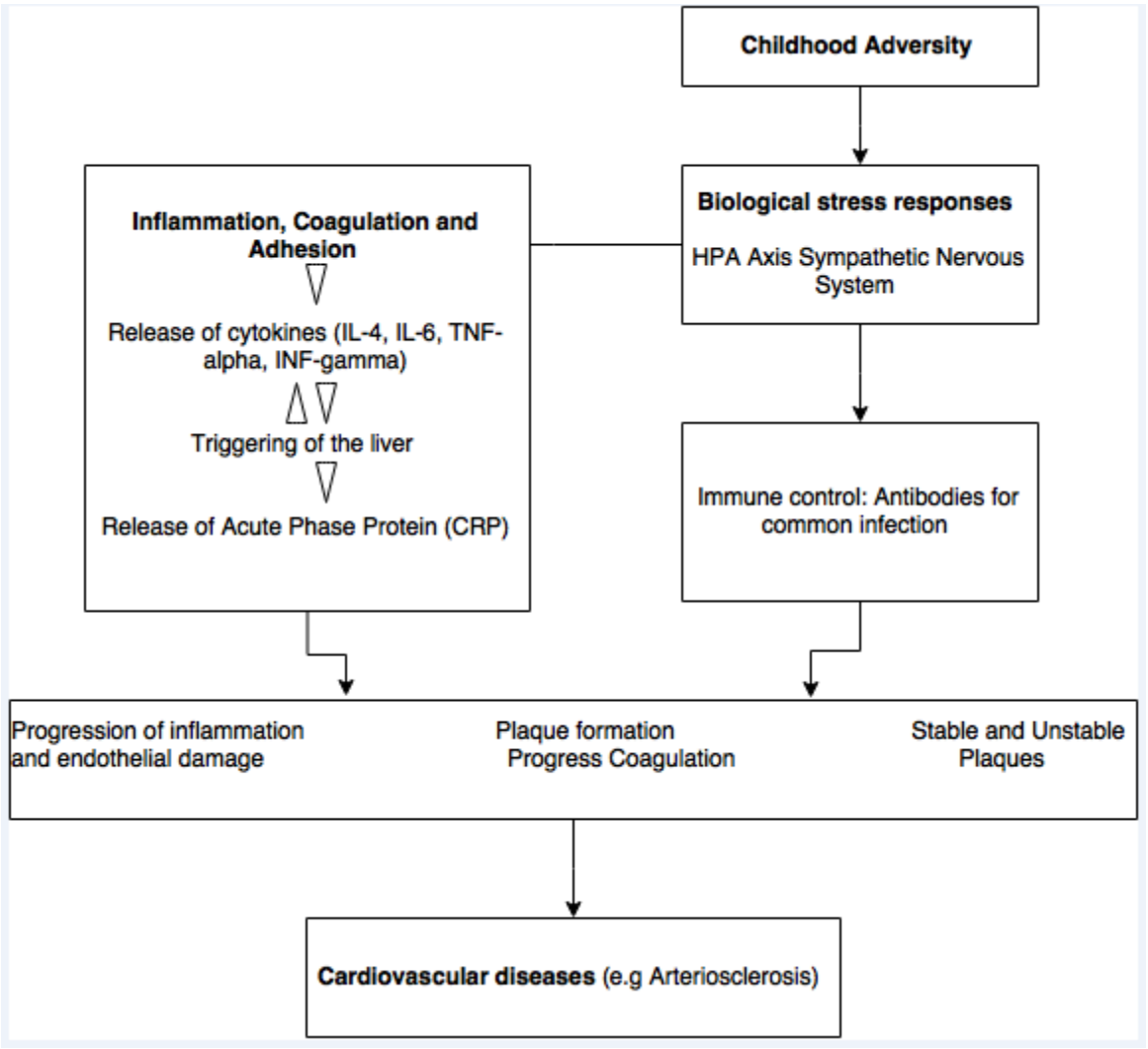


Figure 1.1: *Conceptual model: childhood stress, inflammation, and risk of CVDs (Adaptation from Slopen et al., 2012).*

1.2.4 Prenatal factors and childhood adversity associated with hs-CRP levels

1.2.4.1 CRP a biomarker of chronic low-grade inflammation

Exposure to social adversity during childhood is thought to be a risk factor for CVDs in adulthood through the aforementioned inflammatory mechanisms (Walton et al., 2002). Several inflammatory biomarkers such as CRP, Interleukin-4, interleukin-6, Tumour Necrosis Factor-alpha, and

Interferon-gamma were used to explore the association between childhood adversity and chronic low-grade inflammation in adulthood (Miller et al., 2009c; Herberth et al., 2008; Howe et al., 2010).

CRP has been the biomarker that is given more attention with regard to measuring the levels of low-grade chronic inflammation and future predictions of CVDs. The American Heart Association has proposed cut-off levels of high-sensitivity CRP (hs-CRP) in adults to characterize low-grade chronic inflammation and to define associated risk of CVDs as follow: if the concentration of hs-CRP in blood is between 0-1mg/l the individuals have lower risks of CVD, between 1-3mg/l the individuals have an average risk of CVD and greater than 3mg/l the individuals have high risks of CVD (Pearson et al., 2003). It has been recommended to do the measurement of CRP concentration in the blood to predict CVD risks using High-sensitivity assays (referred to as hs-CRP). In fact, Standard assays for the measure of CRP concentration lack the sensitivity needed to determine levels of CRP at the lower end of the normal ranges and thus the clinical utility of Standard CRP evaluation for vascular risks is extremely limited (Ridker, 2001).

1.2.4.2 Maternal stress and depression during pregnancy

Studies done in humans and animals suggest that developmental conditions during the intrauterine life play a major role in shaping not only aspects of fetal development and birth outcomes but also adult susceptibility for many complex metabolic disorders and health outcomes (Entringer et al., 2010; Barouki et al., 2012; Hanson and Gluckman, 2014). During prenatal development, the fetus is particularly vulnerable to the effects of a broad range of environmental exposures (in particular, maternal distress during pregnancy in the form of exposure to maternal prenatal stressors, or any prenatal chronic/acute stressor) with consequences that can persist into infancy, adolescence, and adulthood. (O'Connor et al., 2002a)

In that context, studies found that maternal distress during pregnancy (exposure to chronic and acute stressors, depression, and/or anxiety) can influence the child's immunologic and psychological traits (Kaplan et al., 2007; O'Connor et al., 2002a). For instance, clinical studies found that maternal prenatal and postnatal depression predicts offspring depression across childhood (1–5 years) and adulthood (18 - 30 years) (Pawlby et al., 2009; Plant et al., 2015; Pearson et al., 2013). Furthermore, Studies suggest that depressed or stressed pregnant women exhibit elevated levels of peripheral

blood inflammatory biomarkers, most commonly C-reactive protein and pro-inflammatory cytokines, as well as urinary cortisol which may remain elevated in the child's biological system (Dowlati et al., 2010; Liu et al., 2012; Zorrilla et al., 2001; Nemeroff et al., 2005, Bhagwagar et al., 2005; Jarcho et al., 2013). In addition, a recent study found an association between maternal prenatal stress and reduced adaptive immunity (the body's immune system acquired due to the first exposure to stimuli thereby preparing an organism for the second encounter to the same stimuli) in their children (O'Connor et al., 2013). Fetal exposure to maternal psychosocial stress during pregnancy together with the resulting inflammatory processes contributes to shaping the child's immune functions characterised by higher levels of circulating inflammatory markers (Bale et al., 2010).

Similarly, studies conducted in high income countries exploring the impact of family socioeconomic status measured by family income suggest that women who have low family incomes during prenatal and postnatal periods are more likely to have children with behavioural problems (such as higher levels of negative moods, or inattention) and less pro-social behaviour (in particular, being unable to interact with other children and adults in a successful or appropriate manner) than children born to mothers with higher family incomes (Dearing et al., 2001; Blau, 1999; Yeung et al., 2002). A more recent study conducted in the United States found that increased family incomes improves the emotional-behavioural symptoms (kids becoming happier and active) compared to children from a less income family (Akee et al., 2015).

Therefore, it is important to consider the potential role of maternal stress during pregnancy in shaping the child's immune functions, in particular, the inflammatory status, and child's later susceptibility to cardiovascular and metabolic disease risks.

1.2.4.3 Factors of postnatal social adversity associated with hs-CRP levels

This last decade, there has been growing interest in exploring whether childhood adversity is associated with low-grade inflammation as early as during adolescence. The first and recent systematic review on the topic showed that most studies have reported positive associations between childhood social adversity and concentration of circulating biomarkers of inflammation in

youth (mainly CRP, cytokines, and fibrinogen) (Slopen et al., 2012). Childhood social adversity was associated with increased concentration of hs-CRP in adolescents of mean age of 18 years with Latin backgrounds in the United States of America (Fuligni et al., 2009) and age 12 to 17 years in the United States of America (Murasko, 2008). Similarly, a study found that harsh family environments, measured using the Risky Families Questionnaire in teenagers aged 15 to 19 years in Canada, predict elevated CRP and enhanced inflammatory responses (Miller and Chen, 2010). The National Health and Nutrition Examination Survey study (conducted in the United State) also reported a significant negative association between parental SES when participants were 3 years of age and CRP level when participants were adolescents aged (16 years of age): high parental SES was associated with low CRP concentration levels and vice-versa (Dowd et al., 2010). On the other hand, a study by Thomas and colleagues (2005) found that boys (mean age of 12.9 years) from schools with high socioeconomic status had elevated CRP levels than boys and girls (mean age of 12.9) from schools with low socioeconomic status (Thomas et al., 2005). Finally, other studies rather found a null association between childhood social adversity and adult inflammation (Cook et al., 2000; Gimeno et al., 2008; Miller et al., 2009c).

These discrepancies can be attributed to methodological limitations such as: the study designs (cohort or cross-sectional), in detail, studies use varying designs to measure the association between early life adversity and CRP therefore, some designs such as cross-sectional lacks the time element (difficult to determine temporal relationship). The methods used to measure early life adversity differs across studies (in detail, indicators or variables used to measure social adversity in childhood differs), the reliability and validity of the sampling methods and sample size used, thus the sample being representative of the target population (Slopen et al., 2012).

Nonetheless, in addition to childhood social adversity, several factors were found to be associated with CRP levels in adolescence. These factors include cigarette smoking (Slopen et al., 2013), alcohol consumption (Costello et al., 2013), body mass index (BMI) (Jarvisalo et al., 2002), socio-economic status (SES) (Dowd et al., 2010), systolic blood pressure (Lande et al., 2008), birth weight (Dritsakou et al., 2015), infections (Póvoa et al., 2005), ethnicity and gender (Khera et al., 2005)

1.3 Problem statement

From a methodological perspective, the few studies which examined the association between stress and hs-CRP were cross-sectional in design, therefore they do not inform on the temporality of events (Guran et al., 2007). Moreover, studies used different methods to measure childhood social adversity which could explain the discrepancies in the results found in the different studies (Hanson and Chen 2010; Carpenter et al., 2012).

In summary, there is a lack of information on: (1) the relationships between early life psychosocial stress and circulating inflammatory markers during adolescence and young adulthood stages, (2) the inflammatory biomarkers during adolescence that would accurately predict the risks of CVDs in adulthood (3) whether elevated circulating inflammatory markers tracks from adolescents to adulthood. So far, only one study reported that raised CRP levels at 18 years of age were associated with raised CRP levels at 39 years of age in a cohort of 1617 subjects from 1980 to 2001 (Juonala et al., 2006).

Finally, most studies on the relationship between early life stress and hs-CRP levels and ultimately CVDs are done in high-income countries. Therefore, there is limited information on the association between early life social adversities, hs-CRP levels, and CVDs in low-and middle income countries such as South Africa (Gaziano et al., 2010). Due to the paucity of studies on the topic, it is recommended that the CRP must be used as a biomarker of inflammation in adolescents in order to continue exploring the association between early adversity and proinflammatory phenotype in adolescence and to provide with more evidence to guide further research (Slopen et al., 2012).

Accordingly, the aim of this project is to explore, using data from a longitudinal cohort, the relationship between childhood social adversity, a proxy of childhood psychosocial stress, and CRP levels during late adolescence in a South African population.

1.4 Justification

Premature deaths caused by blood vessels and heart diseases in individuals of working age (35-64 years) are expected to increase by 41% between 2000 to 2030 and the economic impact which will be experienced as a result of this increase will be enormous (Norman et al., 2006). The overall prevalence of elevated blood pressure in adolescents (18-year-olds) residing in Soweto was found to be 34.9% by Munthali and colleagues (2016). Furthermore, it was also found that birthweight (previously associated with prenatal conditions and adulthood levels of hs-CRP) is associated with elevated blood pressure in adolescents (Munthali et al., 2016). These findings further highlight the need to assess the risk factors and the probable early origins of the inflammatory status of adolescents. To my knowledge, in South Africa, there are currently no studies which were conducted to assess the relationship between childhood social adversity as a proxy of psychosocial stress in childhood and CRP levels in adolescents. The increasing burden of CVDs and the lack of studies highlight the need to document the relationship between childhood social adversity and CRP levels in South Africa.

The current study will use data from the Birth-To-Twenty (BT20) prospective cohort study (children followed up so far from their birth until they turned 25 years of age) which is the largest and longest cohort study ever conducted in Africa. The BT20 cohort was initiated in an area of Soweto-Johannesburg. BT20 started in 1990 when Nelson Mandela, who would become in 1994 the first president of the democratic South Africa, was released from prison and at a period characterised by violent civil unrest in Soweto. Therefore, the children who were born in Soweto in 1990 were born in a period characterised by a stressful environment.
Furthermore, more recently, the newly diagnosed cases of CVDs in Chris Hani Baragwanath Hospital situated in Soweto are high, between 2500 to 4000 cases per annum (Stewart et al., 2006).

These particular social and epidemiological contexts highlight the relevance in investigating the relationship between childhood social adversity and CRP level in young adults who grew up in Soweto-Johannesburg between 1990 and 1992.

Generally, investigating on the identification of raised CRP levels in late adolescence/young adulthood associated with early life social adversity will contribute to bringing evidence on the early onset of high levels of CRP. It will encourage further studies to explore whether high levels

of CRP tracks from adolescence to adulthood and ultimately increase the risk of CVDs in the South African context. The current study may contribute to emphasize on the importance of upbringing and developing children in a healthy psychosocial environment as a public health priority (WHO, 2009).

1.5 Research Question

What is the relationship between social adversities in prenatal and infancy period in the BT20 Cohort living in the urban South African area of Soweto between 1990-1992 and their CRP levels at 18 years of age (in 2008)?

1.6 Research Aim

To determine the association between social adversity from the prenatal period to two years of age and the level of CRP in the same cohort at the age of 18 (from 1990 to 2008)

1.7 Specific research objectives

1. To describe the chosen measures of childhood social adversity, including:
    i. Maternal prenatal stress
    ii. Maternal general feeling during pregnancy
    iii. Maternal postnatal depression score
    iv. Household socio-economic status of the cohort during the first two years of life

2. To assess the relationship between the different measures of childhood social adversity (maternal prenatal stress, general feeling, maternal postnatal depression and SES) and CRP levels at 18 years of age and to determine the factors affecting the relationship between social adversities prenatally and postnatally (during the first two years of life) and levels of CRP at 18 years of age.

CHAPTER TWO: RESEARCH METHODS

2.1 Chapter introduction

This chapter covers the background information about the study area, study design, inclusion and exclusion criteria used for sample selection. It gives a description of the methods and materials used when measuring the exposures, potential confounders, and outcomes. The chapter gives a step guide to data collection, management, and analysis plan followed in obtaining the results and conclusions.

2.2 Study design

The current study is based on secondary data from the BT20 prospective cohort characterized by following-up infants born in 1990 until they turned 25 years of age in 2015.

2.3 Study site

The study was implemented in an urban area of Johannesburg (Gauteng Province, South Africa) called SOWETO (South Western Township). **Appendix 1** shows the location of Soweto in South Africa. Soweto has a total surface area of 200.03 square kilometres with an estimated population of 1 271 628 of whom 98.54% are blacks, 0.11% whites, 0.11% Asians and 0.11% Coloureds (Statistics South Africa, 2011). Soweto was initially set aside to house black labourers who were working in industries in Johannesburg and those working in mines around the Johannesburg area. In 1990, the township was ranked among the poorest areas in Johannesburg; furthermore, the township has been reported to be having high unemployment rate, poor housing, and poor infrastructure (Crankshaw, 2002). Soweto has been popularly known as the township of political unrest since 1948 after the national party won the elections and formed the new government. In 1976, the mass protests by students arose over the government's new policy to enforce education in Afrikaans rather than their native language (Grinker et al., 2014). Children born in 1990-1994 in Soweto were born in a period of political and economic unrest characterised by increased violence activities and the BT20 children are no exception to this.

2.4 Study population

The study population included 3273 children from birth to two years who were followed up in the primary BT20 cohort study until they turned 18 years of age (from 1990 to 2008). **Appendix 2** shows the recruitment process and individuals included in the study. BT20 is a multidisciplinary study, tracking the growth, health, psychological adjustment and well-being of urban children across their life course to young adulthood (Richter et al., 2009). The BT20 is an extension of the Birth to Ten cohort study which focused more on the lifestyle, growth, psychological and biological factors of the birth to ten population associated with sexual and reproductive health, obesity, type 2 diabetes and cardiovascular disease risks. During the enrollment period, pregnant women were recruited into the BT20 study in public hospitals thus there was a selection bias which resulted in a misrepresentation of the white population since more white women attended their antenatal care and gave birth in private hospitals, this was however expected since the Soweto township is predominated by a black population (Richter et al., 2007).

2.5 Sampling

For this study, there was no sampling technique used as it included all the participants (study population) of the primary BT20 prospective cohort.

2.6 Data collection

In the primary BT20 cohort, questionnaires and blood samples were used to collect primary data of interest. The primary data was collected at different time-points in the BT20 cohort.

2.6.1 Measures and variables

2.6.1.1 General overview of the study variables

The flow chart below (**figure 2.1**) is an epidemiological overview of the study variables. The exposure variable is childhood social adversity measured during pregnancy and after giving birth. Proxies/measures of childhood social adversity are: maternal prenatal stress, maternal prenatal

general feeling, maternal depression and household SES. The dependent (outcome) variable is hs-CRP measured when the participants were 18 years of age.

A number of variables which were associated with the outcome (covariates) were used to adjust the multivariate analysis looking at the associations between the childhood social adversity proxies and hs-CRP. The covariates include prenatal maternal/caregiver social support and gestational age, household SES when participants were 18 years of age and participant's birth weight and BMI at 18 years).

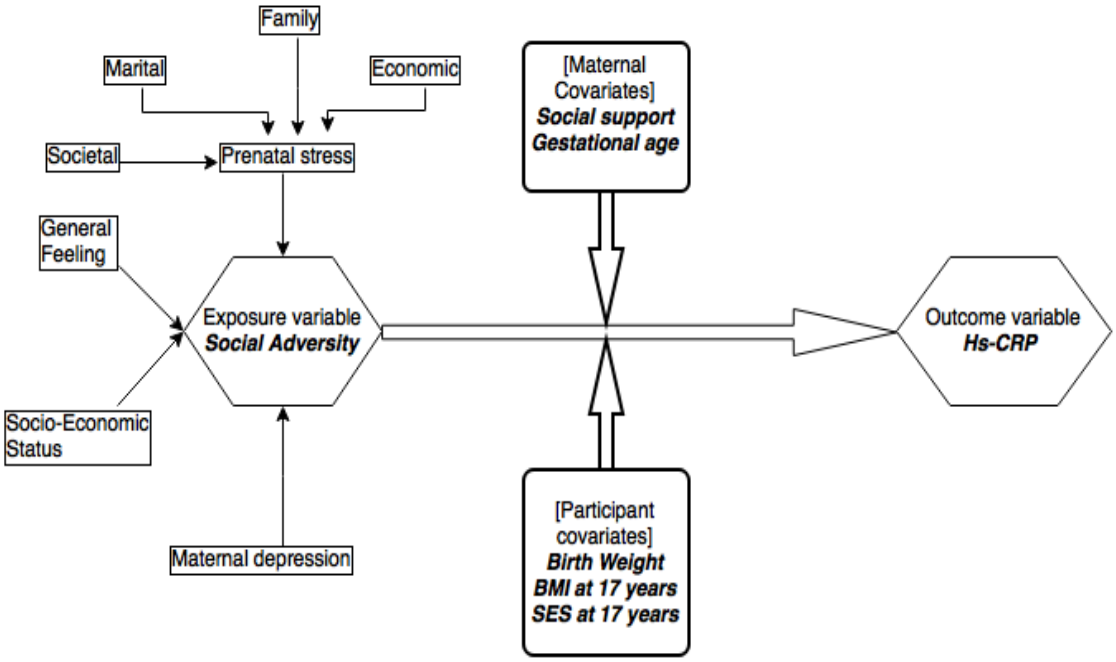


Figure 2.1: General overview of the study variables.

A more detailed description of the variables and their measurement based on the objective of the current study is presented in subsequent paragraphs.

For each objective, the relevant key variables were extracted and are as outlined below:

2.6.1.1.1 Objective One

Independent variable: *Childhood social adversities*

Childhood social adversities were evaluated by measuring the following variables:

i. Maternal Prenatal stress *(Continuous variable)*
ii. Maternal Prenatal General feeling the previous week before the interview *(Categorical variable)*

iii. Maternal postnatal depression *(Continuous variable)*
iv. Household postnatal socioeconomic status *(Continuous variable)*

Prenatal stress in BT20 mothers (**Appendix 3**) was assessed during pregnancy using an interview-administered questionnaire with sixteen questions representing events that could be a potential source of maternal stress. Each question had a yes/no response with the response "yes" meaning that the mother experienced the stress referred to in that particular question. The responses were coded 2 for "yes" and 1 for "no". Scores were summed across all the sixteen responses to yield an overall prenatal stress score. An increase in the overall stress score was indicative of high prenatal stress. The scale has a reasonable internal consistency ($\alpha = 0.64$) and was used in both studies conducted internationally and in South Africa (Weinstock et al., 2008, O'Connor et al., 2003).

For further exploratory analysis, stressful events were separated into four groups to investigate whether specific types/categories (sources) of prenatal stress were associated with hs-CRP. Ten stressful events previously found to be strongly associated with maternal postnatal depression (Ramchandani et al., 2009) were combined in 4 types of stressors. A score for each participant was calculated for each type of stressor. The method used to calculate the scores for each type of stressor followed the methodology used in a previous BT20 study (Ramchandani et al., 2010).

The four types of stressors were as follows:

Marital stress was determined using partner violence and relationship breakdown. These two stressors were given a score of yes (coded 2) and no (coded 1) with "yes" indicating that women experienced the particular stressor and "no" indicating that women did not experience the particular stressor. The responses of all the marital stress questions were added to obtain an overall marital stress score. A higher marital stress score denotes that the participant had a higher prenatal marital stress whereas a lower marital stress score denotes that the participant had a lower prenatal marital stress

Family stress was determined using three stressors (fighting with family, a family member with a drug problem and family member with a disability). These stressors were added to obtain an overall family stress score. A higher family stress score denotes that a participant had a higher prenatal family stress whereas a lower family stress score denotes that a participant had a lower prenatal

Economic stress was determined using three stressors (being in serious debts, having too little money and having to support a family in financial need). These stressors were added to obtain an overall economic stress score. A higher economic stress score denotes that the participant had a higher prenatal economic stress whereas a lower economic stress score denotes that the participant had a lower prenatal economic stress.

Social stress was determined using two stressors (being in danger of being killed and being a witness to a violent crime). The scores were summed to obtain an overall social stress score. A higher social stress score denotes that the participant had a higher prenatal social stress and a lower social stress score denotes that the participant had a lower prenatal social stress.

General feeling was assessed during the prenatal period (pregnancy) by asking one question to the mother of the BT20 participant on her emotional state the week before the interview. The question was phrased: *During the last week, how have you felt generally?* The responses were either "Happy", "Unsure" or "Unhappy/depressed". For the purpose of this analysis, the responses unsure and unhappy were combined to form a single group called Unhappy/Unsure as they reflect that the participant was not consistently happy. Therefore the responses for analysis were coded 1 (Happy) and 2 (Unsure/Unhappy).

Maternal depression (**Appendix 5**) in the postnatal period is basically a 24 items depression screening questionnaire which aimed at measuring the stressful situations encountered by the mother 6 months after giving birth. In the BT20 cohort, maternal depression in the postnatal period was assessed using the Pitt Depression Questionnaire (Pitt, 1968). This questionnaire included 24 questions regarding the current family environment (For details, see **appendix 5**). The Pitt Depression Questionnaire has been used in a number of studies conducted outside western countries and in South Africa (Lawrie et al., 1998; Wolman et al., 1993). It correlates highly with other

measures such as the Edinburgh Postnatal Depression Scale and has a high score stability over time (Ramchandani et al., 2009; Boyce et al., 1993). Each question has a three-point response scale (0 to 2) resulting in a total score for the entire questionnaire ranging from 0 to 48 after summation of the questions. Total scores of 20 and greater are usually taken as indicative of probable cases of depression (Pitt, 1968) as previously defined and validated by Ramchandani and colleagues (2009) using the data from the BT20 cohort. An increase in depression score denotes high levels of maternal depression.

The household **socio-economic status (SES) score** from the prenatal period (pregnancy) until the BT20 participants were 2 years of age was constructed based on household's assets ownership, namely: electricity, television, radio, motor vehicle, washing machine, fridge, and telephone. The socioeconomic status questionnaire was administered during the antenatal period, and when the BT20 participant was 6 months, 1 year and 2 years of age. At each time point, the participants' mothers were asked whether or not they owned each of the assets aforementioned. Having multiple time points at which the ownership of the assets were assessed allowed to complete the information missing when the participants did not show up at one or two time points for data collection. An aggregated SES score was built by summing the number of the aforementioned assets owned in the household during the period of consideration (Ramchandani et al., 2010). The lower the SES score, the lower the SES status of the individual.

2.6.1.1.2 Objective Two

1) ***Independent variable***: Maternal depression, SES, Prenatal stress, Groups of prenatal stress (marital stress, family stress, economic stress and societal stress) and general feeling in the previous week

2) ***Dependent variable***: Hs-CRP concentration

   Fasting blood samples were collected when the participants were 18 years of age (2007 - 2008) into Serum Clot Activator tubes then stored at -70 degree Celsius. CRP concentration was determined from the measurement of the concentration of hs-CRP (in mg/l) in the blood serum collected at the Developmental Pathways for Health Research Unit (DPHRU) laboratory. The samples were then assayed using a full range CRP immunoturbidimetric assay (Randox RX

Daytona Plus, Clinical Analyzer Randox Laboratories, UK, lower detection limit: 0.252 mg/l). This assay can be used to measure CRP levels within high sensitivity and above the normal range.

3) Covariates

Relevant variables that might confound or mediate the association between social adversity in infants and hs-CRP levels in young adults were obtained using questionnaires.

i) Covariates in the prenatal and postnatal period (0 to 2 years):

Birth-weight (*continuous*) – measured in kilograms (kg) was retrieved from the clinical records.

Gender (*categorical variable*) – Either male or female (coded 1 or 2 respectively)

Gestational age (*continuous variable*) – measured in weeks, it was retrieved from the antenatal and birth records. It represents the number of weeks the pregnancy lasted from conception until delivery.

Social support (*continuous variable*) **-** it was enquired about during the prenatal period. Mothers were asked about the help and support provided by family and friends. The social support consisted of an interview-administered questionnaire made of 9 questions (**Appendix 4**). Responses were coded 1 to 3, with the highest coding indicating low levels of social support. The overall social support score was obtained by summing up all the responses. The higher the score, the lower the level of social support

ii) Covariates measured when the participants were 18 years:

Body Mass Index in kg/m^2 (*continuous variable*) –was calculated using weight (measured in kilograms) and height (metres) according to the formula weight/height^2.

Socioeconomic status (*continuous*) – an aggregated score was calculated by summing all the assets owned in the household. The assets include electricity, television, radio, motor vehicle, washing machine, fridge, and telephone. The higher the SES score, the higher the SES.

2.7 Data processing methods and data analysis plan

The relevant data variables were extracted by the principal investigator of the primary BT20 prospective cohort study.

The dataset was obtained in a format compatible for conversion and analysis in STATA. Data management and Statistical analysis were done using STATA version 13IC (StataCorp LP College Station, TX).

2.7.1 Data Management

Data cleaning and management processes were done in order to assess the data for consistency and completeness. In detail, the data was assessed for duplicates, extreme values, distribution and missing values both graphical methods (histograms, scatter plots) and statistical methods (skewness test, tabulations) were used to examine the dataset. Data cleaning and preparation for analytical dataset was performed by recoding variables, categorising variables and labelling values.

2.7.2 Statistical analysis approach

Table 2.1 presents the statistical processes involved in analysing the dataset in measuring the formulated study objective.

Categorising hs-CRP

As per Centre for Disease Control and Prevention recommendations (Pearson et al., 2003) and previous research (Mueller et al., 2002) hs-CRP levels of 10 mg/l or more are indicative of acute inflammation due to microbial infection and the females who are pregnant have raised hs-CRP. Accordingly, participants with hs-CRP of 10 mg/l or more and females who were pregnant during data collection were excluded from the analysis of the current study (N=87 [49 (57%) had a hs-CRP$\geq$10, 34 (38%) were pregnant and 4 (5%) had both a hs-CRP$\geq$ and were pregnant) participants were excluded from the analysis).

The actual hs-CRP was not normally distributed and transformed hs-CRP (log, cubic, square, square root, and inverse) was not normally distributed either. Therefore for statistical analysis the hs-CRP was divided into tertiles (Tertile 1: hs-CRP $\leq$ 0.48 mg/l; Tertile 2: hs-CRP> 0.48 mg/l

and hs-CRP ≤ 1.16 mg/l; Tertile 3: hs-CRP > 1.16 mg/l). Other studies found almost similar hs-CRP categories used in the current study (Korantzopoulos et al., 2005, Nakamura et al., 2010).

Table 2.1: Objectives and statistical analysis process followed

	Objectives	
	Objective 1 (Description of social adversity measures**)**	**Objective 2 (**Relationship between social adversity and CRP**)**
Statistical Analysis plan	The description of maternal **prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household SES** was done using frequency tables. Maternal prenatal stress score, maternal postnatal depression score, and the household SES score were described using the appropriate summary statistic (mean and standard deviations), where necessary assumptions of the summary statistic were tested and the alternative summary statistics were used (median and ranges). In order to describe the prenatal stress in detail, the categories of prenatal stress (family, marital, economic and societal stress scores) were described using median and ranges	Hs-CRP was transformed from continuous to a categorical variable by generating tertile. Spearman correlation analysis was performed among the exposure variables to assess whether they were correlated. Multinomial unadjusted logistic regression models were performed using tertiles of Hs-CRP as the primary dependent variable and independent variables were exposures of interest (maternal pre-natal stress, maternal prenatal general feeling, maternal postnatal depression and household SES, and categories of maternal prenatal stress). Wald test p-values were used. Adjusted multinomial logistic regression models were fitted for primary exposure and covariates which were significant during the univariate (unadjusted) analysis. Only those that were significant at 20% were considered. Both forward and

	Objectives	
	Objective 1 (Description of social adversity measures**)**	**Objective 2 (**Relationship between social adversity and CRP**)**
	The prevalence of the mother's general feeling during pregnancy was assessed using frequency tables and percentages. Due to high loss to follow-up, the participants who had missing data at age 18 (hs-CRP and other confounders) were compared to those who had complete data at age 18 using their demographic variable and an appropriate test of difference (t-test for continuous variables and chi-square test for categorical variables) was used. In instances whereby the assumptions of the test of difference were violated, an appropriate non-parametric test was used.	backward stepwise model building methods were used. 5% alpha significance level was used. Therefore four adjusted multinomial logistic models were fitted; each model had one exposure variable (a measure of social adversity), outcome variable (tertiles of CRP) and covariates/interactions. Furthermore, four more adjusted multinomial logistic regressions were fitted using categories of prenatal stress as exposures and tertiles of CRP as the outcome variable adjusted for covariates. A fitstat post-estimation regression analysis was performed to determine the fitness of each adjusted ○multinomial logistic regression model.

2.8 Ethical clearance

The BT20 prospective cohort study has an ethics approval obtained from the University of the Witwatersrand Human Research Ethics Committee (Medical) (Clearance certificate number: M01-05-56). The current secondary data analysis of the BT20 dataset received ethics approval from the University of the Witwatersrand Committee for Research on Human Subjects (Clearance certificate number: M160469) (**Appendix 6**). A written consent from the BT20 gatekeeper to use the BT20 dataset was granted and submitted to the University of the Witwatersrand Committee for Research on Human Subjects for obtaining ethical clearance for the current study.

Confidentiality: Each record was assigned a unique study number and linked to the BT20 database. Permission was sought, and data extracted by the responsible Data manager. A confidentiality agreement was signed in order to guarantee the security of information pertaining to individual participants and data analysis was done in such a way that no individual could be identified. Furthermore, the dataset received from the BT20 has been granted in an anonymised format (participants primary identifiers removed) and encrypted therefore making it impossible to identify the participants.

CHAPTER THREE: RESULTS

3.0 Chapter introduction

In this chapter, results are presented starting with the statistical difference between the sample used for analysis and participants not included in the analysis. Thereafter, the general characteristics of the study population are presented, then the descriptions of the proxies of social adversity (maternal prenatal stress, general feeling, maternal postnatal depression and SES). The correlation among the proxies of social adversity are then examined, and finally presenting the results of the relationship between the proxies of social adversity (maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household SES) and Hs-CRP (both unadjusted and adjusted associations).

3.1 General description of study participants

3.1.1 Comparisons of the population included in the analysis with the population not included in the analysis

A total of 3273 participants were enrolled in the study. The Hs-CRP data was available for 1068 (32.6%) participants (both males and females combined) for the current analysis while 2205 (64.7%) participant did not have Hs-CRP data, furthermore 87 (2.7%) were excluded from the analysis.

Participants with and without the Hs-CRP (those included in the analysis and those who were not included in the analysis respectively) were compared based on their demographic, anthropometric and maternal psychosocial characteristics. This analysis enhanced the understanding of whether the sample used in the analysis (those with Hs-CRP data) was a true representation of the study population. **Table 3.1** presents the statistical comparison among the two groups. Both groups are not statistically different in terms of the following characteristics: gender (p=0.47), birthweight (p=0.21), SES at 18 years (p=0.08), BMI at 18 years (p=0.51) and maternal characteristic [social support (p=0.4), maternal prenatal general feeling (p=0.53)].

Table 3.1: Comparison of the BT20 participants included and those not included in the univariate/multivariate analysis

		Not Included		Included		Total		*P*-value
		%	N	%	N	%	N	
PARTICIPANTS CHARACTERISTICS		**70.1**	**2381**	**29.9**	**1014**	**100**	**3395**	
Gender								0.47
	Male	48.3	1149	49.6	503	48.7	1652	
	Female	54.7	1232	50.4	511	51.3	1743	
Birthweight (kg)	Median (Range)	3.1 (1, 4.9)	2349	3.1 (1.07, 4.8)	1012	3.1 (1, 4.92)	3361	0.21*
SES status at 0 to 2 years	Median (Range)	4 (0, 7)	1602	4 (0, 7)	822	4 (0, 7)	2424	0.7*
Weight at 18 years	Median (Range)	57.4 (35, 136.6)	999	57.5 (36.3, 125)	1002	57.4 (35, 136.6)	2001	0.71*
BMI at 18 years	Median (Range)	20.7 (14.6, 53.1)	997	20.8 (14, 48.3)	1002	20.7 (14, 53.1)	1999	0.51*
SES status at 18 years	Median (Range)	7 (0, 13)	1018	7 (0, 13)	944	7 (0, 13)	1962	0.08*
Social support score	Median (Range)	19 (11, 22)	706	19 (12, 22)	352	19 (11, 22)	1058	0.4*
General feeling								0.53*
	Happy	74.0	804	75.8	385	74.5	1189	
	Depressed	26.0	285	24.2	123	25.4	406	

* Wilcoxen rank-sum test used on those continuous variables which were not normally distributed.

3.1.2 Description of the study sample

Participants' characteristics

Table 3.2 and 3.3.1 present the general characteristics of the BT20 participant and their mothers respectively. **Table 3.3.2** presents the support and emotional states during the mother's pregnancy period. **Table 3.3.3** presents the prenatal stress and the postnatal depression scores.

3.1.2.1 BT20 Participants and their mothers' characteristics

Participants' characteristics from birth to two years of age:

The study sample was made of more black Africans (N=2574, 75.7%] in comparison with other ethnic groups. There was no statistically significant difference (p=0.51) between males and females in terms of ethnicity. In terms of birth-weight, there was a statistically significant difference in between males and females (p<0.001). Females had a lower median birth weight of 3.02 kg (Range: 1.0 – 4.92) compared to males with a median birth weight of 3.14 kg (Range: 1.1 – 4.8).

The median for the population's household SES score between birth and two years was 4 (Range 0 – 7), no statistically significant differences were observed in the median household SES between males and females (p=0.82).

Participants' characteristics at 18 years

The median age of the population was 17.9 years (Range: 17.1 – 18.9). There was no statistically significant difference in the median age of the study population between males and females (p=0.58). Similarly, at age 18 years, the median SES scores for males and females was also not statistically different (p=0.91). In terms of BMI at 18 years, females had a higher median BMI of 22.0 kg/m^2 (Range: 14.4 – 53.1) than males with a median BMI of 19.8 kg/m^2 (Range: 14.0 – 48.3) and the difference was statistically significant (p<0.001).

There is a statistically significant difference (p= 0.04) in Hs-CRP between males and females, with females having a higher median hs-CRP of 0.93mg/l (range: 0.03 – 30.47) than that of males which is 0.71mg/l (range: 0.04 – 43.60).

Table 3.2: Characteristics of the BT20 participants stratified by the gender

		Male		Female		Total		
		%	*N*	*%*	*N*	*%*	*N*	*P-value*
PARTICIPANTS CHARACTERISTICS		***48.7***	***1652***	***51.3***	***1743***	***100***	***3395***	
BT20 factors at 0-2 years								
Ethnicity (%)	White	10.1	167	9.1	159	9.6	326	0.51
	African black	75.6	1251	75.8	1323	75.7	2574	
	Mixed Ancestry	10.7	177	11.9	207	11.3	384	
	Indian	3.6	59	3.2	56	3.4	115	
Birthweight (kg)	Median (Range)	3.14 (1.1 , 4.8)	1632	3.02 (1 , 4.92)	1729	3.10 (1 , 4.92)	3361	<0.001*
Gestational Age (weeks)	Median (Range)	38 (26 , 44)	1557	38 (26 , 42)	1639	38 (26 ,44)	3196	0.77
Birthweight (z-score)	Median (Range)	-0.44 (-5.59 , 2.63)	1632	-0.47 (-5.85 , 3.23)	1729	-0.46 (-5.85 , 3.23)	3361	0.65
BT20 factors at 18 years								
Weight (kg)	Median (Range)	58 (38.5 , 128.4)	969	56.4 (35 , 136.6)	1032	57.4 (35 , 136.6)	2001	<0.001*
Height (cm)	Median (Range)	171.2 (145.6 , 202.3)	968	159.8 (141 , 184.8)	1034	165 (141 , 202.3)	2002	<0.001*
Height-for-age (z-score)	Mean (SD)	-0.63 (0.93)	968	-0.49 (0.97)	1034	-0.57 (0.95)	2002	0.002*
BMI	Median (Range)	19.8 (14.0 , 48.3)	968	22.0 (14.4 , 53.1)	1031	20.7 (14.0 , 53.1)	1999	<0.001*
BMI-for-age (z-score)	Mean (SD)	-0.64 (1.10)	967	0.30 (1.17)	1030	-0.16 (1.23)	1997	<0.001*
SES at 0-2 years-old	Median (Range)	4 (0 , 7)	1181	4 (0 , 7)	1243	4 (0 , 7)	2424	0.82
Age (years)	Median (Range)	17.9 (17.26 , 18.93)	969	17.91 (17.10 , 18.94)	1034	17.91 (17.10 , 18.94)	2003	0.58
Hs-CRP (mg/L)	Median (Range)	0.71 (0.04 , 43.60)	525	0.93 (0 .03 , 30.47)	543	0.82 (0 .03 , 30.41)	1068	<0.001*
SES at 18 years-old	Median (Range)	7 (1 , 13)	951	7 (0 , 13)	1011	7 (0 , 13)	1962	0.65

****: P-value <0.05***

BT20 mother's characteristics

There were no statistical differences between females and males in terms of their mother's marital status, the level of education, type of house and maternal age (p=0.72, p=0.61, p=0.05, and p=0.62, respectively). Furthermore, no statistical significance was observed between the males and females born to mothers who were living with a sexual partner or not during pregnancy (**Table 3.3.1**).

Table 3.3.1 Demographic characteristics of the BT20 mothers

MATERNAL AND HOUSEHOLD CHARACTERISTICS	**Response category**	**Median (Range) or Percentage**	**N**
Demographics			
Parity	Median (Range)	2 (0 , 10)	3380
Age (years)	Median (Range)	25 (13 , 48)	3385
Marital status (%)	Married/Living together	44.8	1504
	Single/Separated	55.2	1851
Education (%)	No formal education to std 5	15.0	456
	Std 8 to 10	72.0	2186
	Post school training	13.0	395
Type of House Owned (%)	House	68.2	1901
	Not a house	31.80	886
Relation of the person living with the mother (%)	Sexual partner	51.1	779
	No sexual partner	49.0	749

Table 3.3.2 Maternal social support and emotional states during pregnancy

Maternal social support during pregnancy			
People who could help (%)	Nobody	10.3	165
	Unsure	8.1	129
	A number of people	84.4	1348
Partner financial contribution (%)	Yes	88.2	1335
	No / Don’t know	11.8	179
Parents, family or friend to talk to about any problems (%)	Nobody	8.0	128
	Unsure	6.2	99
	A number of people	84.4	1348
Husband or partner to talk to about any problems (%)	Never	13.3	213
	Sometimes	22.5	360
	Always	60.3	964

Helpfulness of sisters at the clinic (%)	Always helpful	87.4	1397
	Sometimes helpful	9.4	150
	Seldom helpful	1.8	28
The father of the child or the partner's acts make it harder (%)	Never	62.8	1003
	Sometimes	24.3	388
Belong to church group or any other organisation (%)	Always	8.2	131
	Yes	69.9	1117
	No	28.1	449
Frequency at social meetings (%)	Once a week	39.6	632
	Once a month	7.5	119
	Irregular	24.2	387
	Not applicable	24.2	386
Have a friend with a baby or about to have a baby (%)	Yes	43.9	702
	No	54.9	878
Frequency of seeing friend with a baby or about to have a baby have (%)	Once a week	18.0	288
	Once a month	5.6	89
	Irregular	18.4	294
	Not applicable	53.6	856
Maternal social support score (high score = low social support)	Median (Range)	19 (11 , 22)	1060
Maternal emotional state during pregnancy			
Felt generally the week before the interview (%)	Happy	74.5	1191
	Unsure/Unhappy	24.5	392
Generally being feeling like this during pregnancy (%)	Consistently happy	71.4	1141
	Unsure	13.4	214
	Consistently unhappy	13.1	209

Table 3.3.3 Maternal prenatal stress during pregnancy and post-natal depression after pregnancy

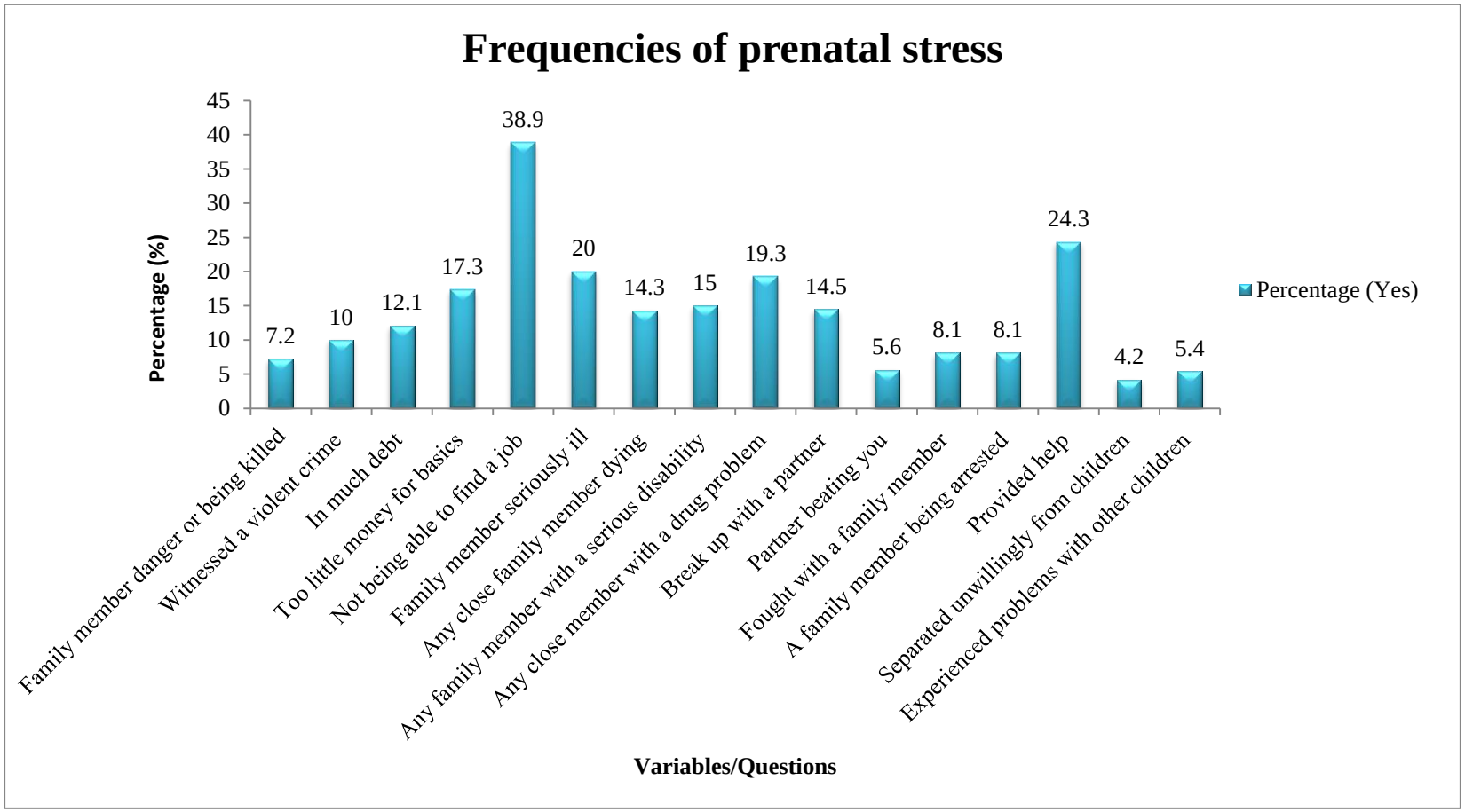
Maternal prenatal stress			
Prenatal stress score (low score = low overall prenatal stress)	Median (Range)	18 (16 , 31)	1233
Prenatal stress score – Marital stress (low score = low marital stress)	Median (Range)	2 (2 , 4)	1525
Prenatal stress score – Family stress (low score = low family stress)	Median (Range)	3 (3 , 6)	1552

Prenatal stress score – Economic stress (low score = low economic stress)	Median (Range)	3 (3 , 6)	1539
Prenatal stress score – Societal stress (low score = low societal stress)	Median (Range)	2 (2 , 4)	1555
Maternal depression status at 6 months post-pregnancy (Pitt depression scale)			
Do you sleep well (%)	Yes	92.5	1725
	Don't know	1.5	28
	No	6.9	129
Do you easily lose your temper (%)	Yes	43.9	820
	Don't know	1.5	28
	No	54.6	1019
Are you worried about your looks (%)	Yes	28.6	534
	Don't know	1.2	23
	No	70.2	1309
Do you have a good appetite (%)	Yes	88.4	1650
	Don't know	0.8	15
	No	10.8	202
Are you as happy as you ought to be (%)	Yes	79.3	1478
	Don't know	2.2	41
	No	18.6	346
Do you easily forget things (%)	Yes	34.4	641
	Don't know	1.0	19
	No	64.6	1205
Have you as much interest in sex as ever (%)	Yes	35.5	660
	Don't know	5.3	98
	No	59.3	1102
Is everything a great effort (%)	Yes	35.0	652
	Don't know	2.5	46
	No	62.5	1166
Do you feel ashamed for any reason (%)	Yes	26.5	493
	Don't know	1.7	31
	No	71.9	1340
Can you relax easily (%)	Yes	81.7	1524

	Don’t know	1.2	23
	No	17.1	318
Can you feel the baby is really yours (%)	Yes	98.8	1843
	Don’t know	0.2	4
	No	1.0	19
Do you want someone with you all the time (%)	Yes	41.2	770
	Don’t know	1.3	25
	No	57.4	1072
Are you easily woken up (%)	Yes	77.7	1448
	Don’t know	0.8	15
	No	21.5	400
Do you feel calm most of the time (%)	Yes	82.8	1546
	Don’t know	1.5	27
	No	15.8	294
Do you feel that you are in good health (%)	Yes	84.7	1582
	Don’t know	1.7	32
	No	13.6	253
Does food interest you less than it did (%)	Yes	35.3	650
	Don’t know	2.3	42
	No	62.5	1166
Do you cry easily (%)	Yes	46.9	874
	Don’t know	1.2	22
	No	51.9	967
Is your memory as good as ever (%)	Yes	78.4	1461
	Don’t know	1.7	32
	No	19.9	371
Have you less dire for less than usual (%)	Yes	52.5	975
	Don’t know	5.9	109
	No	41.7	774
Have you enough energy (%)	Yes	83.4	1557
	Don’t know	2.1	40
	No	14.5	270
Are you satisfied with the way you are coping with things (%)	Yes	76.7	1432
	Don’t know	1.2	22

	No	22.1	413
Do you worry a lot about the baby (%)	Yes	45.5	849
	Don't know	0.8	15
	No	53.7	1002
Do you feel like your normal self (%)	Yes	38.4	716
	Don't know	1.8	35
	No	59.7	1002
Do you have confidence in yourself (%)	Yes	90.9	1697
	Don't know	1.9	35
	No	7.2	135
Depression_Score (high score = high level of depression)	Median (Range)	14 (0 , 42)	1866
Depression score categories	Depressed (Score > 10)	24.4	450
	Not depressed (Score <10)	75.6	1393

Figure 3.1 Bar Chart for frequencies of mother's prenatal stress



3.1.2.2 Description of the BT20 participants hs-CRP

Tertiles for hs-CRP were calculated (**Table 3.4**). Each category had 332 participants after excluding individuals who were pregnant and those with hs-CRP greater than 10mg/l as described in the Methods section (Chapter two).

Table 3.4: Summary statistics of the tertiles of hs-CRP and the distribution

Category	***Hs-CRP ranges***	***Number of participant in each category***
1st Tertile	Less/equal to 0.48 mg/l	332 Participants
2nd Tertile	Greater than 0.48 mg/l and less/equal to 1.16 mg/l	332 Participants
3rd Tertile	Greater than 1.16	332 Participants

3.1.2.3 Description of the measures of social adversity (maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household SES)

Maternal Prenatal Stress

An assessment of the individual maternal prenatal stressors is presented in **Figure 3.1**. Most mothers (38.9%) reported being worried about not being able to find a job. 24.3% of the mothers reported having provided help to someone in need which ultimately served as a stressor to them. In addition, 20% of the mothers reported that they had a family member who was seriously ill. Furthermore, 19.3% of mothers reported having a family member with a drug/alcohol problem. A proportion (14.5%) of mothers reported having broken up with their partner in the last six month. **Figure 3.1** presents the reported frequencies of all the maternal prenatal stressors.

The median of the prenatal score for BT20 mothers was 18 (Range: 16 – 31). Maternal prenatal stress scores were significantly different between the tertiles of hs-CRP (p=0.02). Bonferroni

posthoc analysis showed that the participants in the 2nd tertile of hs-CRP had a higher maternal prenatal stress score (p=0.02) compared to those in the 1st tertile of hs-CRP (**Table 3.5**).

The median score of marital stress, family stress, economic stress, and societal stress scores of the BT20 mothers were 2 (Range: 2 – 4), 3 (Range: 3 – 6), 3 (Range: 3 – 6), and 2 (Range: 2 - 4), respectively. Maternal marital stress scores were statistically different between hs-CRP tertiles (p<0.001). Bonferroni posthoc analysis showed that BT20 participants in the 2nd tertile of hs-CRP had a significantly higher marital stress score (p=0.01) compared to BT20 participants in the 1st tertile of hs-CRP. Those who were in the 3rd hs-CRP tertile tend to have higher maternal marital stress scores in relation to those in the 1st hs-CRP tertile (p=0.07).

Maternal family stress scores were significantly higher in those in the 2nd tertile of hs-CRP compared to those in the first tertile of hs-CRP (p=0.02). There was no statistically significant difference in family stress scores among those in the 3rd hs-CRP tertile in relation to those in the 1st tertile.

No statistically significant difference between hs-CRP tertiles was found for both maternal prenatal economic stress and maternal prenatal societal stress scores (p=0.59 and p=0.84, respectively).

Prenatal General Feeling

A higher proportion (74.5%) of mothers of the participants reported being consistently happy the week before the study interviews were conducted whereas 25.5% women reported being consistently unhappy. There was no significant difference (p=0.26) in the maternal prenatal *general feeling* of BT20 mothers between hs-CRP tertiles.

Maternal Postnatal Depression

BT20 mothers had a median postnatal depression score of 14 (Range: 0 – 42). 75.6% (N=1393) mothers had a depression score of less than 10 (denoting lower levels of depression) whereas 24.4% (N=450) had a depression score of more than 10 (denoting high levels of depression). Assessing individual maternal postnatal depression variables, a higher proportion (46.9%) of mothers reported being losing their temper easily. Moreover, 45.5% of mothers reported being worried a lot about their baby. 41.2% of the mothers reported that they want someone with them all the time.

Furthermore, 34.4 % of mothers reported to forgetting things easily. **Table 3.3** presents all the questions and the frequencies of the responses.

There were no statistically significant differences (p= 0.99) in the maternal depression score among the tertiles of hs-CRP.

Household Socioeconomic status between birth and two years of age

The median household socioeconomic score was 4 (Range: 0 – 7). There were no significant differences between the three hs-CRP tertiles (p=0.73) in the socioeconomic status of the population.

Table 3.5 Statistical differences between tertiles of hs-CRP and measures of social adversity

Measures of Social adversity		***Tertile 1***	***Tertile 2***	***Tertile 3***	***P-value***
Maternal prenatal stress score	Mean (SD)	18.12 (1.77)	18.80 (2.45)	18.59 (1.95)	0.02
Maternal prenatal marital stress score	Mean (SD)	2.12 (0.33)	2.27 (0.54)	2.23 (0.48)	0.01
Maternal prenatal family stress score	Mean (SD)	3.35 (0.56)	3.56 (0.81)	3.50 (0.71)	0.02
Maternal prenatal economic stress score	Mean (SD)	3.54 (0.79)	3.63 (0.89)	3.57 (0.78)	0.59
Maternal prenatal social stress score	Mean (SD)	2.17 (0.42)	2.18 (0.47)	2.15 (0.41)	0.84
Maternal prenatal general feeling	Depressed [n (%)]	33 (29.0%)	42 (36.8%)	39 (34.2%)	0.26*
Maternal postnatal depression score	Mean (SD)	15.07 (6.53)	14.99 (7.03)	14.98 (7.25)	0.99
Household postnatal SES score	Mean (SD)	3.58 (1.70)	3.61 (1.65)	3.69 (1.63)	0.73

ANOVA and Bonferroni used on all score variables, *Chi-square test used.

3.2 Associations between the measures of social adversity (maternal prenatal stress, general feeling, maternal postnatal depression and SES) and the participants' hs-CRP level at 18 years of age.

3.2.0 Introduction

In this section, the correlation between the four measures of social adversity is presented. The relationships between the four measures of social adversity and the hs-CRP (tertiles) are also presented. The results are presented as follows: first, the correlation between measures of social adversity, then, the unadjusted associations whereby the association between each measure of social adversity and hs-CRP is assessed without controlling for any confounding variables, and lastly, the adjusted analysis whereby the associations between each measure of social adversity and hs-CRP are assessed after adjusting for the presence of other confounders.

3.2.1 Bivariate associations between measures of social adversity (maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression, and household SES)

Table 3.6 below presents the results of the correlations between all the proxies of social adversity. The correlations between the proxies of social adversity variables were all statistically significant ($p<0.05$). In detail, there is a positive association between maternal prenatal stress score and maternal postnatal depression score ($p<0.001$). In addition, a negative statistical association ($p<0.001$) was observed between maternal postnatal depression score and household SES score and also between maternal postnatal depression and maternal social support. In detail, a decrease in the household SES score was associated with an increase in maternal postnatal depression score moreover; a decrease in maternal prenatal social support was associated with an increase in maternal postnatal depression score. There was no statistically significant correlation ($p>0.05$) between the household SES score and the maternal social support score, maternal prenatal general feeling, maternal marital stress score and maternal societal stress. There is a negative association between maternal prenatal stress and maternal social support, furthermore, a negative association is also observed between maternal prenatal stress and household SES.

Table 3.6: Spearman correlation results for associations between maternal prenatal stress, general feeling, maternal postnatal depression and SES

	Depression score	Prenatal stress	SES	Maternal social support	Marital stress	Family stress	Societal stress	Economic stress	General feeling past week
Depression score	1.00								
Prenatal stress	0.18*	1.00							
SES	-0.06*	-0.14*	1.00						
Maternal social support	-0.15*	-0.08*	0.06	1.00					
Marital stress	0.10*	0.40*	-0.04	-0.19*	1.00				
Family stress	0.11*	0.56*	-0.07*	-0.09*	0.17*	1.00			
Societal stress	0.08*	0.67*	-0.03	-0.07*	0.13*	0.10*	1.00		
Economic stress	0.11*	0.36*	-0.08*	-0.03	0.18*	0.24*	0.12*	1.00	
General feeling past week	0.19*	0.22*	-0.01	-0.19*	0.22*	0.15*	0.13*	0.11*	1.00

*p< 0.05

3.2.2 Unadjusted associations between maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household SES and BT20 participants' hs-CRP level at 18 years old

Table 3.7 shows detailed results of the unadjusted association between each proxy of social adversity and hs-CRP. One unit increase in the maternal prenatal stress score was associated with an increase by 1.18 in the child's relative risk of being in the 2nd hs-CRP tertile in comparison to being in the 1st tertile. Furthermore, for a single unit increase in the maternal prenatal stress score, the relative risk of the youth being in the 3rd tertile (hs-CRP>1.16 mg/l) in comparison to being in the 1st tertile (hs-CRP≤0.48 mg/l) increased by a factor of 1.13 (p=0.05).

One unit increase in maternal marital stress score during pregnancy was associated with an increase by 1.92 in the relative risk of being in the 3rd tertile (hs-CRP≥1.16 mg/l) as compared to being in the 1st tertile (hs-CRP≤0.48mg/l) (p=0.02). Moreover, the relative risk of being in the 2nd hs-CRP tertile increased by a factor of 2.18 in comparison to being in the 1st hs-CRP tertile for one unit increase in maternal marital stress score (p=0.005).

A one unit increase in the maternal family stress score while the mother was pregnant was associated with 1.40 relative risk of a youth being in the 3rd hs-CRP tertile in comparison to being in the 1st hs-CRP tertile at age 18 years (p=0.04). In addition, the relative risk for a youth to be in the 2nd hs-CRP tertile in comparison to being in the 1st tertile increased by a factor of 1.57 with a single unit increase in the maternal family stress score during pregnancy(p=0.007).

In the unadjusted model, there were no statistically significant associations between the tertiles of hs-CRP and the following proxies of social adversity: maternal postnatal depression score, household SES, maternal prenatal general feeling, maternal prenatal economic stress and maternal prenatal societal stress.

Table 3.7: Multinomial logistic regression results for exposures regressed on the categorized hs-CRP

	CRP ≤ 0.48 (mg/l)	**0.48 < CRP ≤ 1.16 (mg/l)**			**CRP > 1.16 (mg/l)**		
	Ref	***RRR***	***P-value***	***95% CI***	***RRR***	***P-value***	***95% CI***
Depression score	*Ref*	1.00	0.91	0.97, 1.03	1.00	0.90	0.97, 1.03
Prenatal stress score	*Ref*	1.18	0.008*	1.04, 1.34	1.13	0.05	1.00, 1.28
SES at age 0 to 2	*Ref*	1.00	0.78	0.92, 1.12	1.04	0.79	0.94, 1.15
General feeling past week – Unhappy/Unsure	*Ref*	1.52	0.11	0.90, 2.56	1.35	0.27	0.80, 2.27
Categories of post-natal stress							
Marital stress	*Ref*	2.18	0.005*	1.27, 3.74	1.92	0.02*	1.11, 3.30
Family stress	*Ref*	1.57	0.007*	1.13, 2.18	1.40	0.048*	1.00, 1.94
Economic stress	*Ref*	1.14	0.32	0.88, 1.50	1.04	0.78	0.79, 1.36
Societal stress	*Ref*	1.02	0.95	0.62, 1.66	0.88	0.62	0.53, 1.45

***: P<0.05**

3.2.3 Adjusted associations between maternal prenatal stress, maternal prenatal general feeling, maternal postnatal depression and household SES and BT20 participants' hs-CRP level at 18 years old

Table 3.8 presents the adjusted statistical associations between the proxies of social adversity and the hs-CRP at 18 years of age. The reference category of the outcome variable is the 1st tertile of hs-CRP (hs-CRP ≤ 0.48 mg/l). The effect of any covariates was assessed on eight (8) models. Each model included only a single proxy of social adversity. Putting all the proxies of social adversity in one model was avoided due to correlations between the proxies of social adversity (**Table 3.6**).

3.2.2.1 *Associations between maternal prenatal stress and BT20 participants' hs-CRP level at 18 years old*

With one unit increase in the maternal prenatal marital stress score during pregnancy, the relative risk of the youth being in the 2nd hs-CRP tertile (0.48 mg/l<hs-CRP≤1.16 mg/l) is 2.23 times greater in comparison to being in the 1st hs-CRP tertile (hs-CRP≤0.48 mg/l) at age 18 years of age (p=0.03). No statistically significant association was observed between maternal prenatal marital stress score and the relative risk of youths being in the 3rd hs-CRP tertile (p=0.21) in comparison to being in the 1st tertile.

For one unit increase in maternal prenatal family stress score, the relative risk of the youth being in the 3rd hs-CRP tertile (hs-CRP>1.16 mg/l) is 1.61 (p=0.04) times greater in comparison to being in the 1st hs-CRP tertile (hs-CRP≤0.48 mg/l). Furthermore, in comparison to being in the 1st hs-CRP tertile, the relative risk for a youth (at age 18 years) to be in the 2nd hs-CRP tertile is 1.7 times greater in youths whose mothers had a one unit increase in the prenatal family stress score during pregnancy.

Maternal prenatal societal stress scores had no statistically significant association with the relative risk of a youth to have his/her hs-CRP in the 2nd or 3rd hs-CRP tertile, in comparison to being in the 1st hs-CRP tertile (p=0.75).

3.2.2.2 *Associations between maternal prenatal general feeling and BT20 participants' hs-CRP level at 18 years old*

Maternal prenatal consistent feeling of unhappiness, in comparison to prenatal consistent feeling of happiness, was not significantly associated with the youth's relative risk at age 18 years of being in the 2nd or 3rd hs-CRP tertile in comparison to being in the 1st hs-CRP tertile (p=0.45).

3.2.2.3 *Associations between maternal postnatal depression and BT20 participants' hs-CRP level at 18 years old*

Maternal postnatal depression score had no statistically significant association with the relative risk of the youth being in the 2nd or 3rd hs-CRP tertile at age 18 years in comparison to being in the 1st hs-CRP tertile (p=0.72).

3.2.2.4 *Associations between household SES and BT20 participants' hs-CRP level at 18 years old*

Household SES score at 0-2 years-old had no statistically significant association with the relative risk of the youth being in the 2nd or 3rd hs-CRP tertile at age 18 years in comparison to being in the 1st hs-CRP tertile.

3.2.2.5 *Associations between other covariates and BT20 participants' hs-CRP level at 18 years old*

Social support

In comparison to the relative risk of being in the 1st hs-CRP tertile (hs-CRP≤0.48 mg/l), a single unit increase in the maternal prenatal social support score decreased the likelihood of the youth being in the 2nd hs-CRP tertile (0.48 mg/l<hs-CRP≤1.16 mg/l) at age 18 years by 27% (p=0.001) and being in the 3rd hs-CRP tertile (hs-CRP>1.16) by 23% (p=0.006).

Gestational age

Relative to hs-CRP≤0.48 mg/l (1st tertile), one week increase in maternal gestational age at delivery decreased by 24% the relative risk of the youth being in the 3rd hs-CRP tertile (hs-CRP> 1.16 mg/l) (p=0.02).

BMI, Birth weight, Sex, and Household SES at 18 years

1 kg/m^2 increase in BMI at age 18 years was associated with an increase by 29% (p:0.03) in the relative risk for a youth being in the 3rd hs-CRP tertile (hs-CRP>1.16 mg/l) compared to being in the 1st hs-CRP tertile (hs-CRP≤0.48 mg/l). There were no statistically significant associations between sex, birth weight, and household SES at 18 years and the relative risk for a youth to have hs-CRP>1.16 mg/l (3rd tertile) at age 18 years and (p=0.6, p= 0.42, p= 0.73, respectively).

Table 3.8: Adjusted multinomial logistic regression results for exposures regressed on the categorized CRP outcome

	Adjusted						
	0.48 < CRP ≤ 1.16 mg/l			*CRP > 1.16 mg/l*			
	RRR	***P-value***	***95% CI***	***RRR***	***P-value***	***95% CI***	***Model P-value***
Depression score	1.01	0.72	0.96, 1.06	1.01	0.72	0.95, 1.07	<0.001
Gender - Female	1.28	0.53	0.59, 2.73	1.08	0.85	0.47, 2.46	
Social support score	0.8	0.01	0.67, 0.95	0.93	0.44	0.76, 1.13	
Birth weight	1.04	0.93	0.47, 2.31	0.85	0.71	0.36, 1.99	
Gestational Age	0.86	0.29	0.64, 1.14	0.76	0.07	0.56, 1.02	
BMI at 18 years	1.17	0.02	1.03, 1.34	1.39	<0.001	1.21, 1.59	
SES at 18 years	0.98	0.78	0.84, 1.17	1.05	0.57	0.89, 1.23	
Prenatal stress score	1.15	0.075	0.99, 1.35	1.09	0.3	0.92, 1.29	<0.001
Gender - Female	0.95	0.88	0.47, 1.91	0.8	0.54	0.39, 1.65	
Social support score	0.73	<0.001	0.61, 0.87	0.77	0.006	0.64, 0.93	
Birth weight	1.68	0.17	0.80, 3.51	0.87	0.71	0.41, 1.85	
Gestational Age	0.77	0.07	0.58, 1.02	0.73	0.03	0.55, 0.98	
BMI at 18 years	1.17	0.003	1.05, 1.31	1.3	<0.001	1.17, 1.45	
SES at 18 years	0.95	0.44	0.83, 1.09	1.03	0.66	0.90, 1.18	
SES at 0 - 2 years	1.06	0.51	0.88, 1.28	1.08	0.46	0.89, 1.31	<0.001
Gender - Female	1.06	0.84	0.58, 1.96	0.98	0.96	0.52, 1.87	
Social support score	0.79	0.001	0.68, 0.91	0.88	0.13	0.76, 1.04	
Birth weight	1.11	0.76	0.57, 2.17	0.63	0.2	0.31, 1.28	
Gestational Age	0.83	0.12	0.65, 1.05	0.75	0.02	0.58, 0.96	

	Adjusted						
	0.48 < CRP ≤ 1.16 mg/l			*CRP > 1.16 mg/l*			
	RRR	***P-value***	***95% CI***	***RRR***	***P-value***	***95% CI***	***Model P-value***
BMI at 18 years	1.15	0.005	1.04, 1.26	1.29	<0.001	1.18, 1.42	
SES at 18 years	0.94	0.36	0.83, 1.07	1.29	0.91	0.87, 1.13	
General feeling - Unhappy	1.59	0.2	0.78, 3.28	1.34	0.45	0.62, 2.92	<0.001
Gender - Female	1.01	0.97	0.55, 1.86	0.94	0.84	0.94, 1.77	
Social support score	0.81	0.004	0.70, 0.93	0.9	0.19	0.77, 1.05	
Birth weight	1.19	0.61	0.61, 2.31	0.68	0.28	0.34, 1.37	
Gestational Age	0.82	0.11	0.65, 1.05	0.74	0.02	0.58, 0.95	
BMI at 18 years	1.14	0.004	1.04, 1.26	1.29	<0.001	1.17, 1.42	
SES at 18 years	0.94	0.38	0.84, 1.07	1	0.98	0.89, 1.13	
Marital stress	2.23	0.03	1.06, 4.69	1.68	0.21	0.75, 3.75	<0.001
Gender - Female	0.96	0.89	0.51, 1.78	0.9	0.76	0.47, 1.72	
Social support score	0.82	0.01	0.71, 0.96	0.91	0.24	0.77, 1.07	
Birth weight	1.16	0.66	0.59, 2.28	0.7	0.32	0.35, 1.41	
Gestational Age	0.83	0.14	0.65, 1.06	0.74	0.02	0.58, 0.96	
BMI at 18 years	1.15	0.003	1.05, 1.27	1.29	<0.001	1.18, 1.42	
SES at 18 years	0.94	0.31	0.02, 1.06	1	0.97	0.88, 1.13	
Family stress	1.7	0.02	1.09, 2.62	1.61	0.04	1.02, 2.53	<0.001
Gender - Female	0.97	0.93	0.51, 1.83	0.85	0.63	0.44, 1.63	
Social support score	0.82	0.009	0.70, 0.95	0.89	0.15	0.76, 1.04	
Birth weight	1.32	0.43	0.67, 2.60	0.7	0.33	0.35, 1.43	
Gestational Age	0.81	0.09	0.63, 1.04	0.74	0.02	0.58, 0.96	

	Adjusted						
	0.48 < CRP ≤ 1.16 mg/l			*CRP > 1.16 mg/l*			
	RRR	***P-value***	***95% CI***	***RRR***	***P-value***	***95% CI***	***Model P-value***
BMI at 18 years	1.15	0.007	1.04, 1.27	1.3	<0.001	1.17, 1.43	
SES at 18 years	0.94	0.35	0.83, 1.07	1.01	0.8	0.89, 1.15	
Economic stress	0.99	0.97	0.70, 1.41	0.94	0.74	0.64, 1.37	<0.001
Gender - Female	1.09	0.78	0.59, 2.04	0.95	0.89	0.50, 1.83	
Social support score	0.78	0.001	0.67, 0.91	0.86	0.08	0.73, 1.01	
Birth weight	1.38	0.36	0.70, 2.72	0.75	0.42	0.37, 1.52	
Gestational Age	0.83	0.14	0.65, 1.06	1.76	0.032	0.59, 0.98	
BMI at 18 years	1.15	0.005	1.04, 1.27	1.29	<0.001	1.17, 1.42	
SES at 18 years	0.96	0.51	0.85, 1.09	1.02	0.73	0.90, 1.16	
Societal stress	1.08	0.81	0.57, 2.05	0.89	0.75	0.44, 1.81	<0.001
Gender - Female	0.98	0.94	0.53, 1.81	0.84	0.6	0.44, 1.61	
Social support score	0.79	0.001	0.68, 0.91	0.88	0.1	0.75, 1.03	
Birth weight	1.27	0.49	0.65, 2.47	0.71	0.33	0.35, 1.42	
Gestational Age	0.83	0.13	0.65, 1.06	0.76	0.03	0.59, 0.98	
BMI at 18 years	1.14	0.006	1.04, 1.25	1.29	<0.001	1.17, 1.42	
SES at 18 years	0.95	0.5	0.85, 1.08	0.99	0.87	0.87, 1.12	

CHAPTER FOUR: DISCUSSIONS

4.0 Introduction

In this chapter, the results of the present research project will be discussed. Firstly, the main study findings with regard to the descriptions of childhood social adversity and the associations between the proxies of social adversity and hs-CRP will be discussed. Secondly, the study findings will be compared with other findings from previous research studies conducted in different study settings. Finally, the contribution of this research project to our understanding of the association between the proxies of social adversity during the early stages of life and hs-CRP during adulthood and ultimately metabolic and cardiovascular diseases will be identified. The strengths and limitations of the current research project will also be discussed.

4.1 Main findings

During pregnancy, more mothers reported being consistently happy. In addition, the overall prenatal stress score was very low, however, mothers had a higher score of relationship or family-related stressors during pregnancy. Similarly, another study found higher family and relationship related stressors (Bergman et al., 2007). A maternal postnatal depression prevalence of 23.7% was found in the current cohort, the prevalence of depression was very low as compared to other studies which found a higher prevalence (above 50%) of maternal postnatal depression (Pikhart et al., 2009, Brummett et al., 2011)

Marital and Family related stresses encountered by the mother during the pregnancy period were strongly and consistently associated with elevated hs-CRP levels of the child at age 18 years. No significant associations were observed between other proxies of social adversity: reported maternal prenatal general feeling, household SES at 0-2 years of age and maternal postnatal depression and hs-CRP of the child during their late teenage years (18 years). Distinctively, the overall population had a disadvantaged socioeconomic background.

4.2 Associations between social adversity in prenatal and infancy periods and CRP levels in young adulthood

4.2.1 Maternal prenatal stress and CRP levels at 18 years of age

Specifically, of the stressors encountered by the mother during pregnancy, those involving a partner or family appeared to be the most predictive of future elevated hs-CRP levels of their children. To my knowledge, there is currently no literature in assessing the relationship between specific prenatal stressors and the child's elevated hs-CRP during adulthood. However, few studies explored whether specific maternal prenatal stressors are critical in increasing health risks for the developing foetus in utero. Studies by Ramchandani and colleagues and another study by Bergman and colleagues are by far the only studies to date which separated out specific stressors encountered by the mother during pregnancy, the investigators in both studies found that stressors related to family and partner relationship were the most strongly associated with adverse child outcomes and ultimately later chronic disease risks (Ramchandani et al., 2010, Bergman et al., 2007). Furthermore, the current study found that economic and social stress encountered by the mother during pregnancy had no significant association with the child's hs-CRP at 18 years of age.

A study conducted in England found that prenatal stress was associated with over 3 fold elevated odds of the child having hs-CRP concentrations of above 3.0 mg/l (Slopen et al., 2015). Similarly, Pawlby and colleagues found that 10 to 15% of the population-attributable risk for health complications such as CVDs may be due to prenatal maternal stress (Pawlby et al., 2009). Moreover, it was found that maternal prenatal stress during pregnancy is associated with altered cytokine production in young adult children aged 16 years and above (Entringer et al., 2008a). A similar study conducted in the Hispanic population in the United States found that perceived maternal stress assessed during pregnancy had an association with the risk of future chronic diseases such as CVDs and diabetes (Watt et al., 2012). Although none of the supporting studies were conducted in an African context, caution was taken in assuming that population-attributable risks found in developed countries may be applicable in the current study. The consistent findings in studies conducted in different countries highlight the potential impacts of prenatal maternal stress on the development of children. In this particular study conducted in a population born during a period of high levels of political and societal violence, the range of stressors to which the BT20 mothers were exposed to during this period while they were pregnant was very different from those

in Western/developed countries (with reference to the aforementioned studies), therefore confirmation of the association between prenatal maternal stress and elevated hs-CRP values in this particular context is very important.

4.2.2 Maternal prenatal general feeling and CRP levels at 18 years of age

The current study observed no statistically significant association between the mothers' general feeling a week before the interview and the hs-CRP levels of the youths at 18 years of age. To the knowledge of the current research study, no studies were conducted to assess the mothers' feeling of the pregnancy and the youth's hs-CRP levels.

4.2.3 Maternal postnatal depression and CRP levels at 18 years of age

The current study found no significant association between the mother's postnatal depression status and the child hs-CRP at 18 years of age. Similarly, Carpenter and colleagues (2012) also did not find any statistical associations between maternal depression status and higher hs-CRP levels in young adults (above 18 years). On the contrary, previously published research found an association between elevated hs-CRP level in teenagers and higher maternal postnatal depression levels (Pikhart et al., 2009, Liukkonen et al., 2011, Brummett et al., 2011). To my knowledge, there were no studies done in an African setting specifically looking at the relationship between maternal depression and child's hs-CRP levels during the child's teenage years.

4.2.4 Household postnatal SES and CRP levels at 18 years of age

The current study found a null association between the household SES at 0 to 2 years and the hs-CRP levels at 18 years of age. These findings are not in accordance with findings from other studies. In the United States and in Britain, studies found a strong statistical association between mother's SES status during pregnancy and the child's hs-CRP levels during the child's teenage years (Dowd et al., 2010, Thomas et al., 2005). However, findings from these two studies contradicted each other; the study in the US by Dowd and colleagues (2010) found that lower SES during pregnancy is associated with higher CRP levels whereas Thomas and colleagues (2005) found that children who went to higher SES schools had higher hs-CRP levels. These studies used a cross-sectional approach with varying methods for measuring the socioeconomic status which might explain the discrepancy among the results found. All the studies are conducted in high-income countries thereby making it difficult to generalise their results to an African context due to the difference in setting and lifestyle.

Therefore, due to the discrepancies in findings of various aforementioned studies, it is necessary to conduct more studies in an African setting which will explore the association between household SES and hs-CRP in adulthood. The findings of the current study suggest that SES does not play a role in increasing one's hs-CRP levels during their teenage years in an African setting.

4.2.5 Categorisation of hs-CRP

Tertiles of hs-CRP in the current population were low. Moreover, they were less than the recommended hs-CRP categories suggested by the CDC with relative to identifying adults at a higher risk of developing future CVDs. However, the cut-offs were consistent across few studies conducted in developed countries in the very same population of 17 to 18 year olds (Korantzopoulos et al., 2005, Nakamura et al., 2010). It was hypothesised that these cut-offs are due to the developmental stage of the population. The hs-CRP levels are suggested to increase with age.

4.3 Other factors associated with higher hs-CRP levels in young adulthood

Youth who were born to mothers with higher social support during pregnancy had a significantly lower risk of having raised hs-CRP during their young adulthood period. These results suggest that increasing social support of women during pregnancy may protect children from having higher hs-CRP levels in the postnatal period**.** In the United States, Thomas and colleagues (2006) found that social support during pregnancy was not a significant predictor of increased hs-CRP levels in adults aged above 50 years (Thomas et al., 2006). The discrepancy may lie in the methods specifically on the tools used to measure social support between the two studies as they used different tools. In addition, the differences in the context where the current study and that of Thomas and colleagues (2006) were conducted may also explain the discrepancies among the findings. The study by Thomas and colleagues (2006) was done conducted in a high-income setting whereas the current study is conducted in a low-middle income setting, therefore; the population measured by both studies were different.

The mother's gestational age was found to be a significant predictor of increased hs-CRP levels. Children born a week after the average gestational age had decreased risk of having elevated hs-CRP at 18 years. Therefore, these results suggest that being born prematurely enhances the child's

risk of having elevated hs-CRP during early adulthood stages. The findings are contrary to those found in the Philippine setting whereby a null association was observed between gestational age and later hs-CRP levels in the youth (McDade et al., 2010). With regard to birth weight, the current study found no association between birth weight and hs-CRP levels; however, McDade and colleagues (2010) found that children born with a low birth weight are associated with elevated hs-CRP levels. Though the current findings with regard to birth weight and gestational age between the two studies differ, they all illustrate that being born earlier or with less body weight whether due to prematurity or other intrauterine factors is associated with higher hs-CRP in adulthood. More supporting studies rather found an association between low birth weight and hs-CRP in adulthood (Sattar et al., 2004, Tzoulaki et al., 2008, Bhuivan et al., 2011)

In the current study, it was observed that an increased Body Mass Index (BMI) during early adulthood stages was a significant predictor of elevated hs-CRP during early adulthood stages. Similar findings were found in Brazil (Oliveria et al., 2008) and Australia (Denney-Wilson et al., 2008). Studies suggest that one less explored pathway which links higher BMI to elevated hs-CRP is through adiposity which puts individuals at a higher risk of having a chronic low-grade inflammation (Pai et al., 2004; Ridker et al., 2008).

4.4 Strengths and limitations of the study

Considering the findings of the current secondary analysis of the cohort study, it is important to acknowledge the following limitations and strengths.

Limitations

The greatest limitation of the primary BT20 cohort study is a loss to follow-up which occurred due to a number of various reasons (e.g relocation, changing of contacts, and death). As a result, there were some missing data mostly for the dependent variable at age 18. The hs-CRP was measured on a single occasion at 18 years of age; this is a limitation since we cannot characterize the kinetics of CRP concentration over a certain time period (1 to 2 weeks) and determine, in case of higher CRP level on whether it is due to recent exposure to infection (or injury), furthermore for assessing CVD risks, the CDC recommends that the CRP should be measured in 2 separate instances which

occurred 2 weeks apart. However, to overcome this limitation, all participants with CRP level equal or above 10 mg/l (a marker of acute infection) were excluded from the analysis.

Some variables such as a participant's smoking status, alcohol consumptions and contraceptive use which were found to be predictors of raised hs-CRP levels in previous studies (Nazmi et al., 2008, Slopen et al., 2013, Costello et al., 2013) could not be used in the current study because they were not measured in the primary BT20 study. Thus the analysis was restricted to variables which were available in the dataset. The intra-and inter-assay coefficients were not assessed in measuring CRP, therefore were not included in the current study.

Strengths

The study compared the characteristics of those included in the analysis and those not included in the analysis. This element is serves as strength for the current study since there were no statistical differences among the two groups thereby ensuring that there was no sample selection bias. Interestingly, the primary BT20 study is a longitudinal study, and this made it possible to look at the relationship between the social adversity data measured during prenatal and postnatal condition and hs-CRP data measured 18 years later.

The study analysed the effect of each proxy of social adversity on hs-CRP on their own, thus pointing out a specific proxy which can be targeted in an intervention program in South African resource-limited settings. Moreover, all the childhood social adversity variables were adapted from previous studies done in South Africa and the measurements scales were validated and had higher levels of consistency in Africa and in other continents.

The current study is the first study in an African setting assessing the effect of prenatal and postnatal adversity and their association with hs-CRP by using data from a longitudinal study.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

Conclusion

From the current study, it can be concluded that levels of family and marital stress during pregnancy are predictors of elevated hs-CRP in youths. The results of the current study highlight and support the importance of maternal psychosocial conditions during pregnancy (Slopen et al., 2012). Furthermore, these results contribute to the increasing empirical evidence from studies done in animals and humans which suggest that adult's health complications start during the intrauterine period (Talge et al., 2007; Bergman et al., 2007).

According to the current study, maternal prenatal stressors may contribute to the child's risk of having CVDs in the future through elevated hs-CRP levels as proposed by the Centre for Disease Prevention and Control. In addition, the current study further adds to the board of knowledge on the predictors of elevated hs-CRP during an adolescent period in an African setting.

To my knowledge, the current study is the first and only longitudinal study in an African which investigates the association between social adversities (prenatal and postnatal) and hs-CRP during an early adulthood stage. Therefore, there is a need to multiply these studies in the continent in order to understand the overall effect of prenatal and postnatal adversities on the development of children and the ultimate risk of future chronic diseases in an African setting. Henceforth, understanding these mechanisms might identify areas which need public health interventions.

Recommendations

In order to enhance the understanding of the effect of prenatal and postnatal social adversity on hs-CRP and later CVDs, future research should use multi-wave longitudinal/cohort studies and measure the child's hs-CRP levels frequently during the developmental stages in order to understand the kinetics of hs-CRP during the adolescent period. More studies are needed in an African setting to further identify the biological/inflammatory markers which can be used in adolescents to detect the risk of future cardiovascular and metabolic diseases.

It is of humongous importance to have replicated studies to examine the methods and validated tools which can be used to measure prenatal and postnatal social adversities in different settings.

In-depth biological work is needed to determine the biological pathways linking social adversity (prenatal and postnatal) and elevated hs-hs-CRP levels later in life.

The aforementioned recommendations will aid in addressing gaps regarding the development of strategies for early life prevention and interventions to detect and reduce the risk of future metabolic and chronic diseases.

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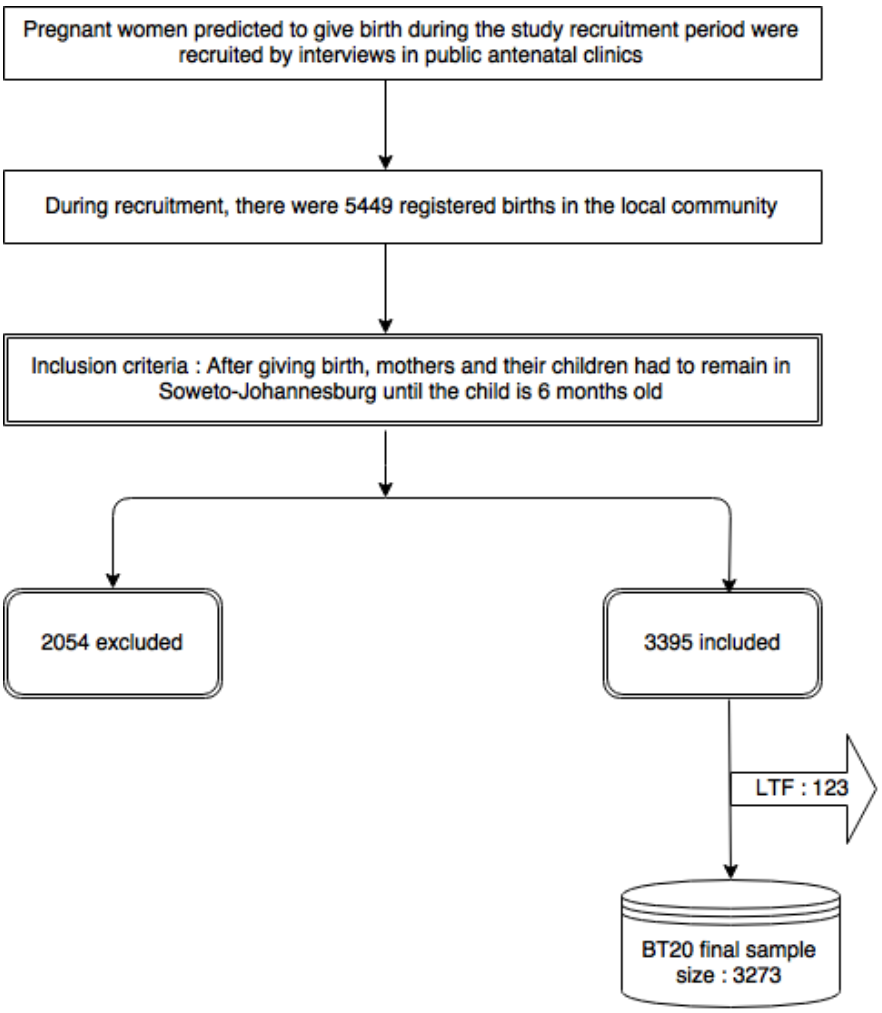
APPENDICES

Appendix 1: Location of the BT20 cohort study in South Africa.

Map showing the location of the birth-to-twenty in South Africa.

**Obtained from Richter et al., 2009*

Appendix 2: Recruitment of study participants



Flow showing the recruitment of study participants (pregnant women) in 1989 to 1990 and the enrolment criteria used to getting the final birth to twenty sample size

**Obtained from Richter et al., 2007*

Appendix 3: Maternal prenatal stress questionnaire

Question	***Response***
1. During the last 6 months have you or a member of your close family been in real danger of being killed, in one of the following ways?	a) By criminals b) By police, army or other officials c) During political activities d) This has not happened at all
2. During the last 6 months did you witness a violent crime (e.g. murder, robbery, assault, rape)?	a) Yes b) No
3. During the last 6 months have you found that you are in so much debt that you don't know how you will repay the money?	a) Yes b) No
4. During the last 6 months have you or your close family ever had too little money for basics, such as food, rent, clothes	a) Yes b) No
5. Have you or one of your close family not been able to find a job for more than 6 months?	a) Yes b) No
6. During the last 6 months have you or anyone in your close family been seriously ill?	a) Yes b) No
7. During the last 6 months did any member of your close family die?	a) Yes b) No
8. Is there anyone in your close family with a serious disability (e.g. epilepsy, mental retardation, deafness, blindness, mental illness)?	a) Yes b) No
9. Is there anyone in your close family who has a problem with drugs or alcohol?	a) Yes b) No

10. During the last 6 months have you had a break-up with your husband or partner?	a) Yes b) No
11. During the last 6 months has your husband or partner hit or beaten you?	a) Yes b) No
12. During the last 6 months have you had any serious fight or alienation from members of your family or your close neighbours?	a) Yes b) No
13. During the last 6 months have you or any member of your close family been arrested, had to go to court or consulted a lawyer on a non-routine matter?	a) Yes b) No
14. During the last 6 months have you given help (money, accommodation etc.) to close family or friends in need?	a) Yes b) No
15. During the last 6 months have you been separated unwillingly from any of your children (excluding holidays)?	a) Yes b) No
16. During the last 6 months have you experienced any problems with your other children (such as schools closing, failure at school, problem behaviour, drugs etc.)?	a) Yes b) No

Appendix 4: Maternal social support questionnaire

Question	*Response*
1. If you had a really big problem and needed help, with money, the children, accommodation and so on, are there people who could help you?	a) Nobody b) Maybe/Unsure c) A number of people
2. Can you talk to your parents, other family members or friends about any problems you may have?	a) Nobody b) Maybe/Unsure c) A number of people
3. Can you talk to your husband or partner about any problems you might have?	a) Never d) Sometimes e) Always
4. Some people think the sisters at the clinic are always helpful, others think only sometimes and some say they are seldom helpful. How do you feel?	c) Always helpful d) Sometimes helpful e) Seldom helpful
5. Do you feel that the father of your child or your partner makes things harder for you because of the way he acts?	c) Never d) Sometimes e) Always
6. Do you belong to a church group or any other organisation?	c) Yes d) No
7. How often do you go to meetings?	c) Once a week d) Once a month e) Irregular
8. Do you have a friend who is also going to have a baby or has just had a baby?	c) Yes d) No
9. If Yes, How often do you see her?	c) Once a week d) Once a month e) Irregular

Appendix 5: Maternal postnatal depression questionnaire

1. Do you sleep well?
2. Do you easily lose your temper?
3. Are you worried about your looks?
4. Have you a good appetite?
5. Are you as happy as you ought to be?
6. Do you easily forget things?
7. Have you as much interest in sex as ever?
8. Is everything a great effort?
9. Do you feel ashamed for any reason?
10. Can you relax easily?
11. Can you feel the baby is really yours?
12. Do you want someone with you all the time?
13. Are you easily woken up?
14. Do you feel calm most of the time?
15. Do you feel that you are in good health?
16. Does food interest you less than it did?
17. Do you cry easily?
18. Is your memory as good as ever?
19. Have you less desire for sex than usual?
20. Have you enough energy?
21. Are you satisfied with the way you are coping with things?
22. Do you worry a lot about the baby?
23. Do you feel unlike your normal self?
24. Do you have confidence in yourself?

*The responses to all the questions above were either "Yes, Don't Know or No"

Appendix 6: Ethical clearance from University of Witwatersrand, Human Research Ethics Committee (Medical)

R14/49 Mr Phuti Dascious Ngwepe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160469

NAME: (Principal Investigator)	Mr Phuti Dascious Ngwepe
DEPARTMENT:	School of Public Health University of the Witwatersrand
PROJECT TITLE:	Relationship Between Social Adversity in Two Year Olds and C-Reactive Protein in Eighteen Year Olds in the Birth-to-Twenty Cohort
DATE CONSIDERED:	06/05/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Rhilatt Said Mohamed
APPROVED BY:	Professor P. Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/05/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially review in April and will therefore be due in the month of April each year.

Principal Investigator Signature

Date 18/05/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES