Body composition and systemic low-grade inflammation in children: the PLAY study

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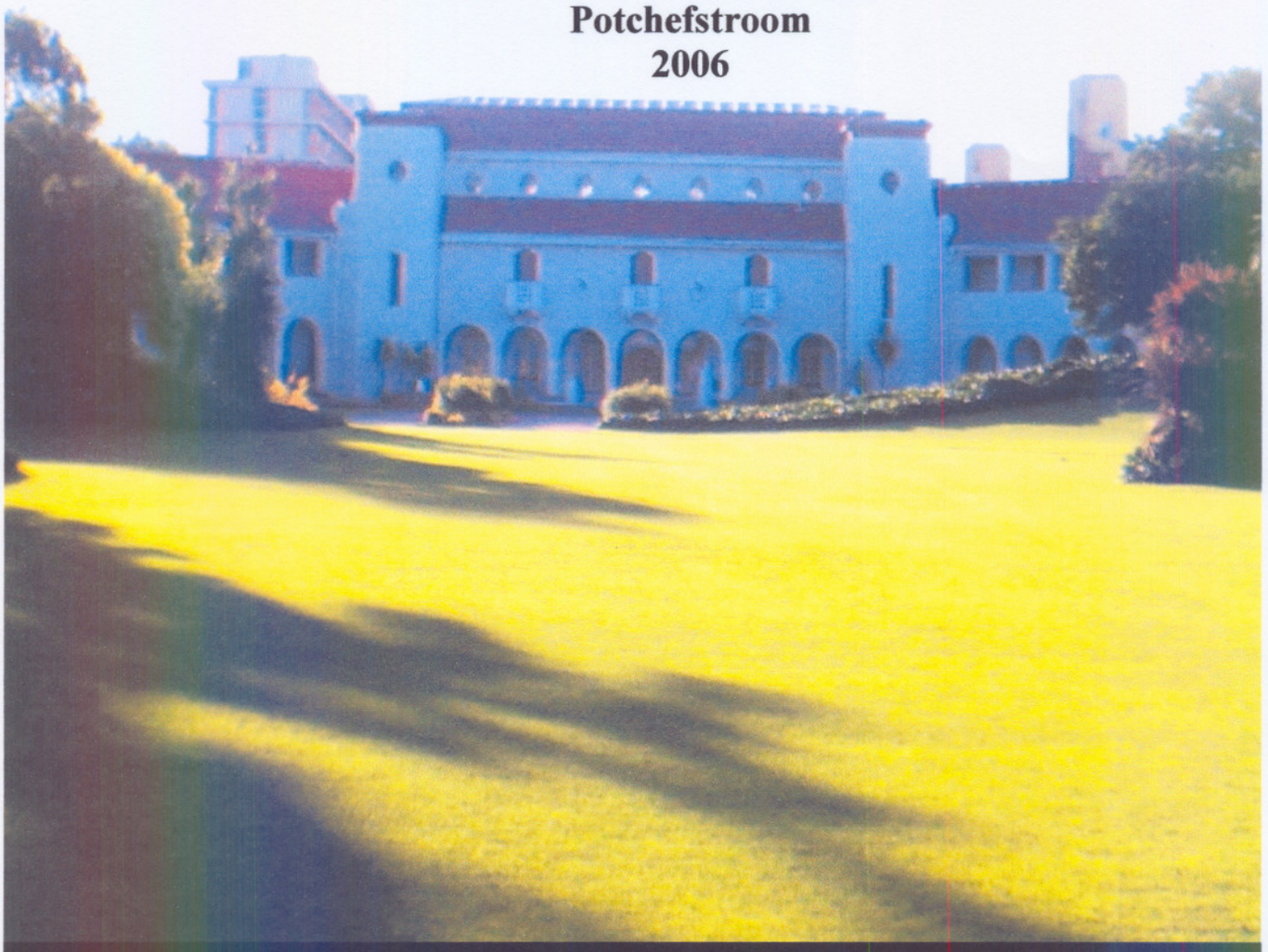

Study leader : Prof. H.S. Kruger
Co-study leader : Prof. A.E. Schutte

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"Om U wil te doen is my een begeerte, U woord is my hele lewe"

Psalm 40:9

OPSOMMING

Agtergrond: Obesiteit-verwante siektes word al meer 'n probleem onder kinders. Inflammasie is onlangs aangespreek as 'n rolspeler in die verhouding tussen die obesiteit- en groei-inkorting-verwante siektes.

Doel: Die doel van die studie was om die verwantskap tussen serum tumor nekrose faktor-alpha (TNF-α), interleukin-6 (IL-6) en C-reaktiewe proteïen (CRP) konsentrasies en 'n verskeidenheid kardiometaboliese en antropometriese parameters van kinders te bepaal in 'n landelike gebied buite Potchefstroom, Suid-Afrika.

Metodes: In die '**P**hysica**L** **A**ctivity in the **Y**oung' (PLAY) studie, wat 'n dwars-deursnit studie was, is die bloedmonsters van 115 meisies en 78 seuns (gemiddelde ouderdom 15.6 ± 1.35 jaar) geanaliseer. Opgeleide veldwerkers het die demografiese inligting, Tanner-groeifases en fisieke aktiwiteit data ingesamel. Fisioloë het die bloeddruk van die kinders gemeet. Antropometriese metings is geneem deur nagraadse studente met 'n vlak 1 of 2 kwalifikasie in antropometrie. 'n Standaard toetsbattery is gebruik deur opgeleide nagraadse studente in Menslike Bewegingskunde om die spierkrag, beweeglikheid en uithouvermoë van die kinders vas te stel. Bloedmonsters is ingesamel, gesentrifugeer en bevrore gestoor totdat dit geanaliseer kon word.

Resultate: Groei-ingekorte meisies het 'n betekenisvol hoër serum TNF-α konsentrasie getoon teenoor die meisies van normale lengte (p=0.03). Die faktoranalise het getoon dat die inflammatoriese status saam groepeer met die lengte-vir-ouderdom-z (LOZ) telling en die middel-heup-verhouding (MHV). Die LOZ-telling van die oor-vet seuns (-1.46,) was betekenisvol kleiner as die maer seuns (-1.14, p=0.01), die oor-vet meisies se LOZ-telling (-1.07) het na 'n kleiner waarde geneig as die LOZ van die maer meisies (-0.89). Geen betekenisvolle verskille is gevind tussen die oor-vet en die maer kinders se inflammatoriese status nie. TNF-α en CRP vlakke het wel geneig om hoër te wees in die oor-vet kinders as in die maer kinders. Die meisies se serum IL-6 en CRP konsentrasies het betekenisvol gekorreleer met die liggaamsmassa indeks (LMI) en MHV ($p<0.05$) en die TNF-α en IL-6 konsentrasie het betekenisvol gekorreleer met die MHV ($p<0.01$ en $p<0.05$, onderskeidelik).

Gevolgtrekking: Wanneer vergelyk word met die meisies van normale lengte, het die groei-ingekorte meisies betekenisvol hoër serum TNF-α konsentrasies gehad. Ongewone vetverspreiding in die oor-vet en groei-ingekorte kinders kan geassosieer word met lae-graadse inflammasie in kinders. Meer navorsing is nodig omtrent hierdie verwantskap met merkers van inflammasie in 'n langtermyn longitudinale studie.

Sleutelwoorde: C-reaktiewe Proteïen, Lengte-vir-ouderdom-z-telling, Groei-inkorting, Inflammasie, Interleukin-6, Kinders, Liggaamsmassa indeks, Obesiteit-verwante siektes, Oor-vet, Tumor nekrosis faktor-alpha.

ABSTRACT

Background: Obesity-related diseases are arising as a major problem among children. Inflammation has recently been identified to play an important role in the relationship between obesity- as well as stunting-related diseases.

Objectives: The aim of this study was to assess the association between serum tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations and a variety of cardiometabolic and anthropometric indices of children in a township outside Potchefstroom, South Africa.

Methods: Blood samples of 115 girls and 78 boys (mean age 15.6 ± 1.35) in the **P**hysica**L** **A**ctivity in the **Y**oung (PLAY) study were cross-sectionally analysed. Trained fieldworkers collected the demographic, Tanner growth stage and habitual physical activity information. Physiologists measured the children's blood pressure. Anthropometric measurements were taken by trained post-graduate students with level 1 or 2 qualifications in anthropometrics. A standard test battery was administered by trained postgraduate students in Human Movement Science to assess muscular strength, flexibility and endurance of the children. Blood samples were collected, centrifuged and stored frozen until further analyses.

Results: Stunted girls had a significantly higher serum TNF-α concentration than the non-stunted girls ($p=0.03$). The factor analyses showed that the inflammatory status clustered with the height for age-z-scores (HAZ) scores and the waist-hip-ratio (WHR). The HAZ-score of the over-fat boys (-1.46) was significantly smaller than the lean boys (-1.14, $p=0.01$), whereas the over-fat girls had a trend for a smaller HAZ-score (-1.07) than the lean girls (-0.89). No significant differences were found between the over-fat and the lean children's inflammatory status. TNF-α and CRP levels tended to be higher in the over-fat children than in lean children. The girls' serum IL-6 and CRP concentrations correlated significantly with their body mass index (BMI) and WHR ($p<0.05$) and their TNF-α and IL-6 concentrations correlated significantly with their WHR ($p<0.01$ and $p<0.05$, respectively).

Conclusion: In comparison to the non-stunted girls, stunted girls had a statistically significantly higher TNF-α concentration. Unusual fat distribution that is found in over-fat and stunted children may be associated with low-grade inflammation in children. More research is needed on these associations with markers of inflammation in a long-term longitudinal study.

Key words: C-reactive protein, Height for age-z-score, Stunted, Inflammation, Interleukin-6, Children, Body mass index, Obesity related diseases, Over-fat, Tumor necrosis factor-alpha.

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ADDENDA

LIST OF ABBREVIATIONS

A	
ADP	Air displacement plethysmography
B	
BMI	Body mass index
BP	Blood pressure
C	
CDC	Centre for disease control
CDL	Chronic diseases of lifestyle
CHD	Coronary heart diseases
cm	Centimetre
COX	Cyclooxygenase
CRP	C-reactive protein
CVD	Cardiovascular diseases
CV	Coefficient of variation
D	
DBP	Diastolic blood pressure
H	
HOMA	Homeostasis model assessment
HPA-axis	Hypothalamic-pituitary-adrenal-axis
HDL-C	High density lipoprotein-cholesterol
I	
ICAM-1	Intracellular adhesion molecule-1
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-3	Interleukin-3
IL-6	Interleukin-6
IL-8	Interleukin-8
IR	Insulin resistance

L

LPL	Lipoprotein lipase

M

MAP	Mean arterial pressure
MI	Myocardial infarction
mmol/L	Millimol per liter
Mn-SOD	Manganese superoxide dismutase
MRC	Medical Research Council
MS	Metabolic syndrome

N

N	Number of subjects
NCD	Non-communicable diseases
NF-κβ	Nuclear factor- κβ
NHANESIII	Third national health & nutrition survey
NO	Nitric oxide

P

P	p-value (significant differences, $p<0.05$)
PA	Physical activity
PACER	Progressive Aerobic Cardiovascular Endurance Run
PDPAR	Previous day physical activity questionnaires
PGE	Prostaglandin
PLAY	PhysicaL Activity in the Young

Q

QUICKI	Quantitative insulin sensitivity check index

R

ROS	Reactive oxygen species

S

SBP	Systolic blood pressure
SD	Standard Deviation

T

Tanner	Sexual Devolpment
T2D	Type-2 diabetes
TNF-α	Tumor necrosis factor-alpha
TNFR1	Tumor necrosis factor receptor-1
THUSA BANA	Transition and Health during Urbanisation in South Africa Children (Bana: Setswana word for children)

V

VCAM-1	Vascular adhesion molecule-1
VO_2max	Maximal oxygen uptake

W

WC	Waist circumference
WHO	World Health Organisation
WHOHAZ	Weight for height Z-scores
WHR	Waist-Hip-Ratio

LIST OF SYMBOLS

α	Alpha
β	Beta
⇧	Increase
⬇	Decrease
%	Percentage
>	Larger than
<	Smaller than

CHAPTER 1:

Introduction

1.1 Background

Obesity has become a global epidemic with an estimated one billion overweight adults, of which at least 300 million are obese (Bullo *et al.*, 2003). Obesity and the metabolic syndrome (MS) have become serious health problems as they are accompanied by cardiovascular risk factors such as dyslipidaemia, hypertension, hyperinsulinemia and diabetes mellitus (Andersson *et al.*, 1998; Hanusch-Enserer et *al.*, 2003). Recently stroke has also become a major health problem amongst black South Africans, possibly because of an increase in hypertension, obesity, smoking habit and hyperfibrinogenaemia during various stages of urbanisation (Vorster, 2002).

Childhood obesity has increased rapidly across the world with approximately 22 million overweight children under the age of five (Dedoussis *et al.*, 2004). Obesity in children is clinically worrisome because it is associated with low levels of physical activity and poor dietary intake leading to increased risk of MS or cardiovascular diseases (CVD) in adulthood (Dedoussis *et al.*, 2004; Nemet *et al.*, 2003). In addition to obesity, stunting is believed to have become just as troublesome. An estimated 38% of children younger than five years of age (Chang *et al.*, 2002) and 34% of males and 28% of females between the ages 15 to 18 years (Lai *et al.*, 1998) are stunted as a result of chronic undernutrition and frequent morbidity. This means that > 200 million young children are stunted (Frongillo, 1999). The high risk of obesity in stunted children has been described in Hispanic-American, Jamaican and Andean populations (Popkin *et al.*, 1996). In order to support the latter, more research is required on the prevalence of obesity and stunting in African children.

The precise mechanism behind the development of the complications with obesity is still not clear, however, it is becoming evident that the increase in adipose tissue mass may produce a variety of cytokines (interleukin-1 (IL-1), interleukin-8 (IL-8), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP)) that might be involved in the obesity-related conditions (Bruun *et al.*, 2002). The latter indicates that

inflammation in overweight as well as in stunted children should be included in studies of obesity and stunting co-morbidities. In obese and physically inactive children some inflammatory parameters, particularly IL-6 and TNF-α levels, are elevated (Halle *et al.*, 2004). It is considered essential to have a clear understanding of the underlying relationship between body composition, systemic low-grade inflammation and the chronic diseases of lifestyle (CDL) in children (Rudin & Barzilai, 2005). The unidentified elevated inflammatory status in children may lead to the development of CVD and the MS later in their life (Ballantyne & Nambi, 2005; Davi & Falco, 2005; Isasi *et al.*, 2003; Ross, 1999) Moreover, screening for CRP may identify individuals at risk of coronary heart disease (CHD) (Kushner *et al.*, 2006). Therefore, early assessment of the inflammatory status of individuals could be considered as a useful method to improve global CVD risks, particularly in patients at intermediate risk (Rudin & Barzilai, 2005; Davi & Falco, 2005).

1.2. Problem statement

Due to the prevalence of obesity as a major medical problem (Halle *et al.*, 2004), interest in obesity-related diseases has prompted renewed research into the physiology of adipose tissue and low-grade inflammation (Gil-Campos *et al.*, 2004). TNF-α, IL-6 and CRP has interrelated roles and have been recognised as pro-inflammatory markers of low-grade systemic inflammation, which may be prognostic in the development of non-communicable diseases (NCD) (Warnberg *et al.*, 2004). The major NCD risk factors to be targeted are blood pressure, cardiovascular response, insulin resistance (IR), fasting glucose levels, lipoproteins, plasma fibrinogen and type 2 diabetes (T2D) (Heilbronn & Clifton, 2002). Therefore, low-grade systemic inflammation is considered to be an indicator of CVD and MS later in life. However, the precise role that inflammation may play in the development of NCD, and the extent or ratio of its influence still remains to be elucidated.

Limited research has been done on the prevalence of inflammatory markers in children, especially black Africans, whether overweight, underweight, stunted, fit or unfit. A few studies have focused on overweight children's inflammatory status, but studies focusing on the co-existence of obesity and stunting with children in respect to their inflammatory status are scares.

1.3. Aim and objectives

The design of the study enables the comparison of low-grade inflammation in children with different body composition variables. Understanding the different associations within these children could provide important significant information on the association between inflammatory status and NCD. The aim of this study is, therefore, to determine the associations between inflammatory markers and cardiometabolic risk markers in a cross-sectional study on African children in a township outside of Potchefstroom in the North West Province, South Africa.

Specific objectives are:

- To assess and evaluate the inflammatory status (TNF-α, IL-6 and CRP) of black African boys and girls,
- to confirm the existence of obesity and stunting-induced inflammation,
- to investigate the relationships between serum CRP, TNF-α, IL-6, obesity, stunting, blood pressure, body mass index (BMI), white blood cell count (WBCC), leptin, plasma insulin, plasma glucose, Homeostasis model assessment (HOMA) and Quantitative insulin sensitivity check index (QUICKI),
- to identify whether the fitness levels of the subjects may influence the inflammatory status of these children.

1.4 Hypothesis

Human adipose tissues express and release the pro-inflammatory cytokines IL-6, TNF-α and CRP, inducing low-grade systemic inflammation in persons with excess body fat (Davi & Falco, 2005). The hypothesis that over-fatness in children is associated with increased serum concentration of these markers of low-grade systemic inflammation was tested. The possibility that stunted children also have increased inflammatory status due to their characteristic central fat distribution was also investigated. Although the harmful effects of obesity are only visible later in adulthood, it is suggested that childhood should be the key period to identify those at risk of becoming obese adults (Mukuddem-Petersen & Kruger, 2004). Recognition of the fact that low-grade local and systemic inflammation accompanies all stages of atherogenesis has led to the identification of a number of novel biomarkers of cardiovascular risk (Khuseyinova & Koenig, 2006). Therefore, detecting

sub-clinical inflammation in early adolescence could be imperative in the prevention of NCD later in adulthood.

1.5 Structure of the dissertation

The effect of obesity and stunting in children and the effect it has on their inflammatory status is explored in this dissertation. This dissertation is a combination of chapters written specifically to comply with the requirements of the North-West University. Chapter 1 is an introductory chapter to present the background of the study, as well as the aim, hypotheses and objectives. Chapter 2 reviews the current knowledge regarding the association between obesity, stunting, CVD and the MS. It especially focuses on inflammation with regards to IL-6, TNF-α and CRP and their association with NCD. This review also examines the therapeutic effect that exercise and weight loss could have on the lowering of inflammatory markers. Chapter 3 informs the reader on the methods used to collect the data. Chapter 4 contains the results accompanied with a discussion and conclusion. Chapter 5 provides the conclusion, exploring recommendations regarding further research and practical applications.

Contribution of the student in this study

Table 1.1 The research team's qualifications and the role they played in the study, with special consideration of the student's contribution towards the study.

Title and affiliation	Contribution to study
Ms. R.A. Pretorius (BSc. Honours in Nutrition and Post-graduate student in MSc. Nutrition) (PNCS)	**Blood sampling collection and preparation. Serum TNF-α and IL-6 sample analyses, anthropometric measurements and writing up the data.**
Prof. H.S. Kruger (Dietician and pharmacist) (PNCS)	Study leader (design, planning and conduct of the study, approval of final protocol) and involved in the statistical analyses and interpretation of results as well as writing up data
Dr. A.E. Schutte (Physiologist) (PNCS)	Co-study leader. Blood pressure measurements. Involved in the statistical analyses and interpretation of results as well as writing up data.
Dr. C. Wessels (Social worker)	Responsible for the demographic data
Dr. C. Underhay (Anthropometrist) (BRS)	Body composition (PodPod), and supervision of anthropometry
Prof. A.E. Pienaar (Child kineticist and Human Movement Scientist) (BRS)	Assessment of fitness and supervision of physical activity data
Sr. M.C. Lessing (Registered general nurse)	Coordinator of subjects and responsible for the collection of blood samples.
Dr. A Monyeki (Human Movement Science) (BRS)	Physical development assessment (Tanner staging)
Dr. L. Mamabolo (Post-doctoral student) (PNCS)	Physical development assessment (Tanner staging) and Serum TNF-α and IL-6 sample analyses. Compiling of the data sheet.
Miss. C. Nienaber (BSc. Honours in Nutrition and Post-graduate student in MSc. Nutrition) (PNCS)	Blood sampling collection and anthropometric measurements
Miss. D. Naude (Post-graduate student in Human Movement Science) (BRS)	Anthropometric measurements

BRS = School for Biokinetics, Recreation, and Sport Science, PNCS = School for Physiology, Nutrition and Consumer Science.

CHAPTER 2:

Literature review

2.1. Introduction

Increased inflammation has recently been identified to play a pivotal role in the close relationship between obesity and non-communicable diseases (NCD) (Basu *et al.*, 2005; Halle *et al.*, 2004). Human adipose tissue expresses and releases pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) (Halle *et al.*, 2004). Increased levels of C-reactive protein (CRP), which is a sensitive marker for systemic low-grade inflammation, is associated with the prevalence of sub-clinical cardiovascular diseases (CVD) (Rudin & Barzilai, 2005; Visser *et al.*, 2001). Therefore, these inflammatory markers have all shown to have a positive association with the development of obesity, indicating an involvement with the low-grade acute phase response in obesity-related disease (Bullo *et al.*, 2003).

Inflammation also plays a central role in all phases of the atherosclerotic disease process, from lesion initiation and progression, plaque rupture and ultimately to the ensuing thrombotic complications of CVD (Ballantyne & Nambi, 2005). The metabolic syndrome (MS) is a cluster of several metabolic abnormalities which are mostly associated with abdominal obesity and the prevalence of insulin resistance (IR), high blood pressure (BP), atherogenic dyslipidaemia, type-2 diabetes (T2D) and pro-inflammatory status (Davi & Falco, 2005; Morange *et al.*, 2004; Raloff, 1999; Rudin & Barzilai, 2005). It is known that CVD and MS affect adults primarily, but a growing body of evidence suggests that a worrisome number of adolescents are also being affected (Klein-Platat *et al.*, 2005). Halle *et al.* (2004) pointed out that the prevalence of elevated inflammation in obese children would be a powerful predictor of CVD in adulthood. As a result, the development of early life style intervention, such as healthy eating and physical activity (PA) regimen during childhood is an important factor in the prevention of CVD and MS in adulthood (Halle *et al.*, 2004).

2.2. Various body compositions among children and young adults

2.2.1. Introduction

South Africa has an extensive diversity of body compositions among children and young adults. In developing countries stunting and underweight often co-exist with obesity, which increases the risk of chronic diseases of lifestyle (CDL) (Coutsoudis & Coovadia, 2001). A comprehensive national survey documented in 1994 indicated that one in four pre-school children in South Africa was stunted and 1 in 10 was underweight (Labadarios *et al.*, 2005). Overweight is listed by the World Health Organization (WHO) as one of 10 leading risk factors for high mortality in developing and developed countries (WHO, 2002). The first South African National Youth Risk Behaviour study showed that 13-19 year old school children are 17% overweight and 4% of them are obese (MRC, 2002).

During obesity the natural energy reserve stored in the fatty tissue of humans and mammals increases to a point where it is thought to be a significant risk factor in certain health conditions (cardiovascular risk factors and metabolic disorders) (Gil-Campos *et al.*, 2004). There is a well-established increase in the risk of cardiovascular death associated with severe overweight as well as a gradient of increasing risk associated with moderate overweight (Warnberg *et al.*, 2004). Since risk factors such as dyslipidaemia, hypertension, hyperinsulinemia and obesity often co-exist in children and adolescents, childhood obesity may lay the metabolic groundwork for adult CVD and T2D (Warnberg *et al.*, 2004). The prevalence of childhood obesity is increasing worldwide, even in countries that have a high prevalence of stunting (Hoffman *et al.*, 2006). The assessment of stunting is integral to public health, clinical and research workers in many fields concerned with the well-being of children and with the biology of growth and development (Frongillo, 1999). The effects of inflammation on bone growth are two-fold. Firstly the systemic inflammatory effects have consequences on hormone, mineral and nutrient metabolism which affect bone growth. Secondly, cytokine mediators of inflammation cause local changes in cell regulation to influence both endochondral processes in growth plate and remodelling activity associated with appositional growth (Skerry, 1994).

2.2.2. The co-existence of stunting and over-fatness among children

Stunting is a phenomenon that is relatively widespread in developing countries with the prevalence ranging from 13% to 24% in Latin Americans to 48% in the east of Africa (Hoffman *et al.*, 2006), and approximately 21.6 % in South Africa (Steyn *et al.*,2005). Stunting is defined as height for age below the 5th percentile on the Centre for Disease Control (CDC) reference growth curve or a height-for-age z-score $\leq$ -2.0 standard (SD) Although children experience a slower phase of linear growth during middle childhood (Tanner, 1962), they will experience one of three patterns of growth during this period, namely: i) catch-up growth, ii) remain stable, growing at approximately the same rate of children in better environments, and iii) they may continue to falter, growing at a slower rate than children in the better environment (Friedman *et al.*, 2005). Poor health due to poor nutrition, deprived dietary quality and environmental problems (inadequate care, food security, lack of education, sanitation and poverty) may be some of the major causes of the development of stunting in these African communities (Van Rooyen *et al.*, 2005; Azevedo *et al.*, 2005). According to Frongillo (1999) the cause and etiology of stunting include the following: i) nutrition (energy, macronutrients, micronutrients and toxic factors), ii) infection (injury, gastrointestinal mucosa, systemic effects and immunostimulation) and iii) mother-infant interaction (malnutrition and stores at birth and behavioural interactions).

Childhood stunting, an indicator of chronic malnutrition (Waterlow, 1994a) and undernutrition (Popkin *et al.*, 1996), has been suggested to be an indicatory factor of elevated rates of obesity (high weight-for-height) in developing countries (Mukuddem-Petersen & Kruger, 2004). It has long been recognized that deficits in macronutrients cause stunting, but there has been increasing attention paid to the role of micronutrients (Frongillo, 1999). Further research is needed to determine for which micronutrients do deficits limit growth and whether stunting is due primarily to deficits in single nutrients or in multiple nutrients simultaneously (Frongillo, 1999). Nevertheless, in the past stunting and the access to food was highly associated, but that linkage may not be as apparent now in countries undergoing the nutrition transition (Popkin *et al.*, 1996). A close relationship exists between stunting and overweight among children from developing countries, including South Africa, but little systematic clinical research has been done on the stunting

period and the subsequent relationship to obesity (Popkin *et al.*, 1996). The co-existence of obesity and stunting in children could be explained through the assumption that an adequate or excessive energy intake could limit linear growth when protein and other nutrient intakes are inadequate, without the fat deposition being affected (Trowbridge, 1982). Stunted children may be programmed to accumulate a greater percentage of body fat during adolescence, especially in the abdominal area (Kruger *et al.*, 2004; Mukuddem-Petersen & Kruger, 2004). Children who are stunted early in childhood are likely to have short stature in adulthood, which has been recognized to lead to the development of heart diseases (Gaskin *et al.*, 2000).

The consequences of stunting accompanied by overweight/obesity in developing countries have been said to be adverse and they could be more prone to chronic diseases such as T2D, hypertension and cardiovascular diseases later in life (Popkin *et al.*, 1996). The precise role that stunting in children may play in the prevalence of low-grade inflammation is still very scanty and warrants further investigation.

2.2.3. The origin of inflammatory markers in obesity

Adipose tissue is not only a simple reservoir of energy, but is also viewed as an active secretory organ, releasing many peptides and cytokines into the circulating blood (Weiss & Caprio *et al.*, 2005). The mechanism behind the development of the obesity-related complications is still not completely elucidated. However, it is becoming increasingly clear that obesity is associated with an abnormal production of pro-inflammatory cytokines, which in turn play a pivotal role in the development of the MS and CVD (Bruun *et al.*, 2002; Hotamisligil, 2003). Weight gain and obesity appear not only to cause inflammation but may be preceded by inflammation (Niskanen *et al.*, 2004). Therefore, it is suggested that obesity could be associated with a state of chronic low-grade inflammation, leading to increased acute phase reactant and activation of inflammatory signalling pathways (Warnberg *et al.*, 2004).

The origin of inflammatory markers in obesity is, according to Trayhum and Wood (2004), a central question. In a review Trayhum and Wood (2004) identified three possibilities for the origin of inflammation in obesity, i) reflects production and release from organs other than adipose tissues, for instance the liver, ii) white adipose tissue

secretes factors that stimulate the production of inflammatory markers from the liver and other organs, for instance in the case of CRP levels of the obese, where it is argued that hepatic production is stimulated by increased IL-6 from expanded fat mass, and, iii) the possibility that adipocytes themselves are the immediate source of some of these inflammatory markers. Davi and Falco (2005) strongly implied that expanded abdominal fat deposition may be responsible for the low-grade inflammatory state through the increased production of IL-6, which in turn is a potent stimulus to CRP synthesis by the liver (Visser *et al.*, 2001; Yudkin *et al.*, 2000). However, it is still unclear whether the mature adipocytes, the preadipocytes or the stromal vascular cells in adipose tissue represent the major cellular source of IL-6 (Yudkin, *et al.*, 2000). TNF-α was initially demonstrated to be markedly increased in obese models (Rudin & Barzilai, 2005), nevertheless, discordant evidence surrounds the truth about TNF-α expression. It is specified that TNF-α is only secreted in omental and not in subcutaneous fat (Reinehr *et al.*, 2005). Despite the conflicting statement, it should be pointed out that TNF-α concentration correlates with the percent body fat, and not necessarily with the amount of adipose tissue (Kirchgessner *et al.*, 1997). Further research is needed in order to elucidate the discordant data on the TNF-α expression through adipose tissue.

2.3. Inflammation

2.3.1. An introduction to inflammation

Inflammation involves a complex battery of enzyme activation, mediator release, cell migration, tissue breakdown and repair (Vane *et al.*, 1996). Hence, inflammation could be defined as the response of living tissue (Vane *et al.*, 1996) to infection or irritation, evolving to protect the organism and repair tissue damage (Moldoveanu *et al.*, 2001; Paul, 1991). Inflammation is commonly divided into three phases, namely acute inflammation, immune response and chronic inflammation (Vander *et al.*, 2001). Acute inflammatory reaction is usually self-timing and resolves quite rapidly with complete removal of the injurious agent and little incidence of tissue damage (Gilroy *et al.*, 2001). Chronic inflammatory reactions on the other hand fail to resolve and persist for longer duration with varying levels of tissue damage (Gilroy *et al.*, 2001) and also involve the release of a number of mediates (Vander *et al.*, 2001). Although the changes in acute-phase reactant are smaller than those in acute infectious diseases, the chronicity of low-grade inflammation may be decisive and might play a role in the progression of obesity, T2D

and CVD (Bullo *et al.*, 2003; Niskanen *et al.*, 2004; Santos *et al.*, 2005). The state of chronic low-grade inflammation typical of obesity and T2D occurs at metabolically relevant site, such as the liver, muscle and most prominently, adipose tissue (Hotamisligil, 2003). Inflammation may also have systemic properties, restricting it not only to a particular tissue but engaging the endothelium (lining of blood vessels) and many other organ systems (Vane *et al.*, 1996). The acute phase response is an example of systemic reaction to inflammation, characterized by vascular permeability, alteration in plasma metal and steroid concentration (Kishimoto, 1989).

The key actors in inflammation are phagocytes and the main phagocytes are neutrophils, monocytes, macrophages and the macrophage-like cells (Vander *et al.*, 2001). When monocytes (released from mast cells) leave the blood and enter tissues during inflammation they will develop into macrophages (Yudkin *et al.*, 2000). These macrophages produce their own mediators (pro and anti-inflammatory cytokines such as IL-6, IL-8, IL-1 and TNF-α) (Yudkin *et al.*, 2000). These inflammatory cytokines may find their way into the cap of atherosclerotic plaque, which leads to the suggestions that they contribute to plaque rupture through the effect of matrix metalloproteinases (Das, 2004; Yudkin *et al.*, 2000). Matrix metalloproteinases are zinc-dependent endoproteases with collagenase and/or gelatinase activity, which are highly expressed in atherosclerosis (Armstrong *et al.*, 2006) and also play key roles in vascular remodelling (Sharony *et al.*, 2005). It is known that atherosclerosis is a complex chronic inflammatory disorder associated with low-grade inflammation (P'ramo *et al.*, 2005).

Cytokines are soluble glycoproteins that are produced by mediate communication between and within organs and organ systems throughout the body (Moldoveanu *et al.*, 2001). The production of cytokines can be up-regulated in response to pro and anti-inflammatory stimuli, and this response can be transient or prolonged (Moldoveanu *et al.*, 2001). In response to an inflammatory stimulus, cyclooxygenase (COX) activity is increased due to new expression of the COX-2 gene (an isoform induced by pro-inflammatory cytokines, present in inflamed tissue and contributes to lesion formation) (Vane *et al.*, 1996). COX is a key regulatory enzyme in eicosanoid metabolism, converting free arachidonic acid to prostagladine (Choi *et al.*, 2006). Therefore, COX is also known as prostagladine H synthase (Vane *et al.*, 1996). COX-2 is an immediate early response gene product in

inflammatory and immune cells and expression is stimulated tenfold to eighteen fold by growth factors, tumor promoters and cytokines (Vane *et al.*, 1996). COX-2 has been detected in macrophages, smooth muscle cells and the endothelial cells in human atherosclerotic lesion (P'ramo *et al.*, 2005) and is widely accepted as a pro-inflammatory enzyme (Choi *et al.*, 2006). COX-2 expression is largely dependent on the activities of transcription factors including nuclear factor (NF)-κβ (Choi *et al.*, 2006). NF-κβ is a transcription factor that plays critical roles in inflammation and immunity (Choi *et al.*, 2006). The NF-κβ pathway is pivotal in the inflammatory response by regulating the expression of pro-inflammatory cytokines, chemokines and inducible enzymes (Gilroy *et al.*, 2004).

2.3.2. Markers that initiate and maintain low-grade inflammation in children

Information about exact noxious agents fuelling inflammatory processes of the vessel wall is scanty, however, abundant evidence shows that low-grade systemic inflammation is a predictive component of cardiovascular events (Marz *et al.*, 2004). The cardiovascular risk factors such as dyslipidaemia, hypertension, CAD and impaired glucose metabolism in young obese children have been strongly associated with low-grade systemic inflammation (Halle *et al.*, 2004; Jinabhai *et al.*, 2003; Reinehr *et al.*, 2005). In order to prevent these diseases in adulthood, it might be important to detect the risk factors for sub-clinical low-grade inflammation as soon as possible.

Key markers of inflammation, such as CRP, IL-6, IL-1 and TNF-α and several cell adhesion molecules have been linked to the future occurrence of myocardial infarction (MI) (Davi & Falco, 2005). Other (non-cytokine) fat derived peptides, such as PAI-1, leptin, adiponectin and resistin, may also play a role in the pathogenesis and/or serve as markers of risk in metabolic syndrome (Rudin & Barzilai, 2005). According to the latter there are a number of markers that serve as risk markers and even indicators of low-grade inflammation. In order to answer the question of which low-grade inflammatory markers are the most prominent and abundantly used in children, a summary of recent studies done on overweight children's inflammatory status is compiled in Table 2.

Table 2.1. A summary of trials that investigated low-grade inflammatory markers in overweight children

Author	Subject	Investigated markers	Major findings
Kelly A.S. *et al.*, 2004	*n*=25 children with a BMI >85th percentile	**CRP**, lipids, BP , VO2 peak, BMI	CRP is independently associated with fasting insulin and BMI.
Lambert M *et al.*, 2004	*n*=2224 9,13 & 16 years of age	**CRP**, lipids, glucose, insulin,	1 standard ⇧ in BMI was associated with 52% of ⇧ CRP. CRP associated with fasting insulin, and worrisome lipid profile
Mangge. H. *et al.*, 2004	148 children with type 1 diabetes, 86 obese children & 142 healthy controls.	**CRP**, juvenile type 1 diabetes, soluble IL-2 receptor, leptin, homocysteine, insulin	CRP ⇧ in type 1 diabetes, and even more ⇧ in obese individuals
Garanty-Bogacka, B *et al.*, 2005	50 children with obesity-related hypertension vs. 143 obese controls.	**CRP, IL-6,** fibrinogen, ICAM-1, VCAM-1	Children with hypertension showed ⇧ in IL-6 and CRP levels
Ford, E.S. *et al.*, 2001	5305 children aged 6-18 years (NHANES III, 1988-1994).	**CRP** & BMI	CRP levels ⇧ with a BMI >85th percentile, with no difference by sex, age, or ethnicity
Reinehr, T *et al.*, 2005	*n*=14 non-obese vs. *n*=31 obese children	**CRP, TNF-α,** BMI, BP, lipids, IR	Obesity correlated with TNF-α and CRP levels, these markers were also independent of lipids, BP, IR
Nemet, D *et al.*, 2003	*n*=30 healthy children	**IL-6, TNF-α,** BMI, HDLC, adiponectin, Insulin	Adiponectin ⇩ whereas TNF-α and IL-6 ⇧ in association with BMI and fat mass, no association between insulin and TNF-α and IL-6
Klein-Platat *et al.*, 2005.	*n*=60 overweight vs. *n=60 normal weight*	**IL-6, CRP**, IR, MS	MS is associated with ⇧ BMI, Inflammatory markers⇧ MS and BMI
Visser *et al.*, 2006	5315 children aged 6-18 years (NHANES III, 1988-1994).	**CRP**, white blood cells	CRP levels ⇧ with a BMI>85th percentile. High white blood cell counts were also detected in overweight subjects, suggesting a state of low-grade systemic inflammation in obese children
Halle, *et al.*, 2004	*n*=197 children aged 10-15 years	**IL-6, TNF-α, CRP,** obesity, physical fitness, fibrinogen	Obese unfit children showed ⇧ in CRP, TNF-α and IL-6. Obese fit individuals had as low levels as lean and fit children

BMI,=Body Mass Index, BP= Blood pressure, CRP=C-reactive protein, HDLC= High density lipoprotein cholesterol, ICAM-1= Intracellular adhesion molecule-1, IL-6= Interleukin-6, IR=insulin resistance, NHANES III, Third National Health and Nutrition survey, TNF-α= Tumor necrosis factor-alpha, VCAM-1= vascular adhesion molecule-1, ⇧ = increase

2.4. An introduction to the investigated inflammatory markers

2.4.1 TNF-α

2.4.1.1. Overview

TNF-α (previously known as lymphotoxin and cachetin) is a multifunctional cytokine, which exerts pleiotropic biological actions in different tissues and species (Liu *et al.*, 1998). Of all the known cytokines expressed in and secreted by white adipocytes, TNF-α was identified first in 1975 by Carswell and colleagues (Trayhum & Wood, 2004). TNF-α belongs to the family whose members share a cysteine-rich common extracellular binding domain, and includes several other non-cytokine ligand like CD40, CD27 and CD30 (Anon, 2006a). CD40 is a transmembrane glycoprotein of the TNF-α family that was initially described on the surface of B cells (Lazaar *et al.*, 1998). The CD40-mediated signaling events include protein tyrosine phosphorylation and activation of NF-κβ (Lazaar *et al.*, 1998). CD40 ligand (CD154) signaling, through its cognate ligand CD40, has since been shown to promote atherosclerosis and plaque instability, which then retains inflammatory effects through promotion of platelets, monocyte and production of reactive oxygen species (ROS) (Armstrong *et al.*, 2006). Thus, CD40-CD40L interaction may provide a molecular mechanism which links inflammation to a prothrombotic state (Davi & Falco, 2005).

Mature TNF-α consist of 157 amino acids glycoprotein peptide hormone, cleaved from a 212 amino acid-long propertied on the surface of macrophages (Vilcek & Lee, 1999). This 17 kD polypeptide is primarily produced and released by mononuclear phagocytes and macrophages (Chu *et al.*, 2002; Hauner *et al.*, 1995). Bruun *et al.* (2006) indicated that TNF-α is produced by the stromal vascular cell fraction (non-adipose) resident in the adipose tissue (Bruun *et al.*, 2006). The abnormal production of TNF-α in obesity is a paradigm for the metabolic significance of this inflammatory response, because when TNF-α activity is blocked in obesity it results in improved insulin sensitivity (Hotamisligil, 2003).

2.4.1.2. Physiological and biological activity

TNF-α has been investigated for its potential role in the development of obesity related co-morbidities (Bruun *et al.*, 2002). It has also been reported that TNF-α induces the expression of two distinct endothelial cell surface receptors, intracellular adhesion molecule 1 (ICAM-1) and E-selectin and that these elevated levels, furthermore, contribute to the development of CVD (Haddy *et al.*, 2003; Moldoveanu *et al.*, 2005; Sterner-Kock *et al.*, 1996). Expression of these cell surface adhesion molecule, leads to enhance pro-coagulant activity, subsequently playing a role in the endothelial dysfunction and vascular pathology observed in hyperinsulinaemic states (Winkler *et al.*, 1999). TNF-α is the main stimulator of IL-6 and IL-8 production (Bruun *et al.*, 2003). The production and release of IL-8, in turn, may be related to the pathogenesis of atherosclerosis and CVD (Bruun *et al.*, 2002; Bruun *et al.*, 2003). IL-8 is a member of the CXC chemokine family, which is produced and released from human mature isolated adipocytes and cultured human adipose tissue fragment (Bruun *et al.*, 2003). It is still speculated whether the circulating levels of IL-8 reflect the release of IL-8 from adipose tissue under normal condition, therefore, more comprehensive research focusing on the harmful effect of IL-8 on vascular function should be conducted (Bruun *et al.*, 2002).

Adiponectin (which is known to be increased after weight loss) inhibits TNF-α secretion, but in obesity the adiponectin levels decrease, diminishing the attenuation of TNF-α (Nemet *et al.*, 2003). Therefore, high levels of adiponectin interfere with the process of atherosclerosis by inhibiting the biological properties of pro-inflammatory TNF-α and increasing anti-inflammatory cytokines such as IL-10 and IL-1 receptor anti-body (Bruun *et al.*, 2006). The effect of adiponectin on TNF-α, as well as the expression of certain endothelial adhesion molecules may serve as evidence that adiponectin and TNF-α are involved in the pathogenesis of T2D and atherosclerosis (Nemet *et al.*, 2003). However, the specific relationship of adiponectin to body fat, fitness and other adipocytokines in children is still unclear (Nemet *et al.*, 2003) and warrants future investigation.

- *Relationship between leptin, TNF-α and the initiation of IR*

According to Mohamed-Ali *et al.* (1997), TNF-α does not influence lipoprotein lipase (LPL) action, lipolysis or insulin signalling through endocrine mechanism. Despite the discordant evidence of TNF-α secretion, the possibility that TNF-α acts as an autocrine or paracrine mediator of IR cannot be excluded (Mohamed-Ali *et al.*, 1997). Data of a large cohort study on nondiabetic subjects, both male and female, spanning a wide range of BMI's indicated that subcutaneous adipose tissue is probably not a central player in human IR (Koistinen *et al.*, 2000). The role of TNF-α in human obesity-related IR is still vaguely understood and the relationship is also controversial (Koistinen *et al.*, 2000; Peraldi *et al.*, 1996). However, the well established association between the amount of visceral fat and IR amplifies the possibility that TNF-α expression in visceral fat might play a role in the pathogenesis of IR (Koistinen *et al.*, 2000; Peraldi *et al.*, 1996).

The ability of leptin and TNF-α to regulate insulin secretion from the pancreatic β cells might contribute to the abnormalities in glucose homeostasis in obesity (Kirchgessner *et al.*, 1997). TNF-α may potentiate its IR effects due to the autocrine and paracrine effects that leptin has on the insulin receptor tyrosine phosphorylation and down regulation of several steps in the insulin signalling pathway (Kirchgessner *et al.*, 1997; Rudin & Barzilai, 2005). Over expression of TNF-α may also lead to the inhibition of signal transduction of the insulin receptor and the down regulation of GLUT4 transporters of adipocyte expression and complete loss of insulin-stimulated glucose uptake, initiating IR (Grohmann *et al.*, 2005; Liu *et al.*, 1998; Winkler *et al.*, 1999). PI3-kinase is an enzyme that plays a pivotal role in the downstream insulin signalling and translocation of GLUT4. It was found that the TNF-α treatment of human adipocytes induces a rapid (60-70%) inhibition of insulin signalling at the level of PI3-kinase (Liu *et al.*, 1998). The inhibition of PI3-kinase could be explained through the ability of tumor necrosis factor-1 (TNFR1) to mimic the inhibitory effect that TNF-α have on insulin signalling (Peraldi *et al.*, 1996). In adults, TNF-α has been shown to manipulate glucose transport in both adipocytes and muscles by the alteration in the insulin signalling pathways and glucose transport protein expression, but no studies to date have been performed in adipose tissue derived from children (Grohmann *et al.*, 2005).

Further studies are required to clarify whether differences in responsiveness to insulin or TNF-α exist in adipose tissue derived from obese children (Grohmann *et al.*, 2005; Lofgren *et al.*, 2000). However, a growing body of data supports the concept that sub-clinical chronic inflammation might be a key player in the pathogenetic factor of IR and T2D development (Sjoholm & Nystrom, 2005).

- *The association between TNF-α, cachexia (wasting syndrome) and malnutrition*

The association of TNF-α with the most extreme states of both catabolism and anabolism could be confusing and warrants careful review (Spiegelman & Hotamisligil, 1993). An elucidation of the potentially important interaction of the TNF-α system with insulin and leptin may provide insight into the pathophysiology of obesity and cachexia in humans (Mantzoros *et al.*, 1997). Both wasting and hyperlipidemia could be due to the loss of lipoprotein lipase, the enzyme responsible for the hydrolysis of circulating triglyceride-rich lipoprotein in muscle and fat (Spiegelman & Hotamisligil, 1993).

Studies concerning TNF-α concentration in weight loss are controversial; some studies reported a decrease in TNF-α concentrations, whereas others described stable TNF-α level in weight loss (Reinehr *et al.*, 2005). Despite the fact that TNF-α increases in obesity and decreases in weight loss, several findings also indicated an increase of TNF-α during cachexia and malnutrition (Hotamisligil *et al.*, 1993). TNF-α induces wasting during acute and chronic illness (Rudin & Barzilai, 2005), causing the delipidation of fat cells and a decrease of the adipose tissue mass (Hauner *et al.*, 1995). Cachexia is a detrimental end point of several diseases and is characterized by severe loss of lean body mass (mainly muscle), protein catabolism and various degrees of depletion of fat depots (Spiegelman & Hotamisligil, 1993). Malnutrition is usually associated with increased environmental exposure to infectious hazards due to deficient sanitation, poor hygiene standards, crowded housing and restricted access to medical care (Azevedo *et al.*, 2005). Therefore, malnutrition during childhood has led to the manifestation of an increase in the production of pro-inflammatory, namely TNF-α (Azevedo *et al.*, 2005). The increased level of soluble TNF-α receptors in malnourished children implies that malnourished children are

capable of producing significant amounts of TNF-α without the presence of increased adipose tissue mass (Azevedo *et al.*, 2005). Nevertheless, it is difficult to determine whether weight loss is directly caused by elevated TNF-α increase *per se* or indirectly to diseases caused by TNF-α expression (Spiegelman & Hotamisligil, 1993). What is known is that TNF-α turned out to be identical to cachetin, mediating wasting during the exposure to chronic infection (Vilcek & Lee, 1999).

The association between anorexia, cachexia and obesity can be explained through the relative amount of TNF-α expressed in the relevant disorders and the milieu of other cytokines and hormones in the different physiological or pathological states (Spiegelman & Hotamisligil, 1993). Cachexia is associated with the anorectic effect of TNF-α in extremely high levels, while experimental obesity is often associated with lower levels of TNF-α without apparent expression of other cytokines (Spiegelman & Hotamisligil, 1993).

2.4.2. IL-6

2.4.2.1. Overview

IL-6 is a circulating pleiotropic cytokine (Dedoussis *et al.*, 2004; Moldoveanu *et al.*, 2001) known to be secreted from a number of different cells including activated macrophages, lymphocytes, cardiovascular components such as endothelial cells, vascular smooth muscles cells and ischaemic myocytes (Poppitt 2005; Yudkin *et al.*, 2000). Mohamed-Ali *et al.* (1997) illustrated that IL-6 is also produced by 3T3-L1 cells, pericardial fat pads and mammary adipose tissue. IL-6 in turn inhibits the differentiation of 3T3-L1 into mature adipocytes, suggesting that it may function locally as an adipostat (Heilbronn & Clifton, 2002). In order to act as an adipostat, a molecule should be released by adipose tissue and be capable of bringing about metabolic changes so as to restore energy balance (Mohamed-Ali *et al.*, 1997). The 20-30 kD IL-6 is translated as a 212 amino acid molecule (Moldoveanu *et al.*, 2001), located on the short arm of chromosome 7, consisting of 5 exons and 4 introns, with a complex transcriptional regulation (Papanicolaou *et al.*, 1998). According to Labcorp®, a blood testing laboratory, the reference interval of IL-6 is between 0.00–14.0 pg/mL (Baron, 2004). Approximately 25-30% of serum IL-6 originates from adipose tissue and the secretion of IL-6 from subcutaneous fat is in

proportion to fat mass (Heilbronn & Clifton, 2002). Omental fat cells secrete approximately 2-3 times more IL-6 compared to subcutaneous adipocytes, implying that abdominally obese subjects have increased IL-6 or CRP (Heilbronn & Clifton, 2002). The latter supports the concept that a positive association exists between BMI, IL-6 and CRP (Connelly *et al.*, 2003).

The use of genetically engineered transgenic and gene knockout mice is one of the most incisive approaches to elucidate the role of IL-6 in inflammation and immunity (Papanicolaou *et al.*, 1998). According to Haddy *et al.* (2003), only IL-6 plays a key role in driving the acute-phase response in gene knock-out models. The production of TNF-α is markedly increased in these mice compared to normal mice and corticosteroids provide feedback suppression on production of TNF-α. These observations suggest that restraints on the production of pro-inflammatory mediators by hypothalamic-pituitary-adrenal (HPA)-axis are blunted in IL-6 knockout mice (Papanicolaou *et al.*, 1998).

2.4.2.2. Physiological and biological activity

IL-6 delegates various effects on cell growth and differentiation, the acute-phase responses and both carbohydrate and lipid metabolism (Mohamed-Ali *et al.*, 2001). Besides the mediation of IL-6 by immunity and infection, other stimuli for the production of IL-6 exist, for instance by the white adipose cells in obesity and cigarette smoking (Yudkin *et al.*, 2000). IL-6 is among the most potent mediators of the acute phase response (Barton, 1997; Moldoveanu *et al.*, 2001) and might play a key role in the development of coronary disease through a number of different mechanisms: metabolic, endothelial and coagulant (Yudkin *et al.*, 2000). The acute phase response reactants include tissue inhibitor of metalloproteinase and other proteins of anti-inflammatory potentials (Barton, 1997). The receptor for IL-6 is composed of two peptide chains called the α and β subunits (Barton, 1997). The α subunit is the ligand-binding chain of molecular weight, approximately 80 kDa, binding IL-6 with low-affinity, the second subunit is a peptide chain of approximately 130 kDa and is called gp130 (Barton, 1997; Yudkin *et al.*, 2000). The gp130 is required for high-affinity binding of gp80-bound IL-6 (Yudkin *et al.*, 2000). IL-6 activates gp130 through a soluble form that consists of the extracellular domain, leaning

it to the activation even on cells that lack the IL-6 receptor on their membrane (Papanicolaou *et al.*, 1998). The gp130 chain is the signal transducing peptide of the receptor complex (Barton, 1997).

IL-6 increases the release of adhesion molecules by the endothelium, increasing the hepatic release of fibrinogen and inducing a pro-coagulant effect on platelets (Yudkin *et al.*, 2000). It has been shown that the administration of IL-6 together with or alone induces a dose-dependent increase in platelet count (Moldoveanu *et al.*, 2001) Therefore, it is implied that an addition of IL-6 to plaque assay results in a higher number of plaque than without IL-6 (Barton, 1997). The platelets have an inflammatory action, secreting inflammatory cytokines (e.g. IL-1β, CD40L) (Davi & Falco, 2005). *In vivo* and *in vitro* studies indicate that IL-6 can act alone or synergise with IL-3 to enhance proliferation of haemopoetic progenitor cells (Moldoveanu *et al.*, 2001). The proliferation of haemopoetic progenitor cell in turn leads to megakaryocytic lineage, which is essential for the production of platelets in the marrow and are normally not present in the blood (Moldoveanu *et al.*, 2001).

The novel findings in a study conducted by Dedoussis *et al.* (2004) postulates that the G-174 C *IL-6* genotype seems to be associated with obesity among prepubertal children (Dedoussis *et al.*, 2004). It has previously been shown that the polymorphism - 174 in the *IL-6* gene is associated with lipid abnormalities. Data from the STANISLAS cohort conducted on 179 healthy families showed a significant correlation between IL-6, high density lipoprotein cholesterol (HDL-C) and apaA1 concentration in women and both apoA1 and HDL-C showed an inverse relationships with IL-6 levels ($P<0.001$) (Haddy *et al.*, 2003). Inverse relationship between IL-6 and apoA1 and (HDL-C) has been found in diabetic women, in old subjects and in patients undergoing abdominal operations (Haddy *et al.*, 2003). The inverse may also then be true, that an increase in IL-6 concentration could lead to an increase in low density lipoprotein concentration (LDL-C) and very low density cholesterol (VLDL-C), but data on this hypothesis is still scanty.

It is known that IL-6 and TNF-α are the cytokines with both metabolic and/or weight-regulated effects (Barton, 1997). Barton (1997) concluded that IL-6 is at least as powerful a cachetin as TNF-α, although he also speculated that IL-6 may be inducing other factors which cause cachexia directly. Both TNF-α and IL-6 inhibits LPL activity and decreases its production in murine adipocyte cell lines, as well as increases lipolysis (Mohamed-Ali *et al.*, 1997). Mohamed-Ali *et al.* (1997) speculates that IL-6 and leptin could act synergistically to maintain adipose tissue energy equilibrium. Therefore, the pro-inflammatory action of IL-6 to induce full acute phase response may also have a role in lipid metabolism.

Factors that play a role in the biological activity of IL-6 (summarised in figure 1)

- *Smoking*

Cigarette smoking is one of the major classical risk factors for atherosclerosis and CVD (Yasue *et al.*, 2006). The development of hypertension has been linked to chronic low-grade inflammation. However, it is not known whether this connection is mediated by features of metabolic syndrome or smoking, or their changes (Nisken *et al.*, 2004). In smokers the increased levels of triglyceride, remnant-like particle cholesterol and apolipoprotein-B may be caused mainly by the elevated IL-6 levels which promote fatty acid synthesis and suppress lipoprotein lipase (Yasue *et al.*, 2006). IL-6 is known to stimulate platelet formation and a strong association exist between smoking and platelet count, even though only few studies up to date have investigated this phenomenon (Yasue *et al.*, 2006). Smoking leads to the over expression of ROS, which then suppresses the endothelial NO activity leading to endothelial dysfunction (Yasue *et al.*, 2006). The latter is supported by Nisken *et al.* (2004) who identified an acute rise in blood pressure and CRP levels of smokers. Smoking in its own right increases inflammation, but smoking cessation may lead to weight gain, which then in turn itself increases the IL-6 levels (Yasue *et al.*, 2006). Therefore, future investigation is needed to identify the exact role that smoking plays in the development of chronic low-grade inflammation.

- *Hormonal regulation*

IL-6 plays a central role in the pathogenesis of the osteoporosis seen in conditions characterized by increased bone resorption, such as sex-steroid deficiency and hyperparathyroidism (Papanicolaou *et al.*, 1998). IL-6 plays a prominent role in skeletal homeostasis, therefore promoting the development of osteoclasts (Papanicolaou *et al.*, 1998). Estrogen loss results in increased production of IL-6 by ex-vivo bone marrow cell cultures and increased production of IL-6 follows the withdrawal of estradiol from primary cultures (Papanicolaou *et al.*, 1998). Estrogen has long been recognized as influencing satiety and adipose tissue distribution. It has also been shown that IL-6 modulates the action of aromatase, a key regulatory enzyme for estrogen metabolism (Mohamed-Ali *et al.*, 1997).

- *Psychosocial stress*

Psychosocial stress can increase the circulating levels of IL-6, perhaps consequent upon the influence that catecholamines have on IL-6 production (Mohamed-Ali *et al.*, 2001; Yudkin *et al.*, 2000). The increase in IL-6 levels stimulates the HPA-axis (Yudkin *et al.*, 2000). The stimulation of the HPA-axis results in a tendency of central obesity, IR and dyslipidemia, in turn the increase of visceral adipose tissue mass increases IL-6 levels even more (Yudkin *et al.*, 2000). The tissues responsible for the adrenergic-induced surge in plasma IL-6 levels have not been identified (Papanicolaou *et al.*, 1998). However, it has been observed that β-adrenergic stimulation induces the expression and release of IL-6 in murine brown adipocytes, implying that adipose tissue could also be an important contributor to the adrenergic-mediated rise in plasma IL-6 levels (Mohamed-Ali *et al.*, 2001; Papanicolaou *et al.*, 1998).

- *Obesity*

As fat mass increases in obesity, adipocytokines become involved in a number of metabolic and hormonal adjustments such as an increase in IL-6 levels which, in turn, induces an acute phase response (Nemet *et al.*, 2002; Dedoussis *et al.*, 2004). Although it has been suggested that about 20% of circulating IL-6 is derived from peripheral blood cells, plasma levels of IL-6 are markedly elevated in obese subjects and is suggested that

up to 30% of this cytokine could be derived from adipose tissue (Mohamed-Ali *et al.*, 2001). It is plausible that IL-6 released from an expanded adipose tissue mass could contribute to certain aspects of the associated pathophysiology, including a pro-inflammatory state predisposing to atherosclerosis (Mohamed-Ali *et al.*, 2001).

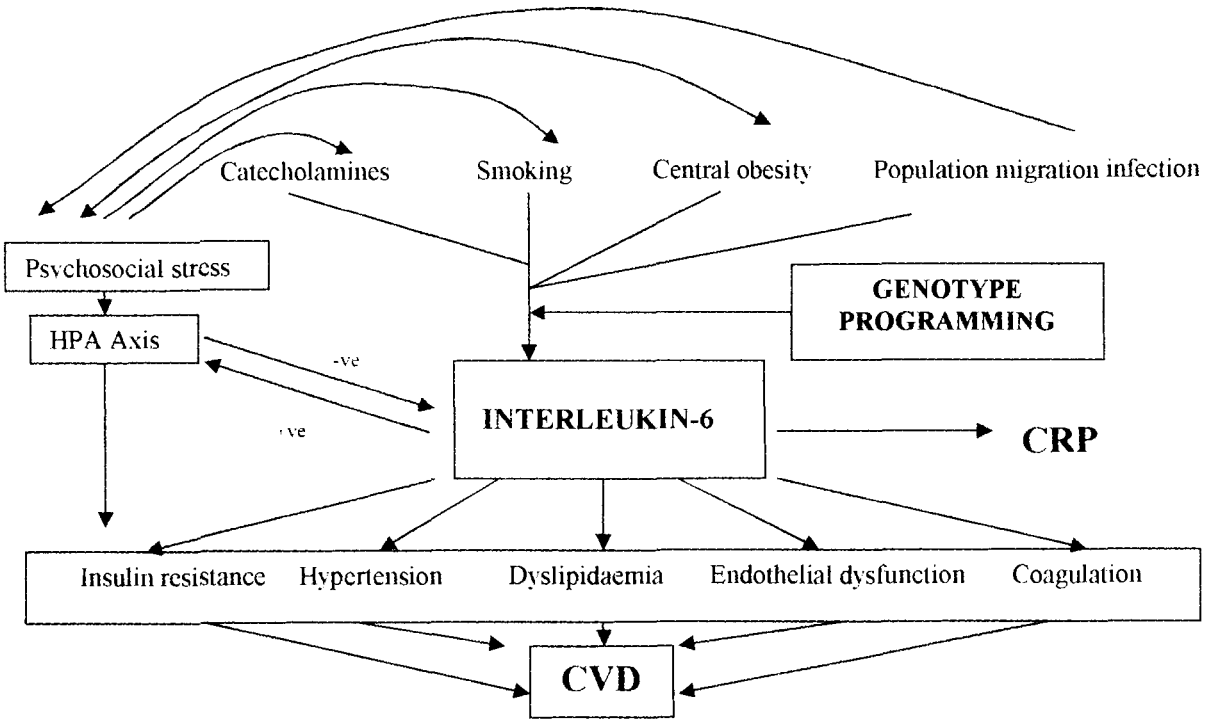

Figure 2.1. The role and regulation of IL-6 (Yudkin et al., 2000)

CRP= C-reactive protein, CVD= Cardiovascular diseases, HPA-axis= Hypothalamic-pituitary-adrenal-axis.

2.4.3. C-reactive protein

2.4.3.1. Overview

The acute-phase response consists of the enhanced production of more than 40 proteins, which have either pro-inflammatory or anti-inflammatory properties (Papanicolaou *et al.*, 1998). CRP is the most prominent marker of the acute-phase response expressed primarily by the liver (Heilbronn & Clifton, 2002; Moldoveanu *et al.*, 2001). In humans CRP can increase up to 1000 fold through increased hepatocyte synthesis after an acute

inflammatory stimulus such as tissue injury or infection (Poppitt, 2005; Rudin & Barzilai, 2005). Minor CRP elevation has been found associated with members in different demographic and socioeconomic groups, with a variety of dietary patterns and with many diverse medical conditions that are not apparently inflammatory (Kushner *et al.*, 2006). Increasing age, female, ethnicities, low socioeconomic status and low-birth weight could be some of the demographic and socioeconomic factors that may be responsible for minor CRP elevation in some individuals (Kushner *et al.*, 2006). Several studies have identified CRP as an independent predictor of coronary heart diseases (CHD) in men and women (Connelly *et al.*, 2003). The low concentration of 3 mg/L in lean individuals may dramatically increase in response to injury, infection and inflammation (Heilbronn & Clifton, 2002). Levels that have traditionally been regarded as the normal range (1-10 mg/L) may reflect chronic low-grade inflammation (Heilbronn & Clifton, 2002). CRP levels within the 'high-normal' range 1-3 mg/L have been shown to predict CVD and development of T2D (Baron, 2004; Jae *et al.*, 2006; Weiss & Caprio *et al.*, 2005). Infection, neoplasm, inflammation, obesity and CVD are all potential reasons for elevated levels of CRP (Hanusch-Enserer et *al.*, 2003). CRP production is increased in obese individuals (Maachi *et al.*, 2004; Mandato *et al.*, 2005) and the reason for the increased production of CRP is most likely due to increased production of IL-6 (Heilbronn & Clifton, 2002).

2.4.3.2. Physiological and biological activity

CRP is the primary inflammatory marker that has been shown to predict future cardiovascular events in individuals with or without established CVD (Ballantyne & Nambi, 2005). Studies of the acute phase response in humans have usually been limited to single causes of tissue injury, infection and surgical trauma (Kushner *et al.*, 1982). Recently, the acute phase phenomena have been associated in obesity, since the liver appears to play a major role in the acute phase response (Kushner *et al.*, 1982). The positive correlation that exists between CRP and weight status could be explained by the fact that the main regulator of the hepatic syntheses of CRP in the liver is the adipocyte-derived pro-inflammatory cytokine IL-6 (Reinehr *et al.*, 2005; Yudkin *et al.*, 2000). However, the mechanisms responsible for increased CRP levels in obese individuals are

not well elucidated (Maachi *et al.*, 2004) and warrant further investigation. Increased levels of the acute-phase inflammatory marker, CRP, are related to IR and the T2D, suggesting a major role for CRP in chronic low-grade inflammation (Sjoholm & Nystrom *et al.*, 2005). CVD has been found to begin in childhood, indicating that increased CRP levels in apparently healthy children affect the arteries, disturbing endothelial function and promoting intima-media thickening (Warnberg *et al.*, 2004).

Poppitt (2005) emphasizes that this pro-atherosclerotic effect of CRP may be caused in part by increased secretion of the endothelium-derived vaso-active factor endothelin-1 and the cytokine IL-6. An increase of CRP to 10 mg/L could enhance endothelial cell adhesion molecule expression (ICAM-1, VCAM-1 and E-selectin) by 10 fold (Heilbronn & Clifton, 2002). Therefore, low-grade inflammation as measured by increased CRP levels may actively promote atherogenesis (developmental process of atheromatous plaques), causing lesion formation through endothelial dysfunction and alteration of plaque architecture, thereby reducing stability and enhancing rupture (Nisken *et al.*, 2004; Poppitt, 2005; Tan, 2004). Moreover, CRP may upregulate angiotensin type-1 receptors, leading to the proliferation of vascular smooth muscle cells and subsequently to the development of hypertension (Nisken *et al.*, 2004). To understand thoroughly the action of CRP on the development of CVD and MS, the cell-surface CRP receptor and signalling pathways should be characterised in the future (Sjoholm & Nystrom, 2005). Minor CRP elevation bears negative prognostic implications for major cardiovascular events later in life therefore, underscoring the need to elucidate the mechanism underlying the CRP response (Kushner *et al.*, 2006).

2.4.4. The relationship between CRP, IL-6 and TNF-α

During inflammation the inflammatory cytokines TNF-α, IL-1, IL-6 and CRP are secreted in that order (Papanicolaou *et al.*, 1998). TNF-α is known to be a potent inducer of the synthesis of IL-6 production (Reinehr *et al.*, 2005). CRP production from the liver in turn is induced by IL-6 which is produced by fat cells and adipocytokines (Isasi *et al.*, 2003). Unlike TNF-α and IL-1, IL-6 does not up-regulate major inflammatory mediators such as prostagladins, NO, matrix metalloproteinase and ICAM-1 (Barton, 1997), but rather

inhibits the synthesis of LPS-induced IL-1 and TNF-α *in vivo* and *in vitro* (Barton, 1997; Reinehr *et al.*, 2005). IL-6 helps in the control of inflammation through the stimulation of the HPA-axis (Papanicolaou *et al.*, 1998) and CRP synthesis (Bullo *et al.*, 2003; Haddy *et al.*, 2003; Klein-Platat, 2005; Papanicolaou *et al.*, 1998; Rudin & Barzilai, 2005; Trayhum & Wood, 2004). In this sense, IL-6 is both a pro and anti-inflammatory cytokine (Papanicolaou *et al.*, 1998). Despite the important role IL-6 plays as a pro-inflammatory cytokine, it is still strongly suggested that IL-6 is abundantly expressed in advanced human atherosclerotic lesions, suggesting that IL-6 has a role in lesion development directly or via CRP (Heilbronn & Clifton. 2002).

TNF-α and IL-6 are cytokines with metabolic and weight-regulating properties (Mohamed-Ali *et al.*, 1997). An important factor of IL-6 and TNF-α is that they are known to regulate a host of physiological processes directly tied to carbohydrate and fat metabolism, including the development of obesity complications such as diabetes, IR and atherosclerosis (Nemet *et al.*, 2003). High levels of TNF-α and IL-6 influence the hepatic synthesis of the acute phase protein such as fibrinogen, ferritin and plasminogen activator inhibitor-1, which are the independent cardiovascular risk factors (Halle *et al.*, 2004). Minor CRP elevations are associated with IR, frank diabetes and the entire MS (Kushner *et al.*, 2006), whereas TNF-α and IL-6 show damaging effects on both glucose homeostasis and β cell function and can disrupt insulin signalling pathways (Gonullu *et al.*, 2005). Therefore, there is a close relationship between circulating concentrations of CRP, IL-6 and TNF-α with the components of the MS, insulin sensitivity, high triglyceride and low HDL-C concentration and elevated blood pressure, with a correlation of r=0.52 between a summary score of two clusters (Gonullu *et al.*, 2005).

Despite the possible role of the TNF-α-system in the low-grade systemic inflammation associated with obesity, few studies have measured TNF-α level to show a positive relationship with CRP serum levels in obesity (Bullo *et al.*, 2003). The possible participation of inflammatory mediators in the pathogenesis of metabolic and cardiovascular events is of intense current interest (Kushner *et al.*, 2006).

2.5. The relationship between body composition, inflammation and the initiation of non-communicable diseases

2.5.1. The concern of obesity-related diseases

The production of adipocytokines during low-grade inflammation is increasingly viewed as a significant component of MS, CVD and high BP (Klein-Platat, 2005; Rudin & Barzilai, 2005; Weiss & Caprio *et al.*, 2005). Several studies, such as the cohort study of 14 719 initially healthy women done by Ridker *et al.* (2003), add support to the concept that a pro-inflammatory state is a component of MS, demonstrating that in all levels of MS, CRP is an important and independent prognostic in terms of future CVD (Morange *et al.*, 2004).

It is widely accepted that most obese individuals have elevated concentrations of IL-6, TNF-α and CRP markers, which are closely associated with diabetes and hypertension (Ramos *et al.*, 2003) and systemic low-grade inflammation (Mandato *et al.*, 2005; Trayhum & Wood, 2004). The balance between these numerous molecules is altered such that enlarged adipocytes produce more pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and less anti-inflammatory peptides such as adiponectin (Weiss & Caprio *et al.*, 2005). Insight into the pathways whereby the MS leads to atherosclerosis and acute coronary syndrome would help to clarify the connection between inflammation and the MS (Morange *et al.*, 2003). Weiss and Caprio (2005) designed a framework for the mechanism that summarises the association between obesity, inflammation and its co-morbidities (Figure 2).

Recently there has been increasing interest in the inflammatory hypothesis as an underlying mechanism of arterial injury (Hanusch-Enserer et *al.*, 2003). As adipose tissue accumulates throughout the body and in other organs, it is possible that the hyperplastic characteristic of adipose tissue over-expresses, becomes more active and then releases more pro-inflammatory cytokines such as IL-6, TNF-α and CRP (Halle *et al.*, 2004; Rudin & Barzilai, 2005). The latter might be related to increased risk of CVD, CHD and the progression of atherosclerosis through the role in plaque progression (Maachi *et al.*, 2004; Poppitt, 2005; Samuelsonn, *et al.* 2003). Halle *et al.* (2004) conducted a study where a

total of 197 children between the ages of 10 and 15 years were investigated in order to assess the relationship between obesity, physical fitness, metabolic cardiovascular risk profile and markers of inflammation. Results obtained in this study indicated that obese children had significantly higher concentrations of inflammatory parameters such as fibrinogen, ferritin, IL-6 and TNF-α than the non-obese subjects ($P<0.01$). Obese children with excessive fatty acid oxidation in the liver generate free radicals, which damage hepatocytes and induce fibrinogenesis through cytokine production (Mandatao *et al.*, 2005). In support of the latter, accumulating evidence indicates that obesity is associated with sub-clinical chronic inflammation (Weiss & Caprio *et al.*, 2005). Das (2002) suggests that obesity is an inflammatory disorder and that inflammation could be the general link between obesity and CVD.

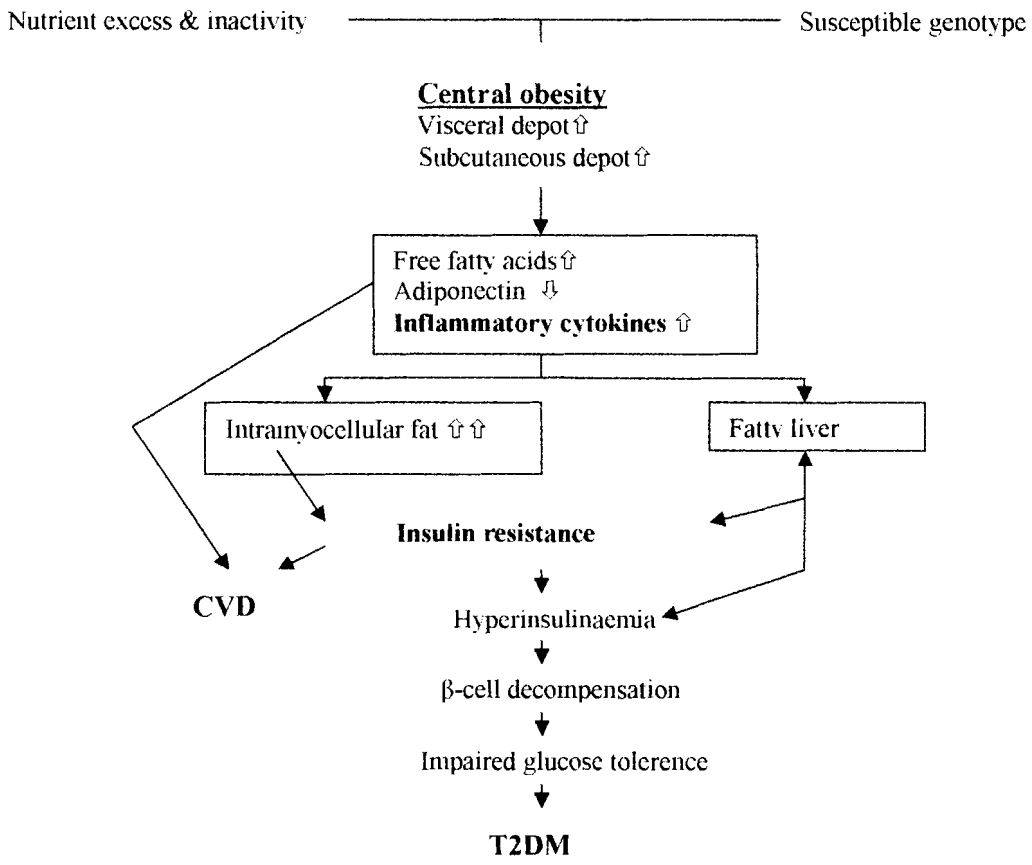

Figure 2.2. Mechanism of obesity-related morbidities (Weiss & Caprio, 2005)

CVD= Cardiovascular diseases, T2DM= Type 2 diabetes mellitus, ⇧= increase, ⇩= decrease

Although clinical hypertension occurs less frequently in children than in adults, ample evidence now supports the concept that the roots of essential hypertension extend back to childhood, thereby supporting the need to track blood pressure in children (Schutte *et al.*, 2003). The majority of patients with high blood pressure are overweight and hypertension is more frequent in obese subjects (Poirier *et al.*, 2006). The increase in BP is greater when the obesity is of abdominal distribution (Poirier *et al.*, 2006). A factor to consider in linking obesity to an increase in BP is the mechanism linking obesity and an increase in peripheral vascular resistance, endothelial dysfunction, IR and substances released from adipocytes (IL-6 and TNF-α) (Poirier *et al.*, 2006).

2.5.2. Obesity, cytokines and endothelial dysfunction

The mechanisms responsible for the increased cardiovascular risk that accompanies T2D remain poorly understood, however, it is commonly held that endothelial dysfunction and systemic low-grade inflammation can explain, at least in part, why deteriorating glucose tolerance is associated with CVD (De Jager *et al.*, 2006). Sub-clinical chronic low-grade inflammation might be an important player in the early stages of atherogenesis, including impairment of endothelial function and the formation of fatty streak and plaque, as well as in the thrombotic events that trigger myocardial infarction (MI) and some strokes (Isasi *et al.*, 2003; Sjoholm & Nystrom, 2005). It is commonly held that endothelial dysfunction and low-grade inflammation are two major key players in the pathophysiology of artherothrombosis (De Jager *et al.*, 2006; Santos *et al.*, 2005). Hanusch-Enserer *et al.* (2003) suggested a correlation between chronic inflammatory response, obesity and endothelial dysfunction. Endothelial dysfunction has been shown to be an early marker of CVD and has, therefore, been associated with atherosclerosis, hypertension, hypercholesterolemia, smoking and diabetes (Loukovaara & Ylikorkala, 2003; Tan, 2003). The latter is supported by a recent study done on 328 T2D patients who were followed up for 9 years. The results showed that chronic inflammatory activity and endothelial dysfunction are interrelated processes that develop in parallel and are independently associated with risk of death (Stehouwer *et al.*, 2002). Therefore, it could be implied that

the inflammatory processes are involved in the pathogenesis of endothelial dysfunction (Tan, 2003).

Endothelial cells are activated on the release of inflammatory cytokines, IL-6 and TNF-α which leads to an upregulation of receptors for vascular adhesion molecule-1 (VCAM-1), ICAM-1, E-selectin and L-selectin for various immune cells (Anon, 2006b). Elevated levels of ICAM-1 and E-selectin have been shown in patients with coronary artery disease (Haddy *et al.*, 2003). Nitric oxide (NO) (main product of endothelial cells in capillaries) and E-selectin are endothelium-derived biomediators whose synthesis is also enhanced through the release of cytokines such as TNF-α and IL-1β (Loukovaara & Ylikorkala, 2003; Sterner-Kock *et al.*, 1996). This molecule (NO) plays major roles in the regulation of cardiovascular, immune and nervous system, with a role in both acute and chronic inflammation (Vane, 1996). ICAM-1 is a member of the immunoglobulin-super family that binds to lymphocyte function-associated antigen-1 and is constitutively expressed at low levels on unstimulated endothelium (Sterner-Kock *et al.*, 1996). NO is an important paracrine vasodilator released by endothelial cells, and is released continuously in significant amounts in the arterioles and contributes to arteriolar vasodilatation (Vander *et al.*, 2001). A decrease in weight may lead to a decrease in cytokines, which in turn is associated with improvements of endothelial function (Haddy *et al.*, 2003). Rural black school children's dietary intakes consisting of low intakes of fruit, vegetable and legumes primarily lead to a deficient intake of essential nutrients, such as vitamin A, vitamin C, vitamin E, folic acid, biotin, magnesium, zinc and iron, that may be responsible for a decrease in arterial compliance through endothelial dysfunction at a young age, leading to hypertension in adulthood (Schutte *et al.*, 2003).

2.5.3. Stunting and its association with inflammation-related diseases

Pro-inflammatory cytokines could be an unmeasured factor that may be related to both lean body mass and progression of stunting (Friedman *et al.*, 2005), and may impede growth through both their anorexia inducing effects and direct inhibitory effects on bone growth. Yudkin *et al.* (1999) investigated the association of IL-6 and TNF-α concentration in urban slum dwellers, urban middle class and village dweller subjects. In

the slum dwellers the IL-6 concentration was around 10-fold higher than the village dwellers, despite being thinner. The possible explanation may be that environmental insults, such as infection or pollutants, or psychosocial factors might compound the effects of increased calorie density and decreased physical activity to increase secretion of IL-6 from macrophages or adipose tissue (Yudkin *et al,* 1999). The latter supports the hypothesis that children from developing countries may have a greater risk of developing low-grade systemic inflammation due to their surrounding environment. There is a lack of information about the association between stunting and inflammation-related diseases; therefore, further research is needed to clarify the association.

2.6. Recommendation

2.6.1 Physical activity and fitness

2.6.1.1 The health benefit of exercise in children and adolescence

Regular exercise enhances general health, improves the individual's mood, insulin sensitivity, plasma lipoprotein profiles and reduces blood pressure, yet many people do not participate in any PA (Das, 2004; Ernst & Pangrazi, 1999). It is evident that PA is a well established factor that may prevent CVD and other chronic diseases of lifestyle indices in individuals (Pate *et al.*, 1997). According to Panagiotakos *et al.* (2004) more than 2 million deaths each year are attributable to lack of PA. The health benefits of regular PA and physical fitness among adults are well established, but less is understood about the health benefits associated among children and adolescents (Pate *et al.*, 1999).

PA in children and adolescents is beneficial for mainly two reasons; firstly, it promotes physical and psychological health and well-being during childhood and adolescence. Secondly, participation in regular PA during childhood or adolescence may increase the probability that children will become active adults (Ernst & Pangrazi, 1999). Exercise improves IR in large part by altering glucose transport in the muscle, but this is only beneficial in the presence of weight loss (Nemet *et al.*, 2003). Taking part in moderate PA may help children recovering from protein-energy malnutrition to enhance linear growth and promote catch-up growth from stunting (Torun & Viteri, 1994).

2.6.1.2. The association between exercise and inflammatory markers

Physical inactivity is recognised as a major risk factor for NCD (Baumann & Craig, 2005). However, PA affects local and systemic cytokine production at different levels (Moldoveanu *et al.*, 2001). When PA is of sufficient vigour to induce an inflammatory response, there is a release, firstly a sequence of pro-inflammatory cytokines (TNF-α, IL-β and IL-6), and then of regulatory, anti-inflammatory cytokines (e.g., IL-4, IL-10 and transforming growth factor-β (TGF-β)) (Moldoveanu *et al.*, 2001). The release of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 through exercise leads to regulation of the migration of neutrophils and monocytes into the areas of injured muscle cells and other tissues to initiate repair (Das, 2004).

Data from a study done in Denmark indicated that after strenuous exercise TNF-α, IL-β, IL-6 and IL-1 receptor antagonists are released in a sequential manner comparable to that observed in physical trauma (Nieman & Pedersen, 1999). According to Das (2004) the CRP and pro-inflammatory cytokines such as TNF-α and IL-6 declined in subjects who exercised for a mean of 2.5 h/wk when compared to control subjects. Short-term muscle contractions induce the release of significant amounts of IL-6 but not TNF-α (Bruun *et al.*, 2006). Exercise generally causes a strong but transient induction of IL-6, however, the magnitude of the response depends on the intensity of the effort (Moldoveanu *et al.*, 2001). The release of IL-6 was moderately increased immediately after exercise, but had reverted to resting values after 20 minutes of recovery (Moldoveanu *et al.*, 2001). Isasi *et al.* (2003) found in a study an inverse association between physical fitness and CRP levels in children and young adults, with a more pronounced relationship in boys than in girls. However, physical fitness and activity have been reported to have a minor independent effect on CRP levels (Reinehr *et al.*, 2005).

The preventive effect of exercise on myocardial damage and atherosclerosis could be explained through the production of manganese superoxide dismutase (Mn-SOD) in the myocardium, arterial tissue and endothelial NO (Das, 2004). The cardio protective effect of exercise works through the induced production of free radicals (superoxide anion, $O_2^{\cdot-}$) and TNF-α, which in turn enhance the synthesis of Mn-SOD activity (Das, 2004).

Panagiotakos *et al* (2004) made a summary of 10 studies that evaluated the effect of physical activity on inflammatory markers and concluded that regular exercise has a lowering effect on several inflammatory markers. All the studies showed an inverse association between CRP levels and PA levels, 2 measured IL-6 and also found a fall below the median. One study identified a contrary correlation between TNF-α and PA. This indicates that long term exercise decreases the atherogenic activity of mononuclear cells in persons at risk of developing CVD (Das, 2004). Therefore, Das (2004) suggested that exercise is anti-inflammatory in nature and has beneficial actions on the human body. In children it is most likely that higher levels of PA with an augmentation of muscle mass and decrease in fat, which in itself would likely lead to increased adiponectin and reduced IL-6 and TNF-α levels (Nemet *et al.*, 2003). Presently it is unclear whether exercise training decreases CRP independently of weight loss, however, it is possible that exercise alone could also decrease CRP without any associated weight loss (Jae *et al.*, 2006).

Despite all these findings, combining moderate long-term exercise training with a hypo-caloric diet would have the most significant effect on the circulating levels of pro-inflammatory cytokines than exercise or diet on its own (Bruun *et al.*, 2006; Das, 2004). Further research around the exercise and the kinetics of cytokine production is needed in order to answer the questions of the duration and magnitude of exercise and the specific effect it has on inflammation.

2.6.2. Weight loss

CVD is strongly associated with obesity (Clifton *et al.*, 2005), therefore, obese individuals are encouraged to lose weight in order to reverse or prevent the adverse health consequences of obesity (Hanusch-Enserer *et al.*, 2003). Circulating levels of IL-6, TNF-α and CRP are directly associated with obesity (Rudin & Barzilai, 2005). Therefore, weight loss represents a safe method for reducing the circulation of pro-inflammatory markers, ameliorating fibrinolytic activity and improving endothelial function (Reinerh *et al.*, 2005; Nicoletti *et al.*, 2003). Sustained weight loss induced by dietary modification and increased physical activity not only reduces IL-6, CRP and IL-8 but also increases

adiponectin levels significantly (Bruun *et al.*, 2002; Das, 2004; Rudin & Barzilai, 2005). The well accepted fact that weight loss decreases CRP in obese patients could be explained through the modulation by reduction in abdominal fat as abdominal fat is related to increased production of inflammatory cytokines, which in turn, stimulates CRP production (Jae *et al.*, 2006). Factors known to modulate CRP include weight loss and physical activity but less is known of the effect of various diets (Poppitt, 2005). However, eating habits of township subjects do not contribute to an improved weight loss. In township areas, street vendors stock only full-cream dairy products, cheap fatty meat, vetkoek and few fresh fruit and vegetables (Kruger *et al.*, 2005).

Although much research is needed to elucidate the mechanism by which weight loss results in decreased inflammation, lowering of CRP levels can be attributed to a decrease in fat mass which lowers IL-6 levels, which in turn, decreases CRP synthesis by the liver and from other cellular sources (Basu *et al.*, 2005).

2.6.3. Therapeutic advances

Interest in the potential use of inflammatory markers in the prediction of future CVD risks raises the possibility that inflammatory factors may serve as targets of therapeutic resolutions. Screening for both CRP and LDL-C in patients may provide better prognostic information than screening for either alone (Rudin & Barzilai, 2005). From a clinical perspective measurements of adipocytokines may become part of the standard evaluation of the obese child and help to manage those overweight children who may be at high risk for the development of T2D and atherosclerosis later in life (Nemet *et al.*, 2003). Understanding the molecular basis of inflammation will lead to the identification of markers that may also serve as new targets of therapy in the management of NCD and CVD.

Prevention of CVD through the evaluation and modification of several risk factors has been the emphasis of health care professionals (Panagiotakos *et al.*, 2004). Statins' predominant effect is to lower LDL-C, thereby reducing cardiovascular morbidity and mortality (Ballantyne & Nambi, 2005). The potential anti-inflammatory effect of statin

needs further research. As a possible drug target, the inflammatory signalling pathway needs to be explored in greater detail (Sjoholm & Nystrom, 2005). Furthermore, recent findings support the notion that the COX-2/PGE2 axis that plays a role in atherosclerosis might be an attractive therapeutic target and warrants further investigation (P'ramo *et al.*, 2005).

2.6.4. Dietary factors to promote or retard inflammation

Limited studies have shown that certain dietary factors such as oleic acid, α-linolenic acid and antioxidant RRR-α-alpha tocopherol reduce biomarkers of inflammation (Basu *et al.*, 2005). However, the role of dietary factors in the prevention of CVD has been the subject of considerable attention (Basu *et al.*, 2005). High fat diets (59% fat) have been shown to promote inflammation in healthy and T2D patients, therefore, dietary fat may influence the pro-inflammatory action of saturated or *trans* fatty acids, which may exert differentiated effects on acute phase proteins and inflammatory cytokines (Basu *et al.*, 2005). In this study of Basu *et al.* (2005) the subjects consuming a Mediterranean-style diet consisting of fresh fruit, vegetables, nuts, whole grains and olive oil showed a decrease in serum concentrations of CRP and IL-6 ($P<0.05$). Future research is needed in order to clarify the exact role that dietary intake has the inflammatory status.

2.7. Conclusion

Evidence is growing that low-grade inflammatory status may be crucial in the progression and severity of atherosclerotic disorders. However, whether CRP or IL-6 plays a causative role or simply acts as a marker of acute-phase reaction is not fully elucidated (Bullo *et al.*, 2003). CRP has shown to be a reliable measure of underlying systemic inflammation and a strong predictor of future cardiovascular events (Rudin & Barzilai, 2005). Interest in this statement stimulates interest in a possible role that inflammatory markers (IL-6, CRP and TNF-α) may have in CVD risk assessment in clinical practice.

Measuring inflammatory markers such as CRP may increase the ability to predict thrombotic events which tend to occur in unstable plaques rich in monocytes, T-lymphocytes and lipids (Heilbronn & Clifton, 2002). Detecting the prevalence of sub-

clinical inflammation in early childhood may prevent the development of CVD in adulthood leading to decreased mortality and morbidity rates. Therefore, further research of children into adulthood is needed to test the benefit of exercise and weight reduction on body composition, metabolic risk factors, inflammatory markers including CRP, TNF-α and IL-6 and endothelial function, as well as subsequent development of atherosclerosis. (Yudkin *et al.*, 2000; Wanberg *et al.*, 2004).

CHAPTER 3:

Methods

3.1 Introduction

The present research study formed part of the PLAY project, which is an acronym for "**P**hysica**L** **A**ctivity in the **Y**oung'. The PLAY study is a multidisciplinary study in which several health parameters were investigated. This was originally a parallel intervention study, but only the baseline measurements were used for the purpose of the present study. Therefore, a single cross-sectional study design was used. All the measurements and questionnaires were taken at the North-West University in Potchefstroom, South Africa.

3.2 Ethical approval

Ethical approval was obtained from the Ethics Committee of the North-West University; under the Ethics number 04M01. Visits to the schools were made in order to explain the study design and ethical procedures to the teachers, parents, guardians and children. The parents or guardians of the children gave written consent and children were only included in the study if the consent form was received (Addendum A).

3.3 Subjects

The participants were only eligible to participate in the study if they were apparently healthy black African boys and girls. Children were excluded from the study if they failed to hand in the consent form or if their parents did not give consent for their participation in the project. The eligible children comprised of Grade 9 pupils in two high schools, Seiphemelo Secondary and Boitshoko High School located in Ikageng, a neighbouring township of Potchefstroom. The children in the Grade 9 group ranged from 13-19 years of age. The age differences are due to the fact that they previously failed a grade or started to attend school at a much later age. The convenience sample size of all scholars was 210 with 192 who consented to give blood. The children from Ikageng were mainly from the Setswana, Sotho, Xhosa and Zulu ethnic groups. Ikageng is a low-income township and

the participants mainly live in a low-income setting with a limited availability in food choices providing a lack in variety in their diet. The setting in the townships leads to rather informal living arrangements where there is not always a sufficient supply of water and electricity (Vorster *et al.*, 1997). External factors, as mentioned above could lead to chronical undernutrition, stunting and increased proneness to infections.

3.4 Collection of cross-sectional data

A green control card was given to each child on the morning of measurements to ensure that the child attended each station and that each measurement was performed (Addendum B).

3.4.1. Demographic data

The demographic questionnaires (Addendum C) were administered by trained field workers. The questionnaire was developed to obtain information regarding the family, their living arrangements and the general circumstances of each of the participants. The data was collected in the childs' preferred language. This information made it possible to gather the most vital demographic data from the subjects, such as age, gender, home language and education level of the parent/caregiver, type of house, accessibility to water and electricity, occupation of bread-winner and general health data. The smoking status of the children was collected via a separate questionnaire (Addendum D) and the questions on the girls' use of contraceptives were not used in the interpretation of the results.

3.4.2. Tanner staging

The Tanner growth stage evaluation serves as a determinant of the physical maturation of the subjects. The determination of Tanner growth stages was done by trained fieldworkers in private rooms (Addendum I & J). Tanner stage estimates the boys' and girls' physical maturity by using 5-stage Tanner scales for breast development in females and pubic hair in males (Tanner, 1962). Questionnaires were administered in an ethical and confidential manner. This system has been established for describing children's biological age in terms of how their bodies are changing and developing sexually (Tanner & Whitehouse, 1982).

3.4.3. Anthropometric measurement

Measurements were taken by post-graduate students in Nutrition and Dietetics with level 1 or 2 qualifications in anthropometrics. Subjects were barefoot and in their underwear during measurement. Boys' and girls' anthropometric measurements were performed separately in a private room (Addendum E). The following measurements were taken:

- ***Height***

Height was measured to the nearest 0.1 cm using a freestanding stadiometer (IP 1465, Invicta length meter, London, UK). Subjects were barefoot and assisted by fieldworkers to keep the head in the Frankfort plane (when the orbital lies horizontal to the tragion). The measurement was taken at the highest point of the skull, the vertex according to the International Society for the Advancement of Kinanthropometry (ISAK, 2001). Two measurements were taken and the mean was recorded.

- ***Weight***

The subjects were weighed in their underwear with a calibrated electronic scale (Precision Health scale, A&D Company, Saitama, Japan). Weight was measured to the nearest 0.01 kg. Two measurements were taken and the mean of the two values was calculated. For the determination of body mass index (BMI), the height and weight measurements were used in a formula (ISAK, 2001) and the subjects were classified according to the age and sex adjusted BMI cut-offs described by Cole *et al.* (2002):

BMI (kg/m^2) = body weight (kg) / body height in m^2

- ***Skinfolds***

Five skinfold thicknesses namely the triceps, sub-scapular, medial calf, supra-spinal and abdominal were taken in duplicate to the nearest 0.1 mm using a John Bull Caliper (British Indicators, London) according to the approved methods of the ISAK (2001). Two measurements were taken and the mean value was calculated and reported.

- ***Circumferences***

Abdominal, waist and hip circumference were measured to the nearest 0.1 cm with a steel tape (Lufkin, Cooper Tools, Apex, NC). The waist-hip-ratio (WHR) was determined by dividing the abdominal circumference (measured around the narrowest part of the abdomen) by the hip circumference (the broadest part of the hip) (Owens *et al.*, 1999):

WHR = abdominal circumference (cm) / hip circumference (cm)

- ***Air displacement plethysmography (ADP)***

In order to calculate the body volume (percentage body fat and lean mass) of the children, an air displacement plethysmography (ADP) and the BODPOD® scale (Life Measurements, Inc, Concord, CA) were used. Measurements were performed according to the manufacturer's instructions and recommendations, with each subject wearing a tight-fitting swimsuit and swim cap. Once the body density was measured the formula of Siri (1993) and Brozek (1963) was used to measure the body fat percentage and mass.

3.4.4. Habitual physical activity questionnaires & fitness test

Habitual physical activity was measured by a validated PDPAR (previous day physical activity recall) questionnaire (Addendum G & H) (Weston *et al.*, 1997). This questionnaire was administered by trained fieldworkers. Field workers recorded events for the previous week and weekend day's physical activities. According to this the subjects were classified as low (1), moderate (2) or highly (3) physically active.

A standard test battery was administered by trained postgraduate students in Human Movement Science to assess muscular strength, flexibility and endurance of the children (Meredith & Welk, 1999). The cardiovascular fitness and endurance of the children were determined through indirect maximal oxygen uptake (VO_2-maximun) through a "bleep-test" (Australian version) (Leger & Lambert, 1982). The results of the "bleep-test" were recalculated to an indirect VO_2max (Meredith & Welk, 1999).

3.4.5. Focus group discussion and questionnaires

At the end of the study the Dietetic students held focus group discussions with the participants to determine how the children experienced the activity programme and reasons for non-compliance.

3.4.6. Blood pressure

Blood pressure was measured after a 5 minute rest before blood samples were taken. A 7-minute continuous measurement of cardiovascular parameters using the Finometer™

device (FMS, Finapres Measurement Systems, Amsterdam, Netherlands) (Imholz *et al.* 1998; Schutte *et al.*, 2004) were taken and analyzed with the Beatscope 1.1 software programme to obtain systolic (SBP) and diastolic blood pressure (DBP), as well as the Windkessel arterial compliance. In its simplest, two-element form, the Windkessel model describes the circulation in terms of parallel resistance and capacitance components. The resistance element corresponds to measured peripheral vascular resistance, while the capacitance element corresponds to the compliance of the arterial circulation (Dart & Kingwell, 2001). The SBP and DBP were used to calculate the MAP using the following formula:

MAP = SBP-DBP x 1/3 + DBP

3.4.7. Biochemical analyses

Subjects were asked to partake in a 12-hour fast the previous day. Four professional nursing sisters took 20 millilitres venous blood from the vena cephalica. A sterile butterfly infusion set (Johnson & Johnson, 21G, 19mm) and syringes were used to collect the venous blood for the preparation of the EDTA plasma and serum. For the preparation of the serum, the tubes were kept aside for approximately 30 minutes in order to coagulate, after this they were centrifuged for 15 minutes at 2000 g at 4°C for the serum and plasma preparation. The serum and plasma was then divided into aliquots-tubes and stored at -84 °C in a Nuaire^{TM} bio-freezer until further analyzed.

Serum was used for the analyses of TNF-α, IL-6, CRP and insulin and whole blood was used to determine the WBCC.

- *Tumor necrosis factor (TNF)-α*

To determine the human TNF-α in serum, the High Sensitivity Human TNF-α / TNFSF1A Immunoassay (ELISA) was used (R&D Systems, Minneapolis, MN). The Quantikine HS TNF-α Immunoassay is a 6.5 hour solid phase ELISA designed to measure TNF- α in serum and plasma (catalogue number HSTA00C). The assay employs the quantitative sandwich immunoassay technique. The sensitivity of TNF-α ranged from 0.06 – 0.32

pg/mL, with a mean minimum detectable dose of 0.12 pg/mL. The intra-assay coefficients of variation (CV) of TNF-α were 8.07 % and the inter-assay was 16.1 %.

- *Interleukin-6 (IL-6) analyses*

The high sensitivity human IL-6 Immunoassay (ELISA) was used for the quantitative determination of the human IL-6 concentration in the serum (R&D Systems, Minneapolis, MN). The assay is a 5.5 hour solid-phase ELISA (Catalogue number HS600B). The assay employs the quantitative sandwich enzyme immunoassay technique. The sensitivity of IL-6 ranged from 0.016 - 0.110 pg/mL, with a mean minimum detectable dose of 0.039 pg/mL. The intra-assay CV of IL-6 were 9.14 % and the inter-assay CV was 20.4 %.

- *C-reactive protein (CRP) analyses*

Serum high-sensitivity C-reactive protein (hs-CRP) was determined by rate turbidimetry with a high sensitivity C-Reactive Protein Kit (CRPH, IMMAGE ®, Immunochemistry Systems, Cat. No. 474630, California, USA) on the Synchron LX System (Beckman Coulter Inc., Fullerton, California, USA). The intra-assay CV's of CRP were 8.6 %.

- *Insulin analyses*

Fasting serum insulin was measured with the αxSYM-method, which is a microparticle Enzyme immunoassay (MEIA) (Abbott, Wiesenbaden, Germany). Insulin sensitivity was measured with the QUICKI-method (Katz, *et al.*, 2000):

Quantitative insulin sensitivity check index (QUICKI) = 1 / [log (fasting venous insulin (μU/ml)) + log (fasting venous glucose (mg/dl))]

Insulin resistance (IR) was measured by HOMA with the following mathematic formula (Matthews *et al.*, 1985):

Homeostasis model assessment (HOMA) = [fasting venous insulin (μU/ml) x fasting venous glucose (mmol/L)] / 22.5

- *White blood cell count (WBCC)*

Whole blood was analyzed within 2 hours of blood sampling with the use of Beckman Coulter $A^c.T^{TM}$ 5 diff Cap Pierce Hematology (Beckman Coulter Company, USA). The machine was calibrated with $A^c.T^{TM}$ 5 diff Cal Calibrator before analysis of the sample, to ensure accurate measurements.

3.5 Statistical analyses

The computer software programme STATISTICA® version 7 (StatSoft, 2004) was used for all statistical analyses. Scatterplots were drawn to identify the possible outliers. Normality was determined using the Shapiro-Wilk's W-test. TNF-α, IL-6, CRP and HOMA were normalized using log transformations. Descriptive statistics (means, standard deviation, medians and 25^{th} and 27^{th} percentiles) were used to present the characteristics and clinical descriptive of the group when divided into gender. Pearson correlation analyses were used to determine whether correlations existed, firstly between HAZ-scores and cardiometabolic variables and secondly between the inflammatory markers, body composition and the cardiometabolic variables. Partial correlations were performed to determine the relationships between the inflammatory markers and different variables, whilst adjusting for smoking and age. T-tests were done to determine differences between groups' samples for parametric data and Mann-Witney-U test for non-parametric data. For the T-test analyses, children were divided according to their body composition. Over-fatness was defined as a percentage body fat of ≥ 25 % in boys and ≥ 30 % in girls (Lohman, 1987) and stunting was defined a height-for-age z-score ≤ -2.0 standard (SD) (Barker, 1992).

Standard linear multiple regression analyses with either TNF-α, IL-6 or CRP as dependent variables were performed within each gender to determine whether there were significant associations with several cardiometabolic and anthropometric variables. Obtained data were also subjected to factor analyses.

The main applications of factor analytic techniques are to reduce the number of variables and to detect structure in the relationships between variables. Therefore, factor analysis is applied as a data reduction or structure detection method (Statsoft, Tulsa, OK). The number of factors to be extracted is determined by using Kaiser's criterion, which chooses only factors explaining more than the average variances of factors (which are always one). Only factors that had variances explained (also called *eigenvalues*) larger than one were extracted. The varimax raw rotation method was chosen.

CHAPTER 4:

Presentation and discussion of research results

4.1. Introduction

The research results from the performed study will be presented and discussed in this chapter. Results were obtained from the scholars in Grade 9 from two secondary schools. Boys and girls of black ethnic groups were included in the study. Blood samples of 115 girls (mean age 15.5 ± 1.33) and 78 boys (mean age 15.9 ± 1.40) were collected for the cross-sectional analyses. The main aim was to identify the association between different inflammatory markers and other cardiometabolic and anthropometric indices in African children.

Values for the serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), fasting serum insulin and HOMA had skewed distributions and, therefore, log transformation was applied. The results will be presented in the following way:

- A brief description of the demographic information of the subjects.
- The descriptive profile of the group is presented in Table 4.1 on the basis of gender. Age, Tanner stage, anthropometric variables, physical activity level, serum insulin and glucose, quantitative insulin sensitivity check index (QUICKI), homeostasis model assessment (HOMA), inflammatory markers and blood pressure are reported.
- Tables 4.2 and 4.4 focuses on the statistical analyses of stunted children, firstly through Pearson's correlations between the height-for-age z-scores (HAZ) and cardiometabolic variables by gender and then the differences between stunted and non-stunted children by means of a Mann-Whitney U-test.
- In table 4.3 unadjusted Pearson's correlations are done among TNF-α, IL-6 and CRP and other variables. Partial correlations were done (while adjusting for smoking and age). The significant data are reported in Tables 4.4 and 4.5.

- The differences between stunted and non-stunted boys and girls are described in Table 4.6 through a Mann-Whitney U-test.
- The differences between over-fat and lean boys and girls are described in Table 4.7 through a Mann-Whitney U-test.
- Tables 4.8 and 4.9 are multiple regression analyses of the boys and girls and a structured matrix of factor analyses of TNF-α, IL-6 and CRP is described in Table 4.10.

A discussion and final conclusion on the most significant findings follows the results section.

4.2 Results

4.2.1. Descriptive profile of the study group

A total of 193 children (78 boys and 115 girls) participated in this cross-sectional study. The study group consisted of school children between the ages of 13 and 20 years, attending either Seiphomelo Secondary School or Boitshoko High School in Ikageng, a township outside Potchefstroom in the North-West Province, South Africa. The two schools were selected for this study by the district nutrition advisor as schools where a relatively high percentage of stunted children would be found. The sample comprised of children from the black ethnic group settled in poor socio-economic conditions. The type of housing was mainly galvanized-zinc or brick houses with a partial water and electricity supply. Subjects in the different schools were in the similar growth phase and socio-economic status and their eating habits and physical activity levels were also similar. Their physical activities mainly comprised of playing with their friends, walking, playing soccer and watching TV. The majority of the children ate porridge with milk or home-made sauce, chicken, cabbage and bread and they drank tea or coffee most frequently. Only a small percentage of the children smoked cigarettes (5.7%), with a median of 6 cigarettes per day (2-6 interquartile range).

The characteristics of the subjects divided on the basis of gender are presented in Table 4.1. When comparing the boys and girls, the following was found:

According to Table 4.1 the boys were significantly taller than the girls ($p<0.001$). The girls' BMI ($p=0.003$), body fat % ($p<0.001$), hip circumference ($p<0.001$), abdominal- ($p<0.001$), triceps- ($p<0.001$) and subscapular ($p<0.001$) skinfold, were significantly higher when compared with the values of the boys. The results indicated that the waist-hip-ratio (WHR) of the boys was significantly higher than that of the girls ($p<0.001$). When looking at the waist and the hip circumferences separately, the girls and the boys' waist circumference fell in the same range, with a greater hip circumference found in the girls. The insulin sensitivity, systolic blood pressure (SBP) and DBP levels were similar between the two genders. When comparing the inflammatory markers, the serum TNF-α, IL-6 and CRP concentrations were similar in the boys and the girls. Due to severely high levels of CRP (>10.0 mg/L) and IL-6 (>14.0 pg/mL) (Baron, 2004: Rallidis, 2006), which are an indication of acute inflammation, five children were excluded from the analyses. WBCC is generally used to determine the state of a person's health and in this subject population one child had a WBCC >11 mm^3, which indicated that this child probably had an infection or was undergoing severe malnutrition and was, therefore, excluded. The results are an interpretation of generally healthy children and these high concentrations could be an indication of acute inflammation or inflammatory-related. The boys' self reported physical activity level (measured by PDPAR) and their cardiovascular fitness (calculated through bleep test and VO_2Max level) were higher than the girls'.

Table 4.1 Characteristics of subjects divided on the basis of gender

Variable	Boys	N	Girls	N	P
Age (y)	15.9 ± 1.40	78	15.5 ± 1.33	115	**0.05**
Tanner stage	3.40 ± 0.72	78	3.43 ± 0.73	112	**0.01**
Weight (kg)	50.3 ± 9.34	78	49.0 ± 6.51	112	0.34
Height (cm)	162 ± 8.69	78	155 ± 6.52	112	**<0.001**
BMI (kg/m^2)	19.0 ± 2.60	77	20.3 ± 3.23	114	**0.003**
Fat (%)	18.3 ± 6.80	55	28.7 ± 6.50	115	**<0.001**
Hip circumference (cm)	79.1 ± 6.13	75	85.1 ± 7.44	115	**<0.001**
Abdominal skinfold (mm)	11.0 ± 7.21	75	18.7 ± 7.14	115	**<0.001**
Waist circumference (cm)	65.7 ± 5.50	75	64.2 ± 5.95	115	0.10
WHR	0.83 ± 0.05	75	0.76 ± 0.05	114	**<0.001**
Triceps skinfold (mm)	8.85 ± 4.30	75	15.8 ± 5.64	115	**<0.001**
Subscapular skinfold (mm)	8.16 ± 4.20	75	12.2 ± 5.28	115	**<0.001**
HAZ	-1.17 ± 0.93	73	-1.02 ± 0.98	107	0.28
Bleep test (number of laps)	6.46 ± 2.00	77	3.48 ± 1.11	111	**<0.001**
VO_2max (mL/kg/min)[1]	34.6 ± 6.82	77	24.5 ± 3.79	111	**<0.001**
Physical activity level[2]	2.15 ± 0.74	75	1.53 ± 0.65	108	**<0.001**
Menarche age (y)	-	-	13.5 ± 1.35	70	0.68
Fasting plasma glucose (mmol/L)	5.27 ± 0.50	78	4.95 ± 0.40	114	**<0.001**
Fasting serum insulin (mmol/L)	6.40 (4.7;10.2)	74	8.45 (6.20;12.5)	114	0.21
QUICKI	0.36 ± 0.03	75	0.35 ± 0.30	113	0.17
HOMA[a]	1.44 (1.04;02.48)	74	1.88 (1.21; 2.83)	113	0.50
TNF-α (pg/mL)	1.10 (0.15; 1.63)	69	1.09 (0.05; 2.12)	104	0.75
IL-6 (pg/mL)	2.61 (1.02; 04.26)	71	1.90 (1.08; 3.55)	111	0.09
CRP (mg/L)	0.36 (0.17; 1.07)	72	0.34 (0.18; 1.04)	112	0.75
WBCC (mm^3)	5.17 ± 1.65	76	5.62 ± 1.74	110	0.12
SBP (mmHg)	99.0 ± 10.9	67	97.2 ± 10.3	101	0.27
DBP (mmHg)	60.6 ± 11.1	67	62.1 ± 10.6	101	0.37

Data are expressed as mean ± SD or [a] median (interquartile range)

BMI = Body mass index; cm = centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; min = Minute; N = number of subjects, QUICKI = Quantitative insulin sensitivity check index; SBP= Systolic blood pressure, SD = Standard deviation; TNF-α = Tumor necrosis factor; HAZ = Height for age Z-score; WHR = Waist-Hip-Ratio, y = Year, WBCC = white blood cell count.

[1]VO_2max calculation based on bleep test; [2]Physical activity level categorised based on PDPAR questionnaire

4.2.2 Correlation analyses

These results determined which variables (HAZ, body composition and fitness levels) showed the strongest correlation with serum TNF-α, IL-6 and/or CRP. Results are shown in Tables 4.2 and 4.3.

The boys' results in Table 4.2 showed significant correlations between WHR (r= -0.26, p=0.03), fat % (r= -0.32, 0.02), CRP (r= 0.28, p=0.02) and the HAZ-scores. In the girls' results HAZ-scores only showed significant correlation with waist circumference (r= 0.21, p=0.03), Tanner stage (r= 0.33, p=0.01), SBP (r=0.28, p=0.01) and DBP (r=0.03, P=0.003).

A Pearson correlation analysis was done between the inflammatory markers (TNF-α, IL-6 and CRP) and different variables in order to determine whether any cardiometabolic risk markers are associated with any of the inflammatory markers (Table 4.3). The boys' results indicated a statistically significant positive correlation between IL-6 and CRP (r= 0.40, p=0.001). CRP in turn correlated negatively with the bleep test (r= -0.25, p <0.05) and VO_2max (r= -0.24, p<0.05), and correlated positively with HAZ-scores (r= 0.28, p<0.05), fibrinogen (r= 0.58, p=0.001), IL-6 (r= 0.40, p=0.01) and WBCC (r= 0.29, p<0.05). No significant correlations were found between any of these variables and TNF-α.

Results showed that serum TNF-α in the girls correlated significantly with WHR (r= 0.36, p=0.001) and IL-6 (r= 0.37, p=0.01), respectively. Serum IL-6 correlated significantly with BMI (r=0.24, p<0.005), waist circumference (r= 0.26, p=0.001), WHR (r= 0.24, p<0.05), fibrinogen (r= 0.20, p<0.05), TNF-α (r= 0.36, p=0.05) and serum CRP (r= 0.19, p<0.05). A positive correlation was found between serum CRP and age (r= 0.21, p<0.05), BMI (r= 0.20, p<0.05), waist circumference (r= 0.23, p<0.05) and fibrinogen (r= 0.42, p=0.001) and a significant negative correlation between CRP and SBP (r= -0.21, p=0.05).

Table 4.2 Pearson correlation coefficients (*r*-values) between height-for-age-z-scores (HAZ) and cardiometabolic variables among girls and boys

	Boys			Girls		
Variable	**HAZ**	**N**	**P**	**HAZ**	**N**	**P**
Age (y)	-0.09	73	0.43	-0.05	107	0.61
BMI (kg/m2)	0.05	73	0.65	-0.02	107	0.80
Weight (kg)	0.45	73	**<0.001**	0.47	107	**<0.001**
Height (cm)	0.75	73	**<0.001**	0.98	107	**<0.001**
Waist circumferences (cm)	0.14	70	0.24	0.21	107	**0.03**
WHR	-0.26	70	**0.03**	-0.19	107	0.06
Fat (%)	-0.32	51	**0.02**	0.01	106	1.00
VO2max (mL/kg/min)[1]	0.06	72	0.61	-0.05	107	0.61
Menarche age (y)	-	-	-	0.06	64	0.65
Tanner stage	-0.09	65	0.50	0.33	105	**0.01**
Fasting plasma glucose (mmol/L)	-0.07	73	0.56	0.09	106	0.34
Fasting serum insulin (mmol/L)	-0.09	70	0.45	0.07	106	0.50
QUICKI	-0.07	70	0.54	-0.1	105	0.30
HOMA	-0.11	70	0.36	0.07	105	0.46
TNF-α (ρg/mL)	-0.12	65	0.34	-0.08	97	0.45
IL-6 (ρg/mL)	0.09	67	0.47	-0.08	103	0.42
CRP (mg/L)	0.28	67	**0.02**	0.01	105	0.92
WBCC (mm^3)	0.03	71	1.00	0.02	103	1.00
SBP (mmHg)	0.08	63	0.55	0.28	95	**0.01**
DBP (mmHg)	0.14		0.28	0.30	95	**0.003**

BMI = Body mass index; cm = centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; MAP= Mean arterial pressure; min = Minute; N = subjects; NS = Non significant; QUIKI = Quantitative index insulin sensitivity check; SD = Standard deviation SBP= Systolic blood pressure; TNF-α = Tumor necrosis factor alpha; WBCC = White blood cell count; HAZ = Height for age Z-score; WHR = Waist-Hip-Ratio; y = Year

[1]VO_2max calculation based on bleep test

Table 4.3 Pearson Correlation Coefficients (*r*-values) between TNF-α, IL-6 and CRP levels and different variables among boys and girls respectively

	Boys						Girls					
Variable	**TNF-α**	**N**	**IL-6**	**N**	**CRP**	**N**	**TNF-α**	**N**	**IL-6**	**N**	**CRP**	**N**
Age (y)	0.11	69	0.15	71	0.048	72	0.18	104	0.12	111	0.21 ❋	112
BMI (kg/m2)	0.16	69	0.28	71	0	72	-0.01	101	0.24 ❋	107	0.20 ❋	108
Weight (kg)	0.09	69	0.1	71	0.1	72	-0.02	103	0.18	110	0.18	111
Height (cm)	-0.01	69	0.15	71	0.19	72	-0.02	102	-0.05	108	0.05	109
HAZ	-0.12	65	0.09	67	0.28 ❋	67	-0.08	97	-0.08	103	0.01	105
Waist circumference(cm)	0.22	67	0.02	68	0	69	0.06	104	0.26 #	111	0.23❋	112
WHR	0.13	67	0	68	-0.16	69	0.37 ¥	103	0.24 ❋	110	0.19	111
Fat (%)	0.1	51	-0.06	52	0.09	53	0.04	99	0.1	105	0.1	106
Bleep test (number of laps)	0.03	68	0.05	70	-0.25 ❋	71	0.16	100	-0.02	107	-0.18	108
VO2max (mL/kg/min)	0.03	68	0.05	70	-0.24 ❋	71	0.13	100	-0.02	107	-0.19	108
Fasting plasma glucose (mmol/L)	-0.06	69	0.02	71	0.03	72	-0.1	103	0.1	110	0.1	111
Fasting plasma insulin (uU/ml)	0.03	68	-0.09	70	0.08	71	-0.13	104	-0.03	111	0	112
QUICKI	-0.04	69	0.08	71	-0.14	72	0.17	103	-0.07	110	-0.07	111
HOMA	0.03	68	-0.09	70	0.07	71	-0.12	103	-0.02	110	0.01	111
Fibrinogen	-0.14	69	0.08	71	0.58 #	72	0.14	102	0.20❋	109	0.42 #	110
TNF-α (pg/mL)	-	-	0.23	68	0.01	69	-	-	0.36 #	102	-0.01	103
IL-6 (pg/mL)	0.23	68	1	71	0.40 #	70	0.36 #	102	-	-	0.19❋	110
CRP (mg/L)	0.01	68	0.40 #	70	-	-	-0.01	103	0.19❋	110	-	-
WBCC (mm^3)	0.11	68	0.23	70	0.29 ❋	71	-0.14	102	0.13	107	0.15	108
SBP (mmHg)	0.07	61	-0.13	62	0.02	63	-0.09	93	-0.08	98	-0.21 ❋	99
DBP (mmHg)	0.18	61	-0.22	62	0.04	63	-0.14	93	-0.16	98	-0.20	99

BMI = Body mass index; Cm = centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; MAP= Mean arterial pressure; min = Minute; N = subjects; NS = Non significant; QUIKI = Quantitative index insulin sensitivity check; SD = Standard deviation SBP= Systolic blood pressure; TNF-α = Tumor necrosis factor alpha; WBCC = White blood cell count; HAZ = Height for age Z-score; WHR = Waist-Hip-Ratio y = Year

❋ P<0.05, ¥ P<0.01; # P<0.001.

After adjustment for smoking and age, significant correlations were only found in the girls between TNF-α, WHR (r=0.37, p=0.05) and IL-6 (r= 0.35, p=0.01), as shown in Tables 4.4 and 4.5. No significant correlations were shown for the boys.

Table 4.4 Partial correlation coefficients between serum TNF-α concentration and different variables among girls (adjusted for smoking and age)

	Girls		
Variable	**TNF-α**	**N**	**P**
WHR	0.37	103	**0.05**
Physical activity level	0.25	98	0.06
IL-6 (pg/mL)	0.35	102	**0.01**

L-6 = Interleukin-6; PA-level = Physical activity level; r = correlation; WHR = Waist-Hip-Ratio

Table 4.5 Partial correlation coefficients between serum IL-6 concentrations and different variables among girls (adjusted for smoking and age)

	Girls		
Variable	**IL-6**	**N**	**P**
BMI (kg/m2)	0.22	107	0.09
Waist circumference(cm)	0.24	111	0.06
WHR	0.24	110	0.06
Abdominal skinfold	0.22	111	0.09
TNF-α (pg/mL)	0.35	102	**0.01**

BMI = Body mass index; r = correlation; TNF-α = Tumor Necrosis Factor-alpha; WHR = Waist-Hip-Ratio

4.2.3 Results of the differences between groups of children with diverse body compositions

Table 4.6 compares the stunted children with the non-stunted children, whereas Table 4.7 compares the over-fat children with the lean children. The stunted boys' waist circumferences, DBP and MAP values were significantly lower than those of the non-stunted boys (p=0.02, p=0.04 and p=0.03 respectively).

The stunted girls had significantly lower weight ($p<0.001$), height ($p=0.01$) and Tanner stage ($p=0.04$) when compared with the non-stunted girls. Of all the inflammatory markers, only serum TNF-α of the stunted girls showed a significantly higher value than in the non-stunted girls ($p=0.03$).

The results in Table 4.7 show that the over-fat boys had significantly higher body BMI ($p=0.03$), height ($p=002$), waist circumferences ($p=0.001$), fat % ($p<0.001$), triceps ($p<0.001$) subscapular- ($p=0.001$) and abdominal skinfold ($p<0.001$), SBP ($p<0.001$) and a lower HAZ-score ($p=0.01$) than the lean children. The over-fat girls' results showed significantly higher values in their BMI, weight, waist circumference, triceps, subscapular and abdominal skinfolds, all with a p-value smaller than 0.001 when compared with the lean girls. The fasting plasma insulin concentration and the HOMA insulin resistance in the over-fat girls were also significantly higher ($p=0.004$, $p=0.01$ respectively), whereas the QUICKI levels in the over-fat girls were significantly lower when compared to the lean girls ($p=0.01$).

Table 4.6 Differences shown as mean ±SD between stunted and non-stunted children among boys and girls according to Mann-Whitney U test

	Boys					Girls				
Variable	**Non-stunted**	**N**	**Stunted**	**N**	**P**	**Non-stunted**	**N**	**Stunted**	**N**	**P**
Age (y)	15.6 ± 1.25	61	16.1 ± 1.01	12	0.17	15.3 ± 1.10	89	15.8 ± 1.38	18	0.16
BMI (kg/m^2)	19.2 ± 2.68	61	18.3 ± 1.93	12	0.43	20.2 ± 2.87	89	20.4 ± 3.26	18	0.57
Weight (kg)	51.7 ± 9.7	61	43.9 ± 5.66	12	**0.01**	49.8 ± 7.88	89	43.3 ± 7.63	18	**<0.001**
Height (cm)	164 ± 8.1	61	155 ± 5.10	12	**<0.001**	157 ± 5.02	89	146 ± 3.94	18	**0.01**
WHR	0.83 ± 0.05	58	0.82 ± 0.06	12	0.80	0.75 ± 0.05	89	0.76 ± 0.04	18	0.48
Waist circumference (cm)	66.4 ± 5.64	58	62.4 ± 4.67	12	**0.02**	64.3 ± 5.44	89	62.0 ± 5.44	18	0.12
Tricep skinfold (cm)	8.93 ± 4.39	58	8.78 ± 4.34	12	0.80	15.8 ± 5.73	89	16.9 ± 6.05	18	0.30
Subscapular (cm)	8.93 ± 4.36	58	7.12 ± 2.02	12	0.22	12.2 ± 5.39	89	12.6 ± 5.60	18	0.74
Fat (%)	18 ± 6.59	40	20.3 ± 7.38	11	0.29	28.7 ± 6.05	84	29.7 ± 7.59	17	0.86
VO2max (mL/kg/min)	35 ± 6.66	60	31.7 ± 7.81	12	0.20	24.2 ± 3.62	85	25.4 ± 4.20	18	0.32
Tanner stage	3.83 ± 073	53	4.1 ± 0.66	12	0.34	3.45 ± 0.66	88	3.05 ± 0.90	17	0.12
Fasting plasma glucose (mmol/L)	5.24 ± 0.44	61	5.48 ± 0.88	12	0.82	4.97 ± 0.40	88	4.93 ± 0.39	18	0.88
Fasting serum insulin (µU/ml)	6.40 (4.8; 10.2)	58	7.50 (4.40; 17.0)	12	0.62	8.50 (6.25; 12.6)	88	7.80 (6.10;11.3)	18	0.51
QUIKI	0.36 ± 0.03	58	0.35 ± 0.05	12	0.49	0.35 ± 0.03	87	0.36 ± 0.04	18	0.34
HOMA	1.45 (1.13; 6.20)	58	1.73 (1.02;4.80)	12	0.54	1.92 (1.29; 2.86)	87	1.62 (1.20; 2.61)	18	0.53
TNF-α (pg/mL)	1.13 (0.94; 4.56)	54	0.87 (0.36; 1.38)	11	0.77	0.69 (0.00; 1.76)	82	1.92 (1.14; 2.69)	15	**0.03**
IL-6 (pg/mL)	3.17 (13.3; 4.56)	56	2.61 (1.23; 3.78)	11	0.67	1.79 (0.88; 3.41)	86	0.46 (0.26; 0.90)	17	0.32
CRP (mg/L)	2..37 (1.13; 6.20)	57	0.31 (0.19; 0.41)	10	0.36	0.31 (0.15; 1.07)	87	0.46 (1.20; 2..61)	18	0.32
WBCC (mm^3)	5.23 ± 1.59	60	5.28 ± 1.94	11	0.92	5.52 ± 1.83	87	5.59 ± 1.18	16	0.88
SBP (mmHg)	100 ± 10.9	52	95 ± 11.7	11	0.08	98.3 ± 11.1	79	92.9 ± 7.80	16	0.07
DBP (mmHg)	62.2 ± 10.9	52	54.1 ± 8.88	11	**0.04**	63.5 ± 8.63	79	60.1 ± 6.17	16	0.12
MAP (mmHg)	74.8 ± 9.42	52	67.7 ± 8.99	11	**0.03**	75.1 ± 0.14	79	71 ± 4.58	16	0.06

Data are expressed as mean ± SD and [a] Median (interquartile range)

BMI = Body mass index; cm = Centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; MAP= Mean arterial pressure; min = Minute; N = subjects; NS= Non significance; P = Significantly different from non-stunted children; QUIKI = Quantitative index insulin sensitivity check; SD = Standard deviation; SBP= Systolic blood pressure; TNF-α = Tumor necrosis factor alpha; WBCC = White blood cell count; HAZ = Height for age Z-score; WHR = Waist-Hip-Ratio; y=year

Table 4.7 Difference shown as mean ± SD between the over-fat and lean children among boys and girls according to Mann-Whitney U test

	Boys					Girls				
Variable	Lean	N	Over-fat	N	P	Lean	N	Over-fat	N	P
Age (y)	16.0 ± 1.40	69	15.4 ± 1.40	9	0.29	15.4± 1.32	80	15.6 ± 1.34	35	0.32
BMI (kg/m2)	18.6 ± 1.97	69	22.5 ± 3.94	9	**0.03**	17.9 ± 2.11	78	21.3 ± 3.10	33	**<0.001**
weight (kg)	49.3 ± 7.48	69	57.7 ± 17.2	9	0.65	43.7 ± 6.23	78	51.3 ± 8.65	34	**<0.001**
Height (cm)	163 ± 8.34	69	158 ± 10.8	9	**0.002**	156 ± 6.26	80	155 ± 6.65	34	0.22
HAZ	-1.14 ± 0.91	64	-1.46 ± 1.07	9	**0.01**	-0.89 ± 0.89	74	-1.07 ± 1.02	33	0.17
Waist circumference (cm)	65.0 ± 4.50	66	71.3 ± 8.71	9	**0.001**	60.2 ± 4.03	80	65.9 ± 5.81	35	**<0.001**
WHR	0.82 ± 0.05	66	0.82 ± 0.04	9	0.50	0.75 ± 0.04	79	0.75 ± 0.05	35	0.24
Fat (%)	16.0 ± 2.57	46	30.0 ± 5.00	9	**<0.001**	20.8 ± 4.70	80	31.5 ± 4.36	29	**<0.001**
Triceps skinfold (cm)	7.81 ± 2.57	66	16.4 ± 6.48	9	**<0.001**	11.6 ± 3.95	80	17.6 ± 5.31	35	**<0.001**
Subscapular skinfold (cm)	7.34 ± 1.67	66	14.1 ± 9.72	9	**0.01**	8.98 ± 2.70	80	13.6 ± 5.47	35	**<0.001**
Abdominal skinfold (cm)	9.26 ± 3.70	66	24.1 ± 12.3	9	**<0.001**	13.2 ± 4.25	80	21.1 ± 6.79	35	**<0.001**
VO2max (mL/kg/min)	35.4 ± 6.42	68	28.4 ± 6.85	9	**0.003**	24.8 ± 3.74	77	24.4 ± 3.83	34	0.50
Tanner stage	3.74 ± 1.50	68	3.56 ± 0.88	9	0.80	3.37 ± 0.77	77	3.45 ± 0.72	35	0.88
Fasting plasma glucose (mmol/L)	5.26 ± 0.54	69	5.30 ± 0.35	9	0.24	4.87 ± 0.35	80	4.98 ± 0.99	34	0.14
Fasting serum insulin (µU/ml)	6.35 (4.65; 10.2)	56	6.60 (4.90; 12.1)	18	0.71	6.40 (4.50; 9.50)	79	8.80 (7.10; 13.5)	35	**0.004**
QUICKI	0.36 ± 0.03	66	0.36 ± 0.04	9	0.81	0.36 ± 0.03	79	0.34 ± 0.03	34	**0.01**
HOMA	1.42 (1.03; 2.41)	56	1.52 (1.13; 2.79)	18	0.68	1.51 (0.94; 2.26)	79	1.93 (1.51; 2.96)	34	**0.01**
TNF-α (pg/mL)	0.78 (0.05; 1.74)	53	0.86 (0.27; 1.41)	16	0.58	1.04 (0.05; 2.10)	72	1.09 (0.09; 2.19)	32	0.89
IL-6 (pg/mL)	2.61 (1.00; 4.56)	53	2.49 (1.16; 4.42)	18	0.91	1.55 (0.47; 3.41)	74	1.99 (1.18; 3.58)	35	0.18
CRP (mg/L)	0.33 (0.15; 0.90)	55	0.59 (0.23; 1.63)	17	0.15	0.30 (0.15; 0.91)	77	0.39 (0.21; 1.23)	35	0.30
SBP (mmHg)	97.3 ± 9.26	59	112.0 ± 13.7	8	**<0.001**	95.4 ± 11.8	72	97.8 ± 10.1	29	0.09
DBP (mmHg)	60.3 ± 10.8	59	62.4 ± 13.6	8	0.80	58.8 ± 13.9	72	63.4 ±8.66	29	0.07

Data are expressed as mean ± SD and [a] Median (interquartile range)

BMI = Body mass index; cm = Centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; MAP= Mean arterial pressure; min = Minute; N = subjects; NS= Non significance; P = Significantly different from non-stunted children; QUIKI = Quantitative index insulin sensitivity check; SD = Standard deviation; SBP= Systolic blood pressure; TNF-α = Tumor necrosis factor alpha; WBCC = White blood cell count; HAZ = Height for age Z-score; WHR = Waist-Hip-Ratio y = Year

4.2.4 Factor analyses of TNF-α, IL-6 and CRP

The structured matrix of the factor analysis shown in Table 4.8 made it possible to investigate the number of various subgroups or factors and to identify what these subgroups represent. The factor analysis extracted 3 groups of variables in both the boys' and girls' groups, each consisting of a cluster of inter-correlated variables. In the boys' group, factor 1 was characterised by their age clustering with their fitness levels and by a negative association with their body fat% and serum leptin concentrations. Factor 2 in the boys indicated a combination of BMI, fat%, leptin, SBP and DBP. This factor forms a pattern of *obesity-related hypertension*. In the boys' group the inflammatory variables and HAZ were grouped together as factor 3, showing that lower values of an inflammatory variable associated with a lower HAZ-score. In the girls group variables grouped as the first factor included the BMI, body fat%, HOMA and leptin. The second factor was the *stunting and inflammation cluster*, with a negative association of HAZ and inflammatory status as well as WHR. The cluster could indicate that stunted children tend to have a higher WHR and that stunting could be associated with an inflammatory state. The HAZ had a positive association with SBP and DBP, and this supports the positive correlation of SBP and DBP with the HAZ-scores in the girls in Table 4.2. In factor 3 only the fitness parameters clustered together.

4.2.5 Multiple regression of TNF-α, IL-6 and CRP

Regression analyses of the association between the inflammatory markers and cardiometabolic or anthropometric indices were done to determine to what extent inflammation is associated with the cardiometabolic and anthropometric variables. The beta coefficient was reviewed in order to evaluate the relative contribution of each predictor to the overall prediction of the dependent variable. Tables 4.9 and 4.10 present the multiple regression analyses within the boys and girls, respectively. In these multiple regression models, either TNF-α, IL-6 and CRP respectively, was the dependent variable and all the cardiometabolic and anthropometric variables were used as independent variables. In the boys' group, significant associations were found between serum TNF-α and IL-6 ($p=0.05$) and IL-6 and CRP ($p<0.001$). Serum IL-6 was significantly associated with MAP ($p=0.01$). Serum CRP was significantly associated with HAZ as an independent variable ($p=0.03$). In the girls group there was a significant association between serum TNF-α as the dependent variable and age ($p=0.04$) and WHR ($p<0.001$) as the independent variables

respectively. Serum TNF-α was significantly associated with IL-6 (p=0.002) and IL-6 was significantly associated with TNF-α (p=0.001). With serum IL-6 as the dependent variable a significant association with BMI as the independent variable (p=0.01) was found. In the group of the girls, serum CRP as a dependent variable did not show any significant association with any of the independent variables. The regression coefficients of MAP in the girls associated with serum CRP as the dependent variable was negative, indicating that the higher the serum CRP level rises, the smaller the MAP will be. All the variables entered into the regression analysis explained in the boys -4.00% of the variation in TNF-α, 21.6% in IL-6 and 23.1% in CRP. In the girls the variables explained 23.2% of the variation in TNF-α, 15.7% in IL-6 and 10.2% in CRP.

Table 4.8 Structured matrix of the factor analysis of TNF-α, IL-6 and CRP in boys and girls

	Boys			Girls		
Variable	**Factor 1**	**Factor 2**	**Factor 3**	**Factor 1**	**Factor 2**	**Factor 3**
Age (y)	0.45					
BMI (kg/m^2)		0.80		0.90		
HAZ			0.47		-0.48	
WHR					0.51	
Fat (%)	-0.64	0.59		0.83		
Bleep	0.92					0.96
VO_2max (mL/kg/min)	0.92					0.96
HOMA				0.47		
Leptin (mg/L)	-0.46	0.74		0.80		
TNF-α (ρg/mL)					0.45	
IL-6 (ρg/mL)			0.72		0.45	
CRP (mg/L)			0.77		0.47	
WBCC (mm^3)			0.5			
SBP (mmHg)		0.73			-0.53	
DBP (mmHg)		0.5			-0.71	
% variance	21.0	15.8	11.8	21.1	15.4	11.0

Centimetre; CRP = C-reactive protein; DBP = Diastolic blood pressure; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; kg = Kilogram; min = Minute; N = subjects; SBP= Systolic blood pressure; TNF-α = Tumor necrosis factor alpha; WBCC = White blood cell count; HAZ = Height for age Z-score; WHR= waist-Hip-Ratio; y=

Table 4.9 Multiple regression of TNF-α, IL-6 or CRP as dependent variable with independent variables in boys. Regression coefficient beta (β) and level of significance, *p* are shown for significant associations only

	TNF-α		IL-6		CRP	
Variable	**R^2 = -0.04**	**N**	**R^2 = 0.216**	**N**	**R^2 = 0.231**	**N**
Age (y)	NS	115	NS	115	NS	78
BMI (kg/m2)	NS	111	NS	111	NS	78
HAZ	NS	107	NS	107	β = 0.09 *p* = 0.03	73
WHR	NS	114	NS	114	NS	75
Fat (%)	NS	109	NS	109	NS	55
VO^2max (mL/kg/min)	NS	111	NS	111	NS	77
HOMA	NS	113	NS	113	NS	74
TNF-α (mg/L)	-	-	β = 0.42 *p* =0.05	104	NS NS	69
IL-6 (mg/L)	β = 0.23 *p* = 0.05	111	-		β = 0.51 *p* 0.002	71
CRP (mg/L)	NS	0	β = 0.44 *p* < 0.001	112	-	-
MAP (mmHg)	NS	101	β = -0.01 *p* = 0.01	101	NS	67

BMI = Body mass index; CRP = C-reactive protein; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; MAP= Mean arterial pressure; N = subjects; NS = indicates not significant (p>0.05). TNF-α = Tumor necrosis factor alpha; HAZ = Height for age Z-score; WHR = Waist- Hip-Ratio; y = Year

Independent variables are considered significant marker when p≤ 0.05.

Table 4.10 Multiple regression of TNF-α, IL-6 or CRP as dependent variable with independent variables in girls. Regression coefficient beta (β) and level of significance, *p* are shown for significant associations only

	TNF-α		IL-6		CRP	
Variable	**R^2 =0.232**	**N**	**R^2 =0.157**	**N**	**R^2 = 0.102**	**N**
Age (y)	β = 0.03 *p* = 0.04	78	NS	115	NS	115
BMI (kg/m2)	NS	78	β = 0.02 *p* = 0.01	111	NS	111
HAZ	NS	73	NS	107	NS	107
WHR	β = 1.53 *p* = 0.001	75	NS	114	NS	114
Fat (%)	NS	55	NS	109	NS	109
VO^2max (mL/kg/min)	NS	77	NS	111	NS	111
HOMA	NS	72	NS	113	NS	113
TNF-α (mg/L)	-	-	β = 0.33 *p* = 0.001	104	NS	104
IL-6 (mg/L)	β = 0.35 *p* = 0.002	71	-	-	NS	111
CRP (mg/L)	NS	72	NS	112	-	-
MAP (mmHg)	NS	67	NS	101	β =-0.01 *p*=0.05	101

BMI = Body mass index; CRP = C-reactive protein; HOMA = Homeostatic model assessment; IL-6 = Interleukin-6; MAP= Mean arterial pressure; N = subjects; NS = indicates not significant ($p<0.05$), TNF-α = Tumor necrosis factor alpha; HAZ = Height for age Z-score; WHR = Waist- Hip-Ratio; y = Year

Independent variables are considered significant marker when $p \leq 0.05$

4.3 Discussion

The most prominent association found in the study indicated that there was a significant association between being stunted and serum TNF-α level in the girls. There were 18 stunted girls out of 115, and according to Table 4.4 the stunted girls' TNF-α value was significantly higher than that of the non-stunted girls ($p=0.03$). TNF-α as a pro-inflammatory cytokine causes specific lean body mass depletion and may also impede growth through both their anorexia inducing effects and direct inhibitory effects on bone growth. (Friedmann *et al.*, 2005). Stunted girls are apparently at risk of having excessive fat stores and seem to be more at risk of accumulating fat on the trunk, which are related to adverse diseases of lifestyle (Benefice *et al.*, 2001; Freedman *et al.*, 1999). The relative fat distribution should be considered and extensively studied due to an increased risk of obesity, metabolic and endocrine disorders, hypertension or cardiovascular diseases in subjects with more subcutaneous or deep fat deposition on the trunk (Benefice *et al.*, 2001). There were no significant differences between fat percentage of stunted and overweight children (Mukuddem-Petersen & Kruger, 2004), nevertheless stunted girls showed a tendency to have more subcutaneous fat than non-stunted girls (Benefice *et al.*, 2001; Mukuddem-Petersen & Kruger, 2004). It has been considered that TNF-α is only secreted in omental (fat found deep in the abdomen behind the muscle) but not in subcutaneous fat (fat beneath the skin surface) (Reinher *et al.*, 2005). In contrast, Ramos *et al.* (2003) indicated that TNF-α messenger RNA's expression increases in subcutaneous fat of obese humans. The results in Table 4.6 support the literature through a significantly higher serum TNF-α concentration in stunted girls who had a similar body fat percentage than the non-stunted girls. Table 4.6 shows that both stunted girls and boys had a significantly lower body weight ($p<0.001$ and $p=0.01$, respectively), but similar fat percentage and skinfold thickness when compared to their non-stunted counterparts. These results indicated relatively higher body fat in proportion to body weight in stunted children. Increased body fat deposits in stunted children may be due to a lower fasting fat oxidation rate, lower habitual physical activity, or both (Kruger *et al.*, 2004). To understand the exact role that TNF-α plays in the linear growth process, more research is needed on the influence of inflammation in a long-term longitudinal study.

The factor analyses in Table 4.10 also serve and support the results of Table 4.6, indicating a negative association between serum TNF-α concentrations and HAZ in factor 2 of the

girls. This factor indicates a clustering of growth-retardation with systemic low-grade inflammation. The WHR also clustered in factor 2, clustering with the inflammatory status and the HAZ, indicating the distribution of fat in stunted children. Circulating TNF-α is extremely low or undetectable in humans, even in obese patients who showed over expression of TNF-α in adipose tissue (Liu *et al.*, 1998). Table 4.2 indicates that the girls' HAZ correlated positively with SBP (r=0.28, p=0.01) and DBP (r=0.30, p=0.003) and in Table 4.4 the stunted boys had lower DBP than the non-stunted boys (p=0.04).

Factor 2 in Table 4.8 also indicates that BP (SBP and DBP) clusters with a positive value with the stunting and negative with inflammation factor. According to Van Rooyen *et al.* (2005), lower diastolic pressure in stunted children as identified in the stunted children of the specific study, could be indicative of early changes in vascular structure of the large conduit vessels. In a follow-up study done by Gaskin *et al.* (2000), it was found that stunted children had a higher SBP than non-stunted children and in the first 2 years of life the stunted children still had increased blood pressure. This is supported by investigation by Van Rooyen *et al.* (2005), who concluded that stunting in early childhood may increase the risk of high SBP and hypertension in later life. However, results in Table 4.6 indicate that both the stunted boys and girls had lower SBP and DBP than the non-stunted boys and girls. The reason for this phenomenon is unclear, but an increase in sample size could clarify the results. Therefore, it is advised that further research should be conducted to investigate the association between BP and stunting.

The income adjusted risk ratio of being overweight for stunted children ranged from 1.7 to 7.8, indicating an important association between stunting and high weight-for-height in a variety of ethnic environmental and social backgrounds (Popkin *et al.*, 1996). The over-fat boys' results in Table 4.7 indicated a significant difference between HAZ when compared to the lean children (-1.14, p=0.01). The over-fat girls also had a trend for a smaller HAZ in comparison to the lean girls (-1.46). This could be a good indication of a risk of stunted children to be over-fat and, therefore, being at the same risk for obesity-related diseases. It is possible that the link between obesity and stunting are biological in origin, but it is still unclear whether the age at which stunting occurs might relate to the development of obesity (Popkin *et al.*, 1996). Table 4.7 indicates that the over-fat boys were shorter than the lean boys (p=0.002) and also had twice the fat percentage than the lean boys (p<0.001). The

over-fat boys' SBP was also found to be significantly higher than the lean boys ($p<0.001$). According to recent studies, serum TNF-α, IL-6 and CRP concentrations are positively associated with measures of obesity (Festa *et al.*, 2001; Jae *et al.*, 2006; Ramos *et al.*, 2003; Weiss *et al.*, 2004). Nevertheless, results in Table 4.7 indicate that there was no significant difference between the over-fat boys' inflammatory status when compared to the lean boys, however, their TNF-α and CRP tended to be higher. When looking at the girls' results in Table 4.7, similar trends were found. The lack of a significant difference between markers of inflammatory status of lean and over-fat children may be due to the small sample size. However, a study on rural African adolescents indicated that over-fatness in the girls increases markedly after menarche and peaks at 17 years, with 11% of girls being over-fat (Cameron & Getz, 1997). Table 4.3 showed that the girls' serum IL-6 and CRP concentration correlated significantly with their BMI measurements ($p<0.005$) and their TNF-α and IL-6 concentrations correlated significantly with their WHR ($p<0.01$ and $p<0.05$, respectively).

Table 4.7 indicates significant differences between the over-fat girls and the lean girls of fasting insulin ($p=0.004$), HOMA ($p<0.01$) and QUICKI ($p<0.01$). Results in earlier studies indicated that inflammation is associated with increased fasting serum insulin concentrations and may be involved in the pathogenesis of insulin resistance (IR) (Kelly *et al.*, 2004). The literature explains that in all races and ethnic groups the severity of glucose tolerance and IR increases directly with the severity of obesity (Liamer *et al.*, 2002; Weiss *et al.*, 2004). Weiss *et al.*, (2004) found that there was a tendency for the prevalence of metabolic syndrome to increase with the severity of obesity, reaching 50% in severely obese children and adolescents. Findings suggest that about one third of the level of IR in obese human adipose tissue is accounted for by secreted TNF-α (Liamer *et al.*, 2002) through its membrane-bound activity (Xu *et al.*, 2002). Fat-derived TNF-α concentrations act on other tissues to impair the function of insulin receptors and hasten the development of IR (Nement *et al.*, 2003). These findings in recent studies could explain the significant increase in HOMA and insulin resistance in the over-fat girls. Both inflammation and IR may mediate or further contribute to endothelial dysfunction, which in turn occurs very early in the pathogenesis of CVD and is an initial marker of atherosclerosis (Kelly *et al.*, 2004). The significant association in Table 4.3 of CRP with the WBCC in boys ($p<0.05$) is consistent with literature of activation of the immune system by inflammation. The

mechanism that links adipose tissue with pathologic process of IR, type 2 diabetes (T2D) and the inflammatory status are poorly understood and further research is needed. In summary, the inflammation score appears to be a helpful tool when evaluating for IR. Strategies that target IR and associated inflammatory activities may be important for CVD risk reduction.

Obesity in children is a clinical problem because it is associated with low levels of physical fitness and increased CVD and metabolic syndrome (MS) (Nemet *et al.*, 2003). Physical activity is important as it is known to reduce cardiovascular morbidity and mortality (Halle *et al.*, 2004). Regular exercise could lead to improve health and lower inflammatory status (Halle *et al.*, 2004). Despite the lack of studies on the association of the inflammatory status and physical activity of children, the results in Table 4.3 indicated a significantly inverse correlation between the fitness levels (both VO_2max and Bleep test) and the CRP values in the boys ($p<0.05$). The boys were also more active than the girls according to the PDPAR, bleep test and VO_2max in Table 4.1. It has been confirmed that higher levels of physical activity in children are associated with an augmentation of muscle mass and decrease in fat (Nemet *et al.*, 2003). Therefore, the inactivity of the girls could be an explanation for their higher fat%, skinfolds and BMI.

Due to the differences between boys and girls at this life stage, it was considered best to interpret the results according to gender. Some important differences between the genders were that the girls had a larger range in fat percentage and skinfold thickness than the boys. The majority of the girls had already reached menarche and their menstrual cycle could have influenced the outcome of the results. Just before menarche, girls accumulate overall fatness and a characteristic regional fat distribution. The menstrual cycle should be taken into consideration when serum concentrations of the inflammatory markers are studied (Benefice *et al.*, 2001). It was not possible to ascertain the stage of the menstrual cycle of each subject accurately and this could have been a limitation of this study. Another limitation of the study could be the small sample size, which consequently limited power and obscured more subtle associations for stunted versus non-stunted and over-fat versus lean children and their inflammatory status. A third limitation could be the absence of endothelial function assessment and inflammatory activity, which may have increased non-differential misclassification, leading to underestimation of present risks factors. There is

also evidence that the smoking of cigarettes may increase serum CRP concentrations (Santos *et al.*, 2005), therefore, possibly acting as a confounder, but the full assessment of all the subjects smoking habits could not have been done accurately during the study. All children did not complete the questionnaire on their smoking habits. From the available results it is clear that only a few children smoked regularly up to a maximum of 15 cigarettes per day.

The cross-sectional study design is not appropriate for drawing causal inferences among the associations. Biochemical indicators are ideal when they reflect long-term or cumulative exposure. Despite the single TNF-α, IL-6 and CRP measurement, which may not accurately reflect long-term inflammatory status, the cross-sectional data only served as a demonstration of the association between inflammation, fitness levels, body fat percentage and HAZ in the different genders. The assessment of the associations between the inflammatory status and cardiometabolic risk markers could provide the knowledge for further research into the field of inflammation, anthropometric indices and related NCD.

Of the initial 193 participants enrolled in the study, 10.4% children were classified as stunted. Randomization was not applied in the study selection, children were chosen as a convenience sample. Chang *et al.* (2002) indicated that an estimated 38% (approximately 226 million children) of children younger than 5 years of age in developing countries are stunted as a result of chronic undernutrition and frequent morbidity. Results in a recent study done on 1915 primary school children (6-12 years) in rural areas of Pakistan indicated that 16.5% children were stunted (Khuwaja *et al.*, 2005). However, the opposite trend was observed in older children (15 to 18 years), whereas 34% in male and 28% in female were classified as stunted (Lai *et al.*, 1998). The timing of stunting is reasonably well understood in that the most stunting occurs before the age of 3 years and stunted children usually become stunted adults (Frongillo, 1999). A recent study showed that the prevalence of stunting fell from 27% at ages 1--5 years to 6% in adulthood (Coly *et al.*, 2006). It could be seen that 10% of adolescents in this study who are at the point of becoming young adults, are showing signs of HAZ close to the <-2 cut-off point (Table 4.1). Some of the consequences of becoming and remaining stunted in adulthood are increased risk of morbidity, delays in motor and mental development and decreased work capacity (Waterlow, 1994b).

Although there is evidence that some markers of inflammation could be directly involved in the formation and progression of atherosclerosis, more definitive proof has to be presented to consider inflammatory markers as prominent indicators of CVD and the MS. Due to the scanty data on children's inflammatory status and the influence in later life, further research is prompted.

4.4 Conclusion

The main aim of the study was to identify whether stunted and over-fat children's inflammatory status associated with any of the cardiometabolic variables or anthropometric indices. Identifying a relevant association could help to identify which of these children may be at risk for CVD or MS later in their lifetime. According to the results of this cross-sectional study, a conclusion is drawn that stunted girls had a statistically significant higher serum TNF-α concentration in comparison to the non-stunted girls, which was positively associated with WHR and the abdominal skinfold, indicating abdominal fat distribution.

There was a significant difference between the HAZ of over-fat and lean children, indicating that stunted children either tends to be over-fat, or that over-fat children tend to be shorter for their age. The inflammatory status in both stunted and over-fat children was similar than the non-stunted and lean children. These results do not support previous studies and literature, namely that obesity is associated with increased levels of inflammation. Reports of the predictive value of minor elevation of serum CRP levels (between 3 and 10 mg/L) for atherosclerotic events have generated considerable interest, as well as a degree of controversy and confusion (Kushner *et al.*, 2006). The findings of significant positive correlation between serum IL-6 concentration and BMI, waist circumference and WHR in the girls support the possible concept that obesity might be the determinant of circulating low-grade systemic inflammatory markers, such as IL-6 (Puder *et al.*, 2006; Trayburn & Wood, 2004).

CHAPTER 5:

Conclusion & recommendations

5.1 Introduction

Childhood obesity is raising rapidly worldwide (Dedoussis *et al.*, 2004), which in turn is associated with significant health problems and it is an early risk factor for much of adult morbidity and mortality (Weiss & Caprio, 2005). Recent accumulating evidence indicates that obesity is strongly associated with sub-clinical chronic inflammation (Wellen & Hotamisligil, 2003). Inflammation in turn plays a pivotal role in all phases of the atherosclerotic disease process (Ballantyne & Nambi, 2005). According to Reinher *et al.* (2005), there are no long-term studies in obese children concerning the relationship between serum CRP and TNF-α, IR, cardiovascular risk factors and changes in weight status. Therefore, more information is needed on the long-term health implications of inflammation and other adipose tissue related factors in over-fat children.

The aim of the study was mainly to identify whether an association exist between low-grade inflammation (TNF-α, IL-6 and CRP), various body composition variables and cardiometabolic indices in children from rural areas in the North-West Province. Dividing the children in sub-groups of non-stunted, stunted, lean and over-fat made it possible to study these associations. Chapter 1 served as a short introduction towards the research question and included the aim, objective, problem statement and hypothesis of the study. In order to achieve the aim and objective of this study, the research question was established, supported by a literature review. Chapter 2 consists of a literature review on inflammation and the various body compositions. The review started with an introduction to the various body compositions, followed by a review on the co-existence of obesity and stunting and the origin of inflammatory markers in obesity. The literature supported the possibility that inflammation and obesity may be positively associated with the development of non-communicable diseases.

A comprehensive explanation of the methods used to collect the cross-sectional data was provided in Chapter 3. The description of the 115 girls and 78 boys between the ages of 13

and 20 years used in the study were presented in this chapter. Body composition, blood pressure, blood analysis, Tanner-stage and cardiovascular fitness levels were measured by trained field workers. A complete discussion of the results obtained in this study was given in Chapter 4. Firstly, the group was divided into gender and the descriptive profile was presented according to their age, Tanner-stage, body composition, inflammatory status, plasma insulin and glucose concentrations, QUICKI and HOMA- index and PDPAR. The results indicated that the stunted girls' serum TNF-α concentration was significantly higher than that of the non-stunted girls. The girls' serum IL-6 and CRP correlated significantly with their BMI measurements and their TNF-α and IL-6 concentrations correlated significantly positive with their WHR. A significant positive correlation was obtained between the boys' serum CRP concentration and the HAZ-score, and a significant negative correlation was obtained between the fitness levels and CRP concentration.

5.2 Conclusion

The conclusion of the study is given in relation to the hypothesis that was set out at the beginning of the study.

Hypothesis 1: The hypothesis that over-fatness in children is associated with low-grade systemic inflammation was tested

This hypothesis is set by the research statement that indicates that human adipose tissues expresses and releases the pro-inflammatory cytokines IL-6, TNF-α and CRP, inducing low-grade systemic inflammation in persons with excess body fat (Rudin & Barzilai, 2005; Visser *et al.*, 2001; Yudkin *et al.*, 2000). The results indicated that significant correlations exist between obesity parameters and inflammatory markers in girls (i.e. between WHR, TNF-α and IL-6, as well as BMI, IL-6 and CRP). No similar correlations were evident in the boys. Despite significant correlations, no significant differences could be found between the inflammatory markers of children with normal percentage body fat and over-fat children.

Therefore in this context, the hypothesis is partially accepted.

Hypothesis 2: It was also hypothesised that stunted children have increased inflammatory status due to their unusual fat distribution.

Limited research has been done to support this hypothesis. However, it was previously stated that stunted children have an increased abdominal fat distribution (Benefice *et al.*, 2001). The results of the present study indicated that stunted girls had a significantly higher TNF-α concentration than their non-stunted counterparts.

Therefore in this context, the hypothesis for the stunted girls and TNF-α value is accepted.

5.3 Recommendations

The results indicate the necessity for further research in the field of systemic low-grade inflammation among African adolescents. The insufficient literature that is available on this research theme, especially literature on stunted children's inflammatory status, encourages further investigation. Increasing the sample size could be valuable to improve the reliability of the research results.

From the existing literature it is clear that studies on the inflammatory status of stunted children is very limited. Recent studies have focused on the inflammatory status of over-fat children (Ford *et al.*, 2001; Garanty-Bogacka, *et al.*, 2005; Halle *et al.*, 2004; Kelly *et al.*, 2004; Klein-Platat *et al.*, 2005; Lambert *et al.*, 2004; Mangge *et al* 2004; Nemet, *et al.*, 2003; Reinehr *et al.*, 2005; Visser *et al.*, 2006), but none of these studies were conducted on an ethnic group in South Africa. In the future, more long term studies could be conducted on the prevalence of low-grade inflammation in African children with different body compositions. The specific effect that exercise and/or a weight loss regimen could have on the inflammatory markers of children could also be investigated in future studies. Therefore, it is necessary to do more intervention studies on black South African children.

The results in this study indicated that TNF-α was higher in stunted girls than their non-stunted counterparts. A number of the inflammatory markers in the girls' results were positively associated with WHR and BMI. More research could be done on the secretion of inflammatory cytokines in stunted children in order to identify the precise role that the distribution of fat may play in these children. Comparing inflammatory markers (TNF-α, IL-6 and CRP) with a range of parameters could be a breakthrough in detecting the possibility of inflammation as indicator for the development of CVD and MS in adulthood. Ample evidence supports the concept that the roots of essential low-grade inflammation extend back to childhood (Visser *et al.*, 2001)

The prevention and management of childhood obesity is important to reduce potential health risks that may arise in early adulthood. Whether the elevated concentrations of CRP among children and adolescents who have the metabolic syndrome predict future adverse health events remains to be determined. Research is needed in order to determine whether the use of inflammatory biomarkers may help to predict which individuals may be at risk for future CVD events, serving as a target of therapy.

CHAPTER 6:

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CHAPTER 7:

Addenda

Addendum A: Consent form

PLAY PROJECT: INFORMATION ON THE STUDY

THE PRPOJECT HAS BEEN APPROVED BY THE ETHICS COMMITTEE OF THE NORTH - WEST UNIVERSITY (Potchefstroom Campus).

I CONFIRM THAT:

It has been explained to me, that:

1. The purpose of the research study is to collect information on growth and activity among Grade 9-10 schoolchildren in Seiphemelo and Boitshoko Secondary Schools, North West Province.
2. The measurements will be done at the beginning (February) and end (October) of the year at the North-West University and children will be transported by bus to the university.
3. I have been told that the researchers will measure me. The participant will be weighed and his/her height as well as circumferences and skinfolds of his/her arm will be measured without causing any pain to the child. For those measurements boys and girls in separate groups will be asked to undress in privacy of a room, because some measurements must be taken with the children dressed in underwear only, or a light shirt and pants/skirt. The researchers will also ask me to indicate my own level of physical maturation from pictures. The different age groups will be measured separately. The researchers and fieldworkers will work in a professional way, not to embarrass the children.
4. Fitness testing will be done.
5. The researchers will ask me about my home environment, the food that I usually eat and activities that I do. None of these questions will be to see if I am clever, or know correct answers. I can just tell them what I usually do.
6. Guidelines for appropriate, culture sensitive, practical and sustainable intervention programmes for children will be developed based on the results.
7. The information I will give shall be kept confidential, only to be used anonymously for making known the findings to other scientists.
8. It was also clearly explained to me that I can refuse to participate in this research study or I can stop answering the questions at any time during the interviews.

The information in this consent form was explained to me by Professor Kruger in English and I confirm that I have a good command in this language and understood the explanations. I was also given the opportunity to ask questions on things I did not understand clearly.

I the participant (child) hereby agree voluntarily to take part in this research survey.

Signed/confirmed at_____on_____2006

Witness_____ Representative of participant (parent/guardian) _____

Addendum B: Green control card

PLAY 2005

Subject name: _____ No:_____ Gender:_____

		CHECK CONTROL
STATION 1	RECRUITMENT	
STATION 2	LIFESTYLE QUESTIONAIRE age.....	
STATION 3	ANTHROPOMETRY : weight:..... height:.....	
STATION 4	ANTHROPOMETRY : Skinfold	
STATION 5	BLOOD PRESSURE : Finometer	
STATION 6	BLOOD PRESSURE : Omron	
STATION 7	TANNER STAGE	
STATION 8	DIETARY QUESTIONNAIRE: 24 HOUR	
STATION 9	PHYSICAL ACTIVITY	
STATION 10	FITNESS TEST	
STATION 0	BACK TO STATION 1	
		SIGNATURE

Addendum C: Demographic questionnaire

DEMOGRAPHIC AND HEALTH SURVEY: PLAY STUDY

IDENTIFICATION			
Number of participant::			
Date of interview:			
Household address:			
Total in household			
Children 1 – 6 years	**Male**	**Female**	
Children 7 – 12 years			
Children 13 – 18 years			
Total adults 18 – 30			
Total adults 31 – 45			
Total adults 46 – 60			
Total adults 61 years +			
Children under 1 year			

LANGUAGE AND ACCULTURATION

Home language of respondent	Tswana1	Sotho2	Xhosa3	Other:4/5/6
Household head speaks:	Home language only 1	Home language + English/Afrikaans 2	English/Afrikaans only 3	

Can YOU read and understand a letter or newspaper in your home language easily, with difficulty, or not at all? Mark the answer.	Easily =1 With difficulty =2 Not at all =3
Can YOU read and understand a letter or newspaper in your English easily, with difficulty, or not at all? Mark the answer.	Easily =1 With difficulty =2 Not at all =3

NEAREST CLINIC			
NAME OF CLINIC		WALK (How many minutes)	Mode of TRANSPORT (Walk1Taxi/2Own car3/Other……)
Potchefstroom clinic	1		
Steve Tshwete	2		
Top City	3		
Boiki Tlapi	4		
Lesego	5		
Promosa	6		
Mohadin	7		

Compiled by Magda Watson (Senior Lecturer: PU for CHE) and recommendations from the Potchefstroom Wellness Forum and Cornelia Wessels.

Addendum D: Habits and medication questionnaire

PLAY STUDY HABITS AND MEDICATION QUESTIONNAIRE

Subject number:

Do you smoke or use any of the following?	YES=1(tick next to what you are smoking and write the amount per day next to it)			NO=2
	TYPE	Tick here (√)	AMOUNT/DAY	
	Cigarettes			
	Tobacco/Pipe			
	Snuff			
	Chewing tobacco			
	Dagga			

If you answered YES in the previous question when (year AND month) did you begin smoking?
How old were you then? ..... ***years***

Have you smoked or used any of the following and then stopped smoking or using the substance?	YES=1(Tick next to what you were smoking and write the amount per day next to it)			NO=2
	TYPE	Tick here (√)	AMOUNT/DAY	
	Cigarettes			
	Tobacco/Pipe			
	Snuf			
	Chewing tobacco			
	Dagga			

If you answered YES in the previous question when (year AND month) did you stop smoking?
How old were you then? ..... ***years***

Do you drink alcohol (beer, wine etc.)?	Yes = 1	No = 2	
If you answered YES for the previous question, state the average amount you drink during 1 week, for example: 3 x 750ml bottles/week	750ml bottles /week	340ml tins/week	200ml glasses /week
Do you drink homemade beer?	Yes = 1	No = 2	
If you answered YES for the previous question, state the average amount you drink during 1 week	500ml carton /week	250ml carton /week	200ml glasses/week

If you drink spirits (brandy, whisky, rum, vodka etc.) how many SHOTS do you drink per week?

If you drink alcohol, when did you start (year AND month) drinking? How old were you then? ..... ***years***	YES = 1	NO = 2

<table>
<tr><td colspan="2">Have you drunk or used any alcohol and stopped drinking?</td><td>YES = 1</td><td>NO = 2</td></tr>
<tr><td colspan="4">If you answered YES to the previous question, when (year AND month) did you stop?
How old were you then? ..... years</td></tr>
<tr><td colspan="2">Do you use any medication chronically (regularly/each day)?</td><td colspan="1">YES = 1 (If YES, specify)</td><td>NO = 2</td></tr>
<tr><td rowspan="6">Do you use any birth control pill?</td><td>YES = 1 (If YES, tick next to the one you take.)</td><td colspan="2" rowspan="6">NO = 2</td></tr>
<tr><td>Ovral</td></tr>
<tr><td>Triphasil</td></tr>
<tr><td>Nordette</td></tr>
<tr><td>Injection (Depo-Provera/ Nur-isterate)</td></tr>
<tr><td>Other (specify)</td></tr>
<tr><td colspan="2">If YES when did you start (year AND month) using the contraceptives (birth control pill?
How old were you then? ..... years</td><td colspan="2"></td></tr>
</table>

Addendum E: Anthropometric data sheet

PLAY-PROJECT

ANTHROPOMETRIC DATA SHEET

Name and Surname: _____ Sexuality:_____

DOB: ____/___/____ Test date: __/__/2006 Age: _____

Subject number: _____

			Measurement 1	Measurement 2	Average
1	Weight	kg			
2	Height	cm			
3	Arm span	cm			
4	Sitting height	cm			
Circumference:					
5	Upper arm - relaxed	cm			
6	Stomach circumference	cm			
7	Calf circumference	cm			
Skin folds:					
8	Triceps	mm			
9	Sub scapular	mm			
10	Calf	mm			
11	Supra spinal	mm			
12	Abdominal	mm			

Addendum F: PLAY data sheet

PLAY-STUDY DATASHEET 2006

PLAY – DATA sheet		Subject no		
Name:				
Age:		Gender	M	F
Birth date:		Grade		
Test date:		Arm span		
		Sitting height		

ANTROPOMETRIC MEASUREMENTS

Stature		Calf circumference	
Body mass		Abdominal circumference	
Sub scapular circumference		BMI	
Triceps circumference		% Body fat	

FITNESSGRAM

Bleep-test (Levels)			Pacer-test (Laps)	
Aerobic capacity				
Curl Up			Trunk lift	
Push Up			Standing long jump	
Sit and reach			Step up test	
Modified sit and reach				
Bent arm hang (girls)			Pull-ups (boys)	
Handgrip strength				

TID TESTS

Basketball throw (m)	1		40 m sprint (sec)	1
	2			2
Vertical jump (cm)	1		Agility (sec)	1
	2			2

Addendum G: PDPAR week

Physical activity questionnaire of the previous week day

Subject nr:

Name:

Race: W | B | C | I

Age:

Gender: M | F

Grade:

School:

Teacher:

Date:

Classification:

Think back about yesterday. For each of the 30 minutes periods, select a primary activity that you performed and write the type of activity in the type of activity column.

Mark the day of the week that you filled this form

Monday | Tuesday | Wed | Thursday | Friday

Time	TIPE	Activity	METS			Very light	Light	Medium	Hard
7:00				•					
7:30				•					
8:00				•					
8:30				•					
9:00				•					
9:30				•					
10:00				•					
10:30				•					
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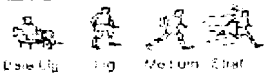

Very Light | Light | Medium | Hard

Addendum H: PDPAR weekend

Physical activity questionnaire of the previous weekend day

Subject no:

Name:

Race: W | B | O | I

Age:

Gender: M

Grade:

School:

Teacher:

Date:

Class first on:

Think back about the weekend. For each of the 30 minutes periods, select a primary activity that you performed and write the type of activity in the type of activity column.

Mark the day of the weekend that you fill in this form

Saturday | Sunday

Time	TIPE	Activity	METS			Very light	Light	Medium	Hard
7:00				•					
7:30				•					
8:00				•					
8:30				•					
9:00				•					
9:30				•					
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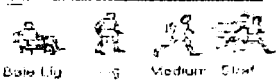


Addendum I: Tanner stage Female

FEMALE SELF-ASSESSMENT OF MATURITY CHARACTERISTICS

Name:	Gender :		F	Age:
Subject number:				

2. When was your last period? (Indicate the number of days, weeks, months).

3. If you have started menstruating, circle the age and the grade when you did

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

4. Do you think you started menstruating at the same time, earlier or later than friends or girls with a similar age than you? Tick √ in the appropriate box.

EARLIER ☐ LATER ☐ SAME TIME ☐

5. If possible, try to recall the exact date when you started menstruating (year, month)

Year:
Month:

6. As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children and you may already be seeing some changes. Doctors use the set of drawings of pubic hair development which is shown to you to determine stage of growth. These changes can be identified in 5 different phases. We want to determine how well you can select your stage of growth from the set of drawings. All you need to do is pick the drawing and description that looks like you do know. Make a tick √ above the drawing that is closest to your stage of development, then put the sheet in the envelope and seal it so your answer will be kept in private.

The following drawings show different amounts of female pubic hair development. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is **closest** to your hair developement.

☐
☐

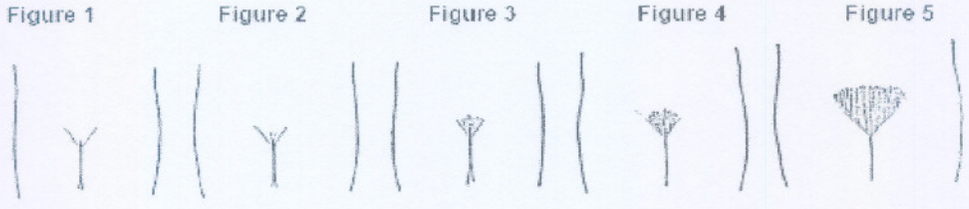
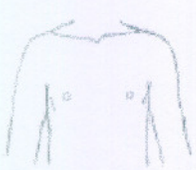
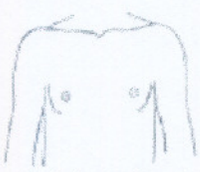
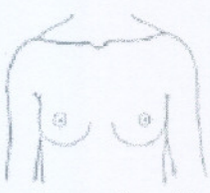
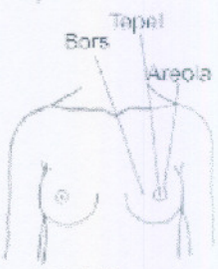


Figure 1	Figure 2	Figure 3	Figure 4	Figure 5
There is no pubic hair at all.	There is small amount of long, lightly coloured hair. This hair mmay be straight or a little curly	There is darker hair, curlier and thinly spread out to cover a somewhat larger area than in stage 2.	The hair is thicker and more spread out, covering a larger area than in stage 3.	The hair now is widely spread covering a large area, like that of an adult female.

7. **In comparison to other girls your age, how would you describe your development with regard to pubic hair development.**

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

8. The following drawings show the amount of breast development. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is **closest** to your breast developement.

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
				
				
The nipple is raised a little in this stage. The rest of the breast is still flat	This is the breast bud stage. In this stage the nipple is raised more than in stage 1. The breast is a small mound. The areola is larger than in stage 1	The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast.	The areola and the nipple make up a mound that sticks above the shape of the breast	This is the mature adult stage. The breasts are fully grown. Only the nipple sticks out in this stage. The areola has moved back to the general shape of breast

9. In comparison to other girls your age, how would you describe your development with regard to breast development.

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

Thank you for your time!

21 June 2001

Addendum J: Tanner stage Male

MALE SELF-ASSESSENT OF MATURITY CHARACTERISTICS

Subject nr:	Name:	Age:

1. Have you already experienced a voice change? Tick √ in the appropriate box

No		Yes
Unbroken	Signs of breaking	Definitely broken/ adult quality

2. If applicable, circle the age/grade in which you experienced signs of breaking of your voice.

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

3. If applicable, circle the age/grade in which you experienced YOUR VOICE DEFINITELY BROKEN.

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

4. Do you think your voice broke at the same time, earlier or later than friends or boys of a similar age than you? Tick √ in the appropriate box?

EARLIER		LATER		SAME TIME	

5. If you have started shaving, in which grade dit it happen?

Grade:
Not Yet

6. Do you think you started shaving at the same time, earlier or later than friends or boys with a similar age than you? Tick √ in the appropriate box?

EARLIER		LATER		SAME TIME	

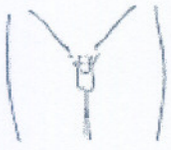
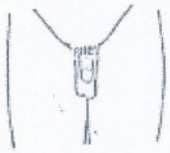
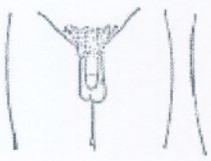
7. The description on this page describes different amounts of male facial hair. Please read each of the descriptions. Then tick √ the appropriate box that describes your stage of facial hair development best.

None	Increase in length, with pigmentation (darkening) at corners of upper lip, spreading medially to complete moustache.	Hair on the upper part of the cheeks and in the midline just below the lower lip.	Hair on the sides and lower border of the chin.

8. As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children and you may already be seeing some changes. Doctors use the set of drawings which is shown to you to determine stages of growth. These changes can be identified in 5 different phases. We want to determine how well you can select your stage of growth from the set of drawings. All you need to do is to pick the drawing and description that looks like you do know. Make a tick √ above the drawing that is closest to your stage of development, then put the sheet in the envelope and seal it so your answer will be kept in private.

The drawings on this page show different amounts of <u>male pubic hair</u>. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is closest to your stage of hair development

☐

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
				
There is no pubic hair at all.	There is small amount of long, lightly coloured hair. This hair may be straight or a little curly.	There is darker hair, curlier and thinly spread out to cover a somewhat larger area than in stage 2.	The hair is thicker and more spread out, covering a larger area than in stage 3	The hair now is widely spread covering a large area, like that of an adult male.

9. In comparison to other boys of your age, how would you describe your development with regard to pubic hair development.

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

10. **The drawings on this page show different stages <u>of growth of the testes, scrotum and penis</u>. A boy goes through each of the 5 stages shown. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is closest to your stage of growth**

☐

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
The testes, scrotum and penis are about the same size and shape as they were when you were a child.	The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum (the sack holding the testes) has gotten lower. The penis has gotten only a little bigger.	The penis has grown in length. The testes and scrotum have grown and dropped lower than in picture 2.	The penis has gotten even bigger. (It is wider. The head of the penis) is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.	The penis, scrotum, and testes are the size and shape of that of an adult man.

11. In comparison to other boys of your age, how would you describe your development with regard to growth of the penis, testes and scrotum.

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

THANK YOU FOR YOUR TIME!