CHAPTER 2 - LITERATURE REVIEW

2.1. INTRODUCTION

The prevalence of cardiovascular disease (CVD) and its risk factors in developed countries has been well documented (Yusuf *et al.* 2001b). However, data are emerging regarding the disconcerting increase in the prevalence of CVD in developing countries. A systematic analysis of population health data found that, when dividing countries into two categories, that is, low- and middle-income countries in one category and high-income countries in the other, ischaemic heart disease (IHD) and cerebrovascular disease (stroke) were the leading causes of death in both of these groups. Together, they were responsible for more than one-fifth of all deaths worldwide (Lopez *et al.* 2006). The large majority of deaths due to IHD (5.7 million of the 7.1 million total deaths) occurred in the lower- and middle-income countries, the number of deaths, expressed as a percentage of the total population, being 0.15% in high-income countries and 0.12% in lower- and middle-income countries (Lopez *et al.* 2006).

Coronary artery disease (CAD) was historically rare in the black South African population (Walker & Sareli, 1997); however, studies are showing an increase in prevalence with urbanisation (Akinboboye *et al.* 2003; Seftel, 1978; Stewart *et al.* 2011). In the early 1990s, approximately 70 black patients with CAD were admitted annually at the Chris Hani Baragwanath Hospital in Soweto (Johannesburg) (Walker, 1991); this increased to around 165 cases in 2006 (Sliwa *et al.* 2008).

Risk factors such as smoking, elevated low-density lipoprotein cholesterol (LDL-C), low high-density lipoprotein cholesterol (HDL-C), high blood pressure (BP), elevated glucose, physical inactivity and obesity, as well as an atherogenic diet, have been proved to be causal risk factors for the development of CVD (Yusuf *et al.* 2001a). A large study which explored the association of acute myocardial infarction (AMI) with these known CVD risk factors was the INTERHEART study. A part of this INTERHEART study was the INTERHEART Africa study, a case-control study in sub-Saharan Africa, among patients with AMI. The results showed that known CVD risk factors account for ≈90% of MI observed in African populations, which was consistent with the global INTERHEART study. There were, however, contrasting gradients found in socio-economic class, risk factor patterns, and AMI risk in the ethnic groups, suggesting that they are at various stages of the epidemiological transition (Steyn *et al.* 2005). Unhealthy lifestyles and the resulting emerging CVD risk factors impart at least the same level of risk for AMI as that found in the overall INTERHEART study (Steyn *et al.* 2005)

In South Africa, during 2006 and 2007, the "Heart of Soweto" research team, based at the Chris Hani Baragwanath Hospital in Johannesburg, screened 1691 volunteers during "Heart Awareness Days" held at various locations in Soweto, such as the taxi rank and shopping malls. The cardiovascular risk profile of the volunteers was examined. The data suggest that, contrary to popular perceptions concerning the cardiovascular health in black Africans, the same risk factors that exist in Western societies are also highly prevalent in Soweto volunteers (Tibazarwa *et al.* 2009). The findings from this study are largely consistent with other similar surveys from other provinces in South Africa (Alberts *et al.* 2005; Oosthuizen *et al.* 2002; Seedat *et al.* 1993; Van Rooyen *et al.* 2000). These data suggest that urban communities in sub-Saharan Africa are at risk of epidemiological transition (Omran, 2001) and at risk of developing more affluent disease states such as coronary heart disease (CHD) (Tibazarwa *et al.* 2009).

According to the World Health Organisation (WHO), as a country develops, the types of diseases affecting the population shift from primarily infectious, such as diarrhoea and pneumonia, to primarily noncommunicable, such CVD and cancers (WHO, 2009). Similarly, the risks that affect the population also shift over time from those for infectious diseases to those that increase noncommunicable diseases. Low-income populations are most affected by risks associated with poverty, such as undernutrition, unsafe sex, unsafe water, poor sanitation and hygiene, and indoor smoke from solid fuels; these are so-called "traditional risks". As life expectancy increases and the major causes of death and disability shift to noncommunicable causes, populations increasingly face modern risks associated with physical inactivity, overweight, obesity and other diet-related factors, and tobacco and alcohol-related risks. As a result, many low- and middle-income countries such as South Africa are now facing a growing burden from the modern risks of life, while still fighting an unfinished battle with the traditional risks to health (WHO, 2009).

Figure 2.1 illustrates the causal chain for IHD according to the WHO. Some elements in the chain, such as high BP or cholesterol, act as a relatively direct cause of the disease, while other risk factors further back in the causal chain act indirectly through intermediary factors (WHO, 2009). In this literature review the risk factors illustrated here, as well as some additional risk factors, will be discussed with particular reference to, or emphasis on, their specific role in the South African black population.

Fat intake and alcohol are the only two dietary components included in the WHO's depiction of the causal chain of IHD. In this literature study, the focus will be on the role of diet in this chain, in a broader context. Diet has been shown to have a dual role in the atherosclerosis process. There are nutrients that play a role in the development of this process, and on the

other hand, nutrients that play a vital role in the prevention of or protection against atherosclerosis. This simplified figure identifies fat intake as a component in this causal chain. However, it is known that the type of fat is also of importance, as different types of fat play different roles in the atherosclerosis process. One should, therefore, be looking at the dual role that nutrients play, focusing not merely on the negative role of diet but rather on the quality of dietary intake. These concepts will be explored in further detail towards the end of this chapter.

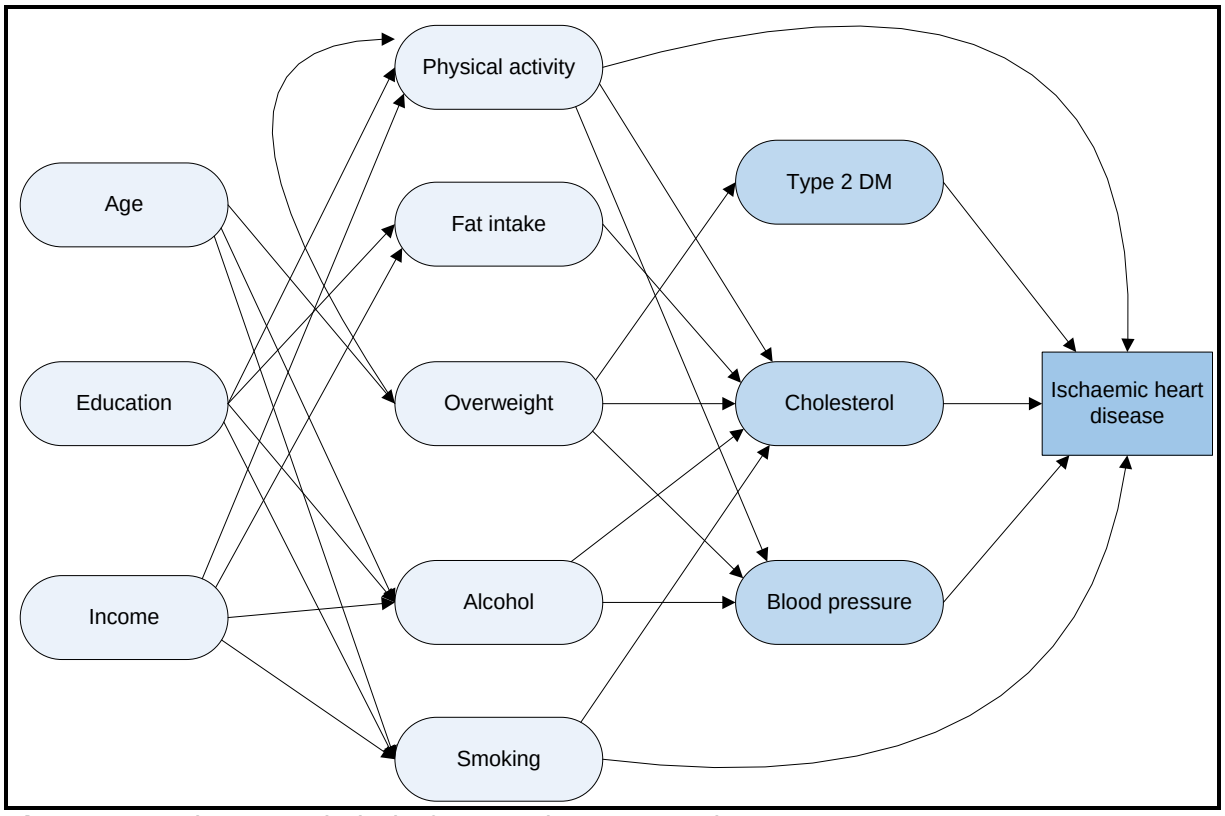


Figure 2.1: The causal chain for IHD (WHO, 2009)

This literature study, therefore, focuses firstly on the various risk factors associated with CVD in the black South African population. This will be followed by a discussion pertaining to assessment of the risk of developing CVD, through the use of risk scores. The role that diet plays in CVD, both causative and protective, will be elaborated on. This will include issues surrounding the use of nutrients against the use of foods, as well as assessing diet quality in a population.

2.2. RISK FACTORS FOR DEVELOPMENT OF CARDIOVASCULAR DISEASE IN THE SOUTH AFRICAN POPULATION

2.2.1. INTRODUCTION

In this section of the literature review, risk factors for CVD will be discussed in the context of ethnicity. Attention will be given to whether the same risk factors that apply to Caucasians also apply to Africans, and if so, whether the same cut-off values should be used for Africans.

2.2.2. HYPERTENSION

The recommendations of the American Joint National Committee (JNC) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure has defined "hypertension" as a systolic blood pressure (SBP) of ≥140mmHg or diastolic blood pressure (DBP) of ≥90mmHg, and "prehypertension" as an SBP of 120 to 139mmHg or a DBP of 80 to 89mmHg (Chobanian *et al.* 2003). It has been estimated that, globally, two-thirds of stroke cases and almost half of all IHD cases are attributable to raised BP (SBP ≥115mmHg) (Ezzati *et al.* 2004). In medium- and low-income countries, high BP caused 17.2% and 7.8% of total deaths respectively (WHO, 2009). Globally, elevated BP was estimated to cause 12.8% of total deaths and 4.4% of total disability-adjusted life years (DALYs) (Ezzati *et al.* 2004), while in South Africa, it was estimated that in the year 2000, high BP accounted for about 9% of all deaths and contributed to 2.4% of total DALYs (Norman *et al.* 2007b).

In South Africa in 2000, high BP was the second leading risk factor (9%), following sexually transmitted diseases resulting from unsafe sex, which accounted for 26.3% of all deaths. If the sexually transmitted diseases such as human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), which strike people while they are much younger, were reduced, it is likely that the proportion of South African deaths related to high BP (9%) would be closer to worldwide estimates (12.85%) (Norman *et al.* 2007b). Between 1995 and 2005, according to the Chronic Diseases of Lifestyle technical report by the South African Medical Research Council (MRC), hypertension was found to be the most common of the CVD risk factors among all ethnicities, but stood out as the risk factor with the highest prevalence in the black South African community (Steyn, 2006). After age and gender standardisation, the overall hypertension prevalence rate was 55%, with 59% of black South Africans and 50% of Caucasians diagnosed with the condition, according to the WHO criteria (BP≥140/90mmHg or having a history of hypertension) (Steyn, 2006). In Soweto, South Africa, screening of

black volunteers (n=1691) during “Heart Awareness Days” revealed that 33% of the participants had high blood pressures (Tibazarwa *et al.* 2009). In a review, Seedat (1999) concluded that, compared with Caucasians, black patients with hypertension in sub-Saharan Africa are more prone to cerebral haemorrhage and malignant hypertension leading to uraemia and congestive heart failure, whereas CAD is relatively uncommon. According to the Chronic Diseases of Lifestyle report, haemorrhagic stroke occurred in about 30% of black stroke patients in hospital-based stroke studies. Owing to the fact that stroke is a heterogeneous condition, not enough is yet known to develop locally relevant interventions (Conner & Bryer, 2006).

In general, there is a strong but complex association between BP and age. Systolic BP and diastolic BP rise in tandem until about 50 years of age. Systolic BP continues to rise steadily after the age of 50 years, whereas DBP tends to fall. The prevalence of systolic hypertension is thus directly proportional to the age of the population (Chobanian *et al.* 2003; Rosendorff *et al.* 2007). The prevalence, impact and control of hypertension differ across racial and sub-ethnic groups of the US population. In African Americans, hypertension is more common, more severe and develops at an earlier age (Cooper & Rotimi, 1997). The pathogenesis of hypertension in different ethnic groups may differ with respect to the contributions of such factors as salt and potassium intake, stress, cardiovascular reactivity, body weight, nephron number, sodium handling or hormonal systems, but in all subgroups the pathogenesis is multifactorial. Differences in socio-economic conditions, access to healthcare services, attitudes and beliefs regarding health care, and deficits in accurate health-related information contribute to much of the variance in hypertension-related diseases across racial or ethnic groups (Cooper & Rotimi, 1997; Douglas *et al.* 2003). In South Africa, several studies have indeed revealed that there are differences in BP between urban and rural subjects in various ethnic groups (Addo *et al.* 2007; Seedat *et al.* 1982; Sever *et al.* 1980). Table 2.1 gives a brief summary of hypertension in the sub-Saharan black population.

Table 2.1 Hypertension in Sub-Saharan Africans (Opie & Seedat, 2005; Steyn, 2006)

Incidence	Lower in rural blacks
	Increasing with urbanisation
	Becoming similar to African Americans
	Higher than Caucasians
Multiple causative factors	Lower plasma rennin
	Sodium cellular abnormalities
	Epithelial sodium channel changes
	Altered genes regulating the RAAS
	Increased peripheral resistance
	Increasing obesity
	Socio-economic stress
	Underweight phenotype
Trends in therapy	Low-dose diuretics
	Calcium channel blockers
	Less response to ACE inhibitors, β-blockers and clonidone as first line agents
	Compelling indications need specific drugs, e.g., ACE inhibitors for diabetic nephropathy and renal disease

RAAS: Renin-angiotensin-aldosterone system; ACE: angiotensin-converting enzyme

Another possible reason for this variance may be that black individuals are more salt-sensitive than Caucasians, which is due to a tendency to retain sodium in the kidney (Lindhorst *et al.* 2007). Genetic factors, personal characteristics, autonomic nervous system function, cardiac function and various environmental factors have all been examined in hypertensive black subjects in comparison with hypertensive Caucasian subjects (Seedat, 2000). Table 2.2 summarises the biochemical and hormonal differences seen between black and Caucasian individuals. There is no complete explanation for these differences and further research is required (Lindhorst *et al.* 2007).

Table 2.2 Differences between blacks and Caucasians in biochemical parameters and hormones related to hypertension (Seedat, 2000)

FEATURE	IN BLACKS
Total cholesterol	Lower
Triglycerides	Lower
High-density lipoproteins	Higher
Low-density lipoproteins	Lower
Very low density lipoproteins	Lower
Response to Na^+ load	Delayed
Urine Na^+/K^+ ratio	Higher
Plasma Na^+/K^+ ratio	Higher
Transport across cell membrane	High intracellular Na^+
Quinidine Na^+ pump activity	Reduced
Plasma rennin activity	Lower
Plasma noradrenaline	Equal
Dopamine β-hydroylase	Lower
Aldosterone	Higher
Kallikrein	Lower
Circulating inhibition of Na/ATPase	Higher

Na^+: Sodium; K^+: Potassium; ATP: Adenosine triphosphate

The question pertaining to which single measurement of BP can best be used to predict risk is an on-going one. According to a meta-analysis of 61 prospective studies, if just one single measurement of BP is to be used to predict risk, irrespective of age, the measured SBP is slightly more informative than the measured DBP, their average (i.e. the mid-blood pressure) is slightly more informative than either alone, and their difference (i.e. pulse pressure (PP)) is much less informative (Lewington *et al.* 2002). The PP is actually inversely correlated with risk (because the DBP is inversely correlated with risk) among people of a given age whose measured SBP is the same. Using mid-BP ensures that any random measurement errors that affect either SBP only or DBP only are halved in calculating the average (Lewington *et al.* 2002).

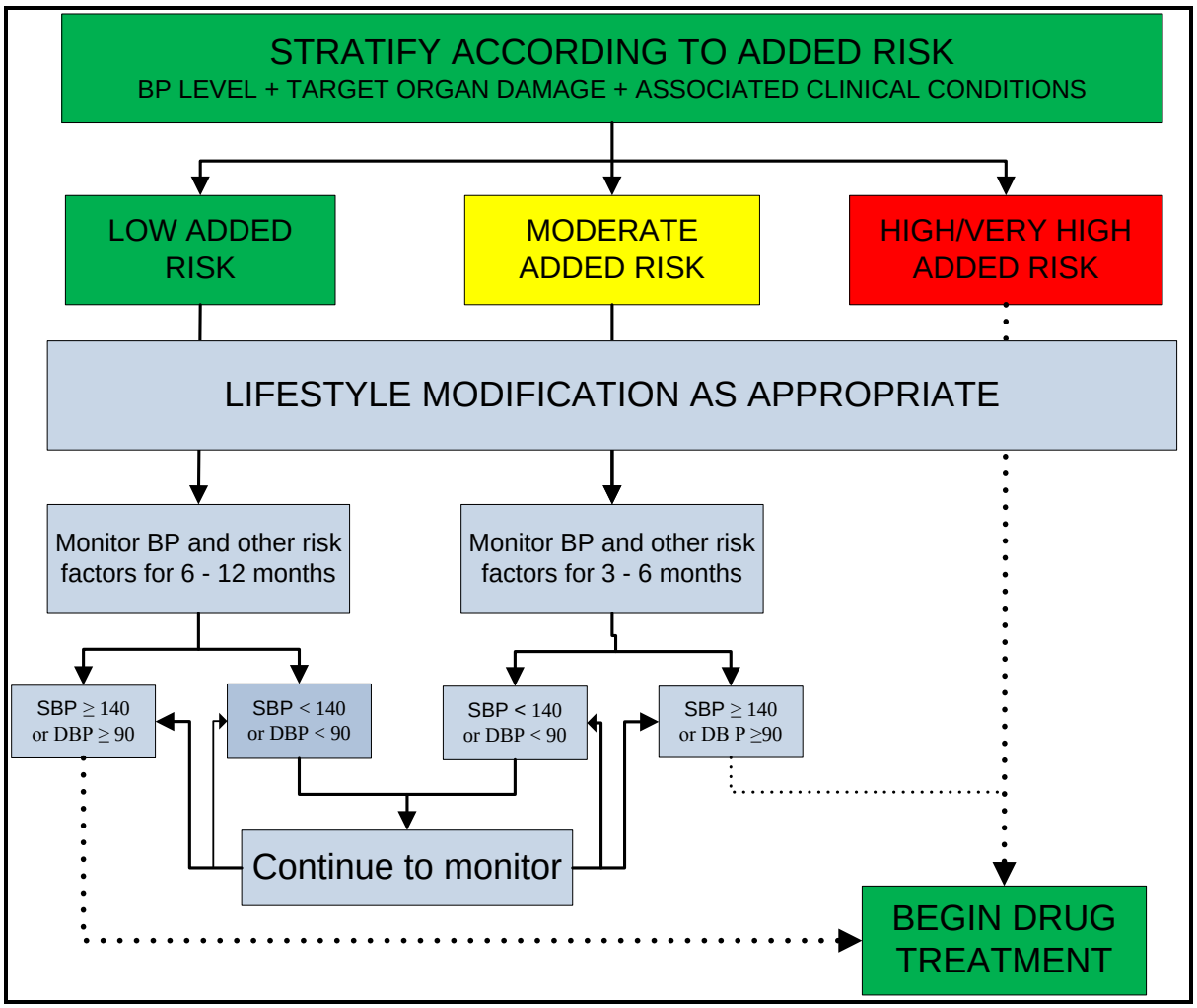
The meta-analysis by Lewington *et al*. (2002) also confirms that there is a continuous relationship with risk of CVD throughout the normal range of BP (down at least as far as 115/75mmHg), but they also demonstrate that within this range the usual BP is even more strongly related to vascular mortality than has previously been supposed. Lowering BP can produce rapid reductions in vascular risk; for example, a 10mmHg lower usual SBP or

5mmHg lower usual DBP would, in the long term, be associated with a 40% lower risk of stroke death and a 30% lower risk of death from IHD or other vascular causes in middle age (Lewington *et al.* 2002).

Because of the high prevalence of hypertension and the fact that lowering BP decreases risk of death from cardiovascular-related diseases, the Southern African Hypertensive Society (SAHS) and the National Department of Health have developed guidelines for the management and, therefore, the lowering of BP in South Africa. The most recent South African hypertension management guidelines were published in 2011 (Seedat *et al.* 2011). Figure 2.2 shows the hypertension management flow diagram based on added cardiovascular risk, while Table 2.3 shows the stratification of risk to quantify prognosis (Seedat *et al.* 2011). The current consensus for target BP is less than 140/90mmHg in general and 130/80mmHg in individuals with diabetes mellitus (DM) or chronic kidney disease (Chobanian *et al.* 2003). For primary prevention of CAD in hypertension, aggressive lowering of BP is appropriate, with a target BP of <130/80mmHg in individuals with any of the following: DM, chronic renal disease, CAD and CAD risk equivalents (carotid artery disease, peripheral arterial disease, abdominal aortic aneurism) and for high-risk patients, defined as having a Framingham risk score of ≥10%; and a target BP of 140/90 mmHg in individuals with none of the above. This is rated as Class IIa, level of evidence B, by the American Heart Association (AHA), which means that the weight of evidence is in favour of efficacy and the level of evidence was derived from a single randomised study or from non-randomised studies (Rosendorff *et al.* 2007).

There are different approaches for defining hypertension and for recommended treatment of it. The British and New Zealand Hypertension Societies use an approach which is based on absolute risk. In this approach the absolute CVD risk is estimated on the basis of the number and severity of all major risk factors. Treatment decisions are then based on the choice of level of risk above which it is reasonable to attempt to lower BP with medications (Gaziano *et al.* 2005). The South African hypertension guidelines, which are based on American JNC VI and VII guidelines, use the BP-level approach, which uses different cut-off points in BP level to define hypertension and recommend treatment. Gaziano *et al*. (2005) formulated a population-based simulation model to assess the cost-effectiveness of the current South African guidelines. They concluded that hypertension guidelines based on absolute risk for CVD are both more effective at saving lives and less costly than those based on BP level. The author suggests that South Africa should rather use a chart that is based on the Framingham risk equation. If the cholesterol levels are not known, then one can assume the average level for the population. According to Gaziano (2006), this would

still be an improvement on Table 2.3, which counts absence of cholesterol information as a "zero" for major risk factors.



Adapted WHO cardiovascular disease-risk management package for low-medium settings

Figure 2.2 Southern African hypertension management flow diagram based on added cardiovascular risk (Seedat *et al.* 2011)

Table 2.3 Stratification of risk to quantify prognosis* (Seedat *et al.* 2011)

	BP (mmHg)				
Other risk factors and disease history	**Normal** SBP 120-129 or DBP 80 - 84	**High-normal** SBP 130-139 or DBP 85 – 89	**Stage 1** **Mild hypertension** SBP 140-159 or DBP 90 – 99	**Stage 2** **Moderate hypertension** SBP 160-179 or DBP 100 - 109	**Stage 3** **Severe hypertension** SBP > 180 or DBP > 110
No other major risk factors	Average risk	Average risk	Low added risk	Moderate added risk	High added risk
1-2 major risk factors	Low added risk	Low added risk	Moderate added risk	Moderate added risk	Very high added risk
≥3 major risk factors or target-organ damage or diabetes mellitus	Moderate added risk	High added risk	High added risk	High added risk	Very high added risk
Associated clinical conditions	High added risk	Very high added risk	Very high added risk	Very high added risk	Very high added risk

*Based on the European Society of Hypertension/European Society of Cardiology guidelines

2.2.3. DIABETES MELLITUS

The global number of individuals with DM in 2000 was estimated to be 171 million (2.8% of the world's population), a figure projected to increase in 2030 to 366 million (6.5%), 298 million of whom will be living in developing countries (Wild *et al.* 2004). The estimated prevalence in Africa is 1% in rural areas and up to 5% to 7% in urban sub-Saharan Africa and between 8% and 13% in more developed areas such as South Africa and in populations of Indian origin (Sobngwi *et al.* 2001). By 2025, the prevalence of DM in sub-Saharan Africa is expected to be more than double the current figures (Wild *et al.* 2004). In South Africa, it is estimated that in 2000, 5.5% of adults aged 30 years and older had DM, which increased with age. DM was estimated to have caused 4.3% of all deaths in South Africa in 2000 (Bradshaw *et al.* 2007). Overall, about 14% of IHD, 10% of stroke, 12% of hypertensive disease and 12% of the renal disease burden in South Africa were attributable to DM (Bradshaw *et al.* 2007). There are, however, no national prevalence statistics for diabetes available in South Africa. The South African Demographic and Health Survey (SADHS) from 1998 provides information on self-reported prevalence of diabetes in males and females 15 years and older. The Asian Indian group had the highest self-reported prevalence, followed by coloured, Caucasian and black African groups, indicating differences in prevalence of DM between ethnic groups (Mollentze & Levitt, 2006). The criteria for the diagnosis of DM, according to the American Diabetes Association, are listed in Table 2.4.

Table 2.4 Criteria for the diagnosis of diabetes mellitus (ADA, 2011)

- HbA1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardised to the DCCT assay.*
  OR
- Fasting plasma glucose ≥7.0mmol/L. Fasting is defined as no caloric intake for at least 8 hours.*
  OR
- 2-hour plasma glucose ≥11.1mmol/L during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.*
  OR
- In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose concentration of ≥11.1mmol/L.

*****In the absence of unequivocal hyperglycaemia, criteria 1 – 3 should be confirmed by repeat testing. HbA1c: Glycated haemoglobin; NGSP: National Glycohemoglobin Standardization Program; DCCT: Diabetes Control and Complications Trial; OGTT: Oral glucose tolerance test; WHO: World Health Organisation

According to Kengne *et al.* (2005), there are various causes for the increased prevalence of DM in sub-Saharan Africa, such as adoption of western lifestyle, possible genetic changes making them more prone to development of DM, absence of or low physical activity at leisure time, and obesity. According to Van der Merwe and Pepper (2006), the pathogenesis of type 2 DM (T2DM) in black South Africans is related to two factors, namely insulinopenia and insulin resistance. With regard to insulinopenia, it has been suggested that this is because of a reduced beta-cell mass, while insulin resistance is an important consequence of obesity, which is highly prevalent in black South African women (Section 2.2.5). Black South Africans possibly either inherit or acquire (as a result of childhood malnutrition and other environmental factors) a decreased pancreatic beta-cell mass, the functional ability of which is exhausted rapidly when glucose intolerance supervenes (Joffe *et al.* 1992). There is then also a down-regulation of the insulin receptor, which results in a reduced lipolytic effect of the decreased insulin concentration on the adipocytes. This may partially explain the elevated free fatty acids (FFA) also found in the black population. These metabolic effects predispose obese black patients to the development of T2DM (Van der Merwe & Pepper, 2006).

Diabetic patients are at greater risk of CVDs, compared with non-diabetic patients, and always have a poor prognosis after cardiovascular events (James, 2001). Type 2 DM is associated with a cluster of lipid abnormalities: elevated plasma triglycerides (TGs), reduced HDL-C, and smaller and denser LDLs which are all associated with an increased risk of CVD (Krauss & Siri, 2004). It is important to remember that the incidence of hypertension in diabetics is also much higher than in non-diabetics (two-fold higher than in age-matched subjects without the disease. Up to 75% of cases of CVD in patients with diabetes can be attributed to hypertension (Sowers, 2003). Diabetes in black Africans is characterised by a high rate of acute and long-term complications (Sobngwi *et al.* 2001). Cardiovascular complications of DM, particularly the macrovascular varieties, are the result of chronic hyperglycaemia in association with classic and acknowledged cardiovascular risk factors (Kengne *et al.* 2005).

Kalk and Joffe (2007) used a hospital-based study (Johannesburg Hospital) to ascertain the prevalence of CHD in black and Caucasian patients with DM, and to evaluate the contribution of classical risk factors to the development of CHD. They found that of the 744 diabetic patients (448 blacks and 296 Caucasians), the prevalence of CHD in the blacks was much lower (4%) than in the Caucasians (23%). Several of the traditional risk factors were significantly lower than their in white counterparts, namely age, total and LDL-C and TG concentrations, while HDL levels were similar. Despite the low frequency of CHD among the diabetic black patients, 25% were at a high risk for CHD in the next 10 years, as estimated from the prevalence of conventional risk factors. However, the black diabetic patients were

diagnosed with DM at a younger age, and were younger than the Caucasian diabetic patients at the time of the study (Kalk & Joffe, 2007). This is an indication that black patients tend to develop T2DM at a younger age, and that they could therefore possibly still develop CHD when they reach the same age as the Caucasian patients.

Deaths from CVD, including those attributable to higher-than-optimum blood glucose levels, do generally occur at younger ages in low- and middle-income countries than in higher-income countries, resulting in a larger loss of healthy life years (Danaei *et al.* 2006). A study done by Danaei *et al.* (2006) to determine global and regional mortality from IHD and stroke attributable to higher-than-optimum blood glucose concentration, found that one in five deaths from IHD and one in eight from stroke was attributable to higher-than-optimum glucose levels. More than three-quarters of cardiovascular deaths attributable to high blood glucose occurred in low- and middle-income countries, where detection and effective management of diabetes is likely to be constrained by resource limitations (Danaei *et al.* 2006).

The increasing prevalence of T2DM worldwide is of concern, but in sub-Saharan Africa, it presents an added challenge, where diabetes must compete with communicable diseases as well as other non communicable diseases for resources. A scarcity of financial resources and appropriate staff means that people living with T2DM have complications such as retinopathy, and those with type 1 DM have an extremely short life expectancy, whether or not they have been diagnosed with the disorder (Beran & Yudkin, 2006). Investigations into the management of hypertension and diabetes in public sector healthcare centres in the Cape Peninsula and KwaZulu-Natal found primary care for these conditions to be sub-optimal (Rotchford & Rotchford, 2002; Steyn *et al.* 2008). Also of importance in the South African setting is the fact that HIV-positive individuals are at an increased risk of insulin resistance. This is due to the pro-inflammatory process of HIV, the direct effects of anti-retroviral therapy (ART) and also indirect effects as consequences of ART (e.g. changes in body fat distribution) (Aboud *et al.* 2007). The morbidity and mortality from HIV has been greatly decreased by treatment with ARTs; however, there are some unintended consequences of ART, which may result in a rise in metabolic syndrome, DM and heart disease that will put a greater burden on resources (Young *et al.* 2009).

2.2.4. DYSLIPIDAEMIA

In 2004, hypercholesterolaemia contributed to 4.5% of total deaths globally (sixth leading cause of death), while in low- and middle-income countries it was the tenth and seventh leading cause of death respectively (WHO, 2009). Worldwide, 56% of the IHD mortality and disease burden was attributable to cholesterol levels of more than 3.8mmol/L, which translated to 3.6 million deaths in 2000 (Ezzati *et al.* 2004). In South Africa, 59% of IHD was attributable to raised cholesterol (Ezzati *et al.* 2004). There are, unfortunately, no nationally representative data on total cholesterol (TC) levels in South Africa, but there are data from several community studies. From these studies it can be seen that estimated mean TC was highest in the Caucasian group, followed closely by the coloured and Indian groups. The black African population had the lowest mean level for males and females at all ages, with levels in urban areas only slightly higher than rural levels (Norman *et al.* 2007a). In Soweto, South Africa, screening of black volunteers (n=1691) during "Heart Awareness Days" revealed that 14% of the participants had elevated (non-fasting) blood cholesterol levels (Tibazarwa *et al.* 2009). Studies in the nineties on black South African populations showed a low prevalence of dyslipidaemia (Mollentze *et al.* 1995; Oelofse *et al.* 1996; Steyn *et al.* 1997). The Transition in Health during Urbanisation of South Africans (THUSA) study in the North West Province, conducted in 1996-1998, showed, however, that serum lipid levels in black South Africans increased with urbanisation in both men and women. The main factor associated with the increase in lipid levels seemed to be obesity, probably due to decreased physical activity. The lipid levels for all strata were, however, still within the normal ranges. The progression to IHD in this population may be slower because of low LDL-C and high HDL-C levels, but with continued urbanisation may become an important health problem in the future (Oosthuizen *et al.* 2002).

Despite the fact that black South Africans have been known to have lower lipid levels, with continued urbanisation, the diabesity pandemic (a dramatic increase in obesity and diabetes (Astrup & Finer, 2000)) is expected to affect sub-Saharan Africa even more than it will First World countries (Motala, 2002; Zimmet *et al.* 2001). Associated with this rise in diabesity, there is an increase in insulin resistance syndrome in patients who have not developed dysglycaemia but who are classified as prediabetic (Kramer *et al.* 2003). This insulin resistance brings along with it a specific and characteristic form of dyslipidaemia that is partially qualitative rather than quantitative in nature (Reaven, 2001). The pattern of dyslipidaemia associated with insulin resistance is characterised by elevated TG and decreased HDL-C. This is also often additionally characterised by postprandial lipaemia, extremely atherogenic, small, dense LDL particles and increased levels of apolipoprotein B (ApoB), and is associated with endothelial dysfunction, including hypercoagulability, resulting

from, among other things, increased plasminogen activator-inhibitor 1 (PAI-1) and fibrinogen (Maritz, 2006). This tendency is now being seen in black South African volunteers, where urbanisation has been associated with increased TG levels (Bourne *et al.* 2002; Oelofse *et al.* 1996). Very strong associations with features of the metabolic syndrome and its other co-morbidities were found in black volunteers in a study in the North West Province (Schutte *et al.* 2003). A study looking at urbanised black South Africans with CAD in Johannesburg found that 60% of the study participants had the metabolic syndrome, despite the fact that DM was an exclusion criterion (Ntyintyane *et al.* 2006). Abnormal lipid profiles are also reported in HIV-positive individuals before and after the onset of ART. This dyslipidaemia is characterised by hypertriglyceridaemia, hypercholesterolaemia and low serum HDL-C, all features of defective lipoprotein metabolism. Triglycerides tend to increase, particularly with the use of protease inhibitors (Chen *et al.* 2002).

When it comes to the role of dyslipidaemia in risk assessment for CVD, there is a continuing debate as to which lipid fraction or ratio is best associated with risk. Low-density lipoprotein cholesterol is a well-established atherogenic factor for CHD; however, in the presence of high TG levels, LDL alone does not sufficiently represent the risk associated with atherogenic dyslipidaemia (NCEP, 2002). Another possibility is the surrogate measure of atherogenic particle concentration, non-HDL-C, which is calculated as TC minus HDL-C or as very low-density lipoprotein cholesterol (VLDL-C) plus LDL-C. The National Cholesterol Education Program (NCEP) has identified non-HDL-C as a secondary target of therapy in patients with TG levels ≥5.7mmol/L, after achieving the primary LDL-C target goal (<2.59mmol/L) (NCEP, 2002). Non-HDL-C includes the cholesterol in all of the apoB-containing lipoproteins, including TG-enriched lipoprotein particles, chylomicrons and chylomicron remnants, VLDL and VLDL remnants, intermediate-density lipoproteins (IDL), LDL and lipoprotein(a) (Lp(a)) (NCEP, 2002).

Small, dense particles in the LDL sub-fraction have also been implicated in atherogenesis (Packard, 2006). The reason for this is that small, dense LDL particles are more susceptible to oxidation than large, buoyant LDL particles and they are retained to a higher degree in the arterial wall. Small, dense LDL particles display a reduced binding to LDL receptors and remain in the circulation for longer periods of time; increasing the LDL concentration in the blood (Berneis & Krauss, 2002). Ntyintyane *et al*. (2008) found that small dense LDL particles were present in 29 out of 40 South African urban black CAD patients.

Another approach to assessing risk for CVD is to look at apolipoproteins, which are classified as the structural components of lipoproteins. Apolipoprotein A-1 (apo A-1) and apoB are the main constituents of HDL and LDL respectively. Apolipoprotein B is the major protein of all

the atherogenic lipoproteins and has been shown to have strong predictive power for severity of coronary atherosclerosis and CHD events. Apolipoprotein A-1 is carried in HDL and is usually low when HDL is reduced. A low apoA-1 is therefore associated with increased risk for CHD but independently of low HDL (NCEP, 2002). A contentious issue in lipidology is whether ApoB and ApoA1 are better markers of risk of vascular disease than their cholesterol counterparts. All the major guideline groups previously recommended a cholesterol-based approach, effectively excluding apolipoproteins from routine clinical use (McQueen *et al.* 2008). The American Diabetes Association and the American College of Cardiology have stated, however, that ApoB is the test of choice to assess adequacy of statin treatment and should therefore be introduced into routine clinical practice (Brunzell *et al.* 2008).

Yet another marker is Lp(a) particles which contain apoA and apoB in a 1:1 molar ratio. A meta-analysis of prospective studies indicated that plasma Lp(a) concentration is an independent risk factor for CHD in both men and women (Craig *et al.* 1998). There are limitations, however, with the measurement of Lp(a), and serum Lp(a) is, furthermore, relatively resistant to therapeutic lowering. Nevertheless, an elevated Lp(a) does present the option of increasing a person's CVD risk (NCEP, 2002). A more recent approach is the use of ratios of lipid sub-fractions in risk assessment. It had been proposed that, for practical purposes, the independent effects of LDL and HDL cholesterol in coronary risk can be summarised by the total cholesterol/HDL ratio or the LDL/HDL ratio. Data used from the Lipid Research Clinics follow-up cohort, however, showed that LDL/HDL ratio alone may not fully capture the complex interaction between LDL and HDL and the relation to coronary risk (Grover *et al.* 2003).

The INTERHEART study investigators compared apolipoproteins and cholesterol as indices for risk of AMI. They found that non-fasting ApoB/ApoA1 ratio was superior to any of the cholesterol ratios for estimations of risk of AMI in all ethnic groups, in both sexes and in all ages (McQueen *et al.* 2008). The atherogenic elements of the lipid profile of the controls in the black African group (INTERHEART) were lower than those of the other two groups (coloured and European / other Africans). This included lower levels of total cholesterol, LDL cholesterol and ApoB/ApoA-1 ratio (Steyn *et al.* 2005). The TG/HDL ratio has also since then been investigated in men (208 cases), and was found to be a significant predictor of first coronary events across all categories of body mass index (BMI). The authors claim that these data demonstrate that the TG/HDL ratio is a simple determination that describes a deeply altered lipid and glucose metabolism, and identifies subjects at high risk for a first coronary event, independently of BMI (Cordero *et al.* 2009). Reasons for the high predictive value of this ratio include the fact that this ratio has been identified as an accurate marker of

insulin resistance. Secondly, as the TG/HDL ratio increases, LDL particles are smaller and denser, which correlates strongly with the initiation and progression of atherosclerosis. Lastly, higher values of this ratio are associated with higher risk of cardiovascular events even if LDL-C is low or lowered by treatment (Cordero *et al.* 2009).

Dyslipidaemia, therefore, definitely has a major role in risk assessment for CVD, and it appears that using ratios of lipid fractions that assess various aspects of the role of lipids in atherogenesis is the better option for risk assessment. Although black South Africans have been known to have lower lipid levels than Caucasians, with urbanisation and the increase in obesity, diabetes and subsequently, the metabolic syndrome, lipid levels are expected to increase in this population.

2.2.5. OBESITY

Obesity is associated with numerous co-morbidities such as CVD, T2DM, hypertension, certain cancers and sleep apnoea, and has been classified as an independent risk factor for CVD (Poirier *et al.* 2006). The estimated years of life lost in America as the result of obesity differ among races and between genders, but it is estimated that the optimal BMI for adults aged 18 to 85 years is 23 to 25kg/m^2 for Caucasians and 23 to 30kg/m^2 for blacks (Fontaine *et al.* 2003). In the African Americans, a consistent reduction in life expectancy was not observed until a BMI of 37 to 38kg/m^2 for women and 32 to 33kg/m^2 for men (Fontaine *et al.* 2003). Globally, obesity has been ranked as the fifth risk factor cause of death, causing 4.8% of total deaths (WHO, 2009). The WHO Comparative Risk Assessment study (Global CRA) estimates that in adults aged ≥30 years, a BMI above 21kg/m^2 is associated with an estimated 58% of T2DM, 21% of IHD, 39% of hypertensive disease, 23% of ischaemic stroke (Ezzati *et al.* 2004). In the South African general population, in 2000, 87% of type 2 DM, 68% of hypertensive disease, 45% of ischaemic stroke and 38% of IHD was attributable to a BMI from as low as 21kg/m^2 and upwards . Excess body weight is estimated to have caused 7% of all deaths in South Africa in 2000 and 2.9% of all DALYs (Joubert *et al.* 2007a).

The 1998 SADHS showed high levels of excess body weight among South Africans, particularly women. The mean BMI in adult women and men ≥15 years was 27.3kg/m^2 and 23.4kg/m^2 respectively. High proportions of adult men (29%) and women (56%) were overweight or obese, with the prevalence of obesity being particularly high among women (30%), and higher in urban (33%) than non-urban (25%) areas. Black women had the highest prevalence of overweight and obesity (58.5%), followed by women of mixed ancestry (52%), Caucasian women (49.2%) and then Indian women (48.9%). In men however, the prevalence of overweight and obesity was highest in Caucasian men (54.5%), followed by

Indian men (32.7%) and men of mixed ancestry (31%), with the lowest prevalence in black men (25%), indicating differences in the prevalence of obesity among different ethnic groups (Department of Health *et al.,* 2002). Also of concern in the South African setting is HIV lipodystrophy, which is seen in patients with long-term survival of the HIV infection, most of whom are receiving ART. This syndrome consists of both metabolic abnormalities as well as body fat redistribution (central adiposity with peripheral wasting) (Falutz, 2007).

In Soweto, South Africa, screening of black volunteers (n=1691) during the "Heart Awareness Days" held in the community revealed that by far the most prevalent risk factor for heart-related disease was obesity. Not only were 70% of the participants overweight, but the majority (43% overall) of these were technically obese (BMI ≥30kg/m^2). The prevalence of obesity was also significantly higher in women (55%) than in men (23%) (Tibazarwa *et al.* 2009). This confirms the estimates of the burden of disease (attributable to obesity) as approximately double in females compared with that in males (Joubert *et al.* 2007a). Furthermore, hypertension is about six times more frequent in obese subjects than in lean men and women (Stamler *et al.* 1976).

The "Heart Awareness" days study also reflected a poor awareness among participants of the common modifiable risk factors for heart disease and other forms of CVD (irrespective of previous contacts with the healthcare system). For example, study personnel frequently noted participant surprise when the link between obesity and increased risk of CVD was explained (Tibazarwa *et al.* 2009). The concept of 'benign' or 'healthy' obesity was thought to be relevant in the black population, due to the notion that IHD and dyslipidaemia were less prevalent (Walker *et al.* 1989; Walker *et al.* 1990). It was therefore assumed that obesity in this population was without consequence. However, as a result of studies published in the last decade, it has been clearly documented that in black ethnic groups obesity does in fact also predispose to hypertension, glucose intolerance and diabetes (Van der Merwe & Pepper, 2006).

A large number of epidemiological studies conducted in developed and developing countries have shown that small size at birth in full-term pregnancies is linked to the subsequent development of major features of the metabolic syndrome, including obesity, T2DM, increased BP, dyslipidaemia and increased mortality from CVD (Yajnik, 2003). The evidence points to undernutrition of the foetus during intrauterine life as a major determinant for these features (Barker *et al.* 1989; Hales *et al.* 1991; Yajnik, 2003). This is possibly a very relevant issue in sub-Saharan Africa, where the problem of undernutrition and overweight or obesity exist in the same communities and even households (Doak *et al.* 2005; Jehn & Brewis, 2009; Popkin, 2002b). However, it must be pointed out that as long as a (previously) stunted

individual remains lean and is exposed to a non-obesigenic lifestyle, he/she can remain metabolically healthy. In contrast, it is when a previously undernourished individual recovers food abundance and becomes overweight or obese, that there is a greater risk of noncommunicable diseases, such as insulin resistance in adulthood. This may be relevant in the black South African population undergoing urbanisation and its associated lifestyle changes (Van der Merwe & Pepper, 2006).

As mentioned earlier, obesity is classified as an independent risk factor for CVD (Poirier *et al.* 2006). Adipose tissue is not simply a passive storehouse for fat but an endocrine organ that is capable of synthesising and releasing into the bloodstream an important variety of peptides and nonpeptide compounds that may play a role in cardiovascular homeostasis. Adipose tissue is a significant source of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), PAI-1, resistin, lipoprotein lipase, acylation-stimulating protein, cholesterol-ester transfer protein, retinol-binding protein, oestrogens, leptin, angiotensin, adiponectin, insulin-like growth factor-I (IGF-I), insulin-binding protein 3 (IGFBP3) and monobutyrin. Of clinical consideration, circulating concentrations of PAI-1, angiotensin II, C-reactive protein (CRP), fibrinogen, and TNF-α are all related to BMI. Interleukin-6 modulates CRP production in the liver and CRP may be a marker of a chronic inflammatory state that can trigger acute coronary syndrome (Poirier *et al.* 2006).

There has recently been much speculation over which measure of overweight and obesity is best able to discriminate those individuals who are at increased cardiovascular risk. A meta-analysis was conducted to determine which of the four simple indices of overweight and obesity (BMI, waist circumference (WC), waist-hip ratio (WHR) or waist-to-height ratio (WHtR)) is the best discriminator of hypertension, T2DM and dyslipidaemia. Measures of central obesity, in particular, WHtR, provided a superior tool for discriminating obesity-related cardiovascular risk compared with BMI (Lee *et al.* 2008). Unlike BMI, WHtR takes into account the distribution of body fat in the abdominal region, which is known to be more associated with cardiovascular risks than body weight *per se* (Cikim *et al.* 2004). Body mass index is unable to distinguish between someone with excess adipose tissue and someone with high muscle mass (Lee *et al.* 2008; Yajnik & Yudkin, 2004). Although WC is a simple measure of abdominal obesity, it assumes that people with the same WC would have the same cardiovascular risk regardless of differences in height. Waist-hip ratio can stay the same even when there is a change in body size because WC and hip circumference (HC) can increase or decrease proportionately, whereas WHtR will only change if there is a change in waist circumference (in adults) (Lee *et al.* 2008). A matter still to be debated is whether ethnic-specific cut-off points should be used (Lee *et al.* 2008).

Intentional weight loss in obese individuals can improve or prevent many of the obesity-related risk factors for CHD, such as T2DM, increased LDL-C, hypertension, elevated CRP (Klein *et al.* 2004). Intentional weight loss (from 33.5 to 27.7kg/m^2) was associated with a 25% reduction in mortality rates in overweight patients with diabetes (Williamson *et al.* 2000). The Nurses' Health Study from 1980 to 2000 showed that obesity and physical inactivity independently contribute to the development of CHD in women. This emphasised the importance of maintaining a healthy body weight and of regular physical activity in preventing CHD (Li *et al.* 2006). The primary target of weight loss should not be weight normalisation, but rather some weight loss which can lead to substantial improvements in risk factors (Kraus *et al.* 2002). Regular exercise with minimal weight change has, however, also been shown to have broad beneficial effects on the lipoprotein profile (Kraus *et al.* 2002). Even if weight loss is minimal, obese individuals with a good level of cardiovascular fitness show a reduced risk for cardiovascular mortality as compared with lean, poorly fit individuals (Lee *et al.* 1999).

The high prevalence of obesity among black South Africans, particularly the women, is a matter of concern, particularly as a risk factor for CVD. It has been seen, however, that African-American women tend to have less visceral adipose tissue than Caucasian women, despite having similar waist circumferences (Conway *et al.* 1995). In addition, the ARIC study showed that obesity and fat distribution had a greater impact on the odds of hypertension in Caucasians than in African American women (Harris *et al.* 2000). Similar data is not available for black African women. Visceral adiposity is known to add more risk for CAD than subcutaneous adipose tissue. This could possibly be a reason why the incidence of CAD, although on the rise, is not as high as one would expect in this obese group of women, and definitely warrants further investigation.

2.2.6. SMOKING

The use of tobacco is one of the most important avoidable causes of CVD (Holbrook *et al.* 1984). According to the WHO, in 2003 the number of smokers worldwide was estimated to be 1.3 billion, of which 82% were in developing countries (Thun *et al.* 2003). During the twentieth century, 100 million individuals died worldwide as a result of tobacco-related diseases and this number is expected to increase to one billion during the twenty-first century (Mackay & Ericksen, 2002; Peto & Lopez, 2001). The use of tobacco was the leading cause of death in high-income countries in 2004, the second leading cause of death in middle-income countries, while in low-income countries it was the seventh cause of death (WHO, 2009). In South Africa, in 1995, smoking prevalence among adults was 30.2%; it fell to

24.1% by 2004. In the 10 years to 2001, cigarette consumption had decreased by one-third (Saloojee, 2006). The black South African population has the lowest level of smoking prevalence within the country (22.7% in 2000, down from 28.1% in 1993) (Van Walbeek, 2002) and also smokes the fewest cigarettes per day (average of seven per day in 1998) (Steyn *et al.* 2002). In South Africa, in 2000, smoking accounted for 8–9% of deaths and 3.7 to 4.3% of DALYs (Groenewald *et al.* 2007).

Current and current/former tobacco smoking were among the strongest risk factors in the African sample of the INTERHEART study; however, the risk of AMI associated with current smoking in the black African group was significantly lower than that found in the global INTERHEART study (Steyn *et al.* 2005). Stein *et al.* (2008) found that among urban black South African smokers there was a two-fold risk for CVD, compared with non-smokers. Sitas *et al.* (2004), in a national study of tobacco-related deaths, found that if smokers had the same death rate as non-smokers, 23% of vascular deaths could be avoided.

Cigarette smoking promotes the initiation and progression of atherosclerosis by inhibiting vasodilation, increasing vasoconstriction, stabilising thrombus, thickening of intima-media thickness (IMT), initiating inflammation and modifying lipid profiles. It also increases superoxide production. Superoxide inactivates the primary vasodilator, nitric oxide (NO), thereby producing endothelial dysfunction by reducing NO bioavailability (Rahman & Laher, 2007; Tsiara *et al.* 2003). When compared with non-smokers, active smokers have on average a 25% lower level of circulating concentrations of ascorbic acid, α-carotene, β-carotene and cryptoxanthin, weakening one of the lines of defence against oxidative stress (Alberg, 2002). The links between smoking, inflammation and CVD are also well established (Reichert *et al.* 2009).

The global INTERHEART study showed a clear dose-response relation between the number of cigarettes smoked per day and the risk of AMI. The odds for AMI were nine-fold higher in those who smoked 40 or more cigarettes a day than in never-smokers (Teo *et al.* 2006). When the never-smokers and former smokers were compared, the former smokers had a moderately higher risk of developing AMI. However, risk of AMI did fall progressively with time after cessation of smoking, but even in people who had quit 20 or more years before, there was a residual excess risk of about 20% (Teo *et al.* 2006). The risk of AMI was higher in the younger than the old men and women. This was because of the higher prevalence of smoking among the younger men and women, when compared with the older age groups (Teo *et al.* 2006). The excess risk associated with smoking in women was similar to that in men. This study also looked at the harmful effects of second-hand smoke (SHS). Non-smokers exposed to a spouse's SHS also had an increased risk of AMI (Teo *et al.* 2006).

Cigarette smoking is the main preventable cause of death worldwide. It accounts for a large burden of preventable disease in South Africa. There is evidence that smoking exerts harmful effects on several cardiovascular risk factors (Tsiara *et al.* 2003). While the South African government has taken bold legislative action to discourage tobacco use since 1994, it still remains a major public health priority (Groenewald *et al.* 2007).

2.2.7. GENDER

Coronary heart disease is the leading cause of death among women in developed and developing countries (Reddy, 2004). However, the incidence of CHD is markedly lower among women than men prior to the age of 50 years, after which time CHD increases and, by the eighth decade, approaches levels seen among men (Shaw *et al.* 2006; Sytkowski *et al.* 1996). It has been hypothesised that this later onset of CVD in women is due to the cardioprotective effect of endogenous sex hormones, primarily oestrogen (Barrett-Connor, 1991). The global INTERHEART study found that women experience their first AMI on average nine years later than men. The risk factors associated with AMI were, however, found to be the same in men and women. The difference in age of first MI was largely explained by higher risk factor levels at younger ages in men compared with women (Anand *et al.* 2008). In the African INTERHEART study, men also presented with AMI at a younger age than women and there were no age differences seen between the ethnic groups (Steyn *et al.* 2005). In South Africa in 2000, it was seen that IHD mortality rate was consistently higher for males than females in all ethnic groups (Bradshaw *et al.* 2006)

Key risk factors in women include age, post-menopausal status, smoking, family history of premature coronary disease, depression, sedentary lifestyle and the metabolic components – dyslipidaemia, hypertension and T2DM. Except for postmenopausal status and sex hormone-related risk (low testosterone in men), these risk factors are the same for men and women, but differ in degree of associated risk (Evangelista & McLaughlin, 2009). As mentioned earlier in section 2.2.5, in South Africa, black women have the highest prevalence of obesity, higher than that of black men as well as Caucasian men and women. However, there does not seem to be a difference in the role of gender *per se* in the development of CVD between different ethnic groups.

2.2.8. HAEMOSTATIC VARIABLES

The acute phase of clinical CVD consists of rupture of an atheromatous plaque and the formation of an acute thrombus (Rosenson & Lowe, 1998). It is now recognised that a hypercoagulable and hypofibrinolytic state predisposes to such clinically manifest CVD (Juhan-Vague & Vague, 1991). Fibrinogen and PAI-1_{act} are two of the haemostatic factors that are considered to be known risk markers for CVD (Mertens & Gaal 2002).

Fibrinogen is an acute-phase protein, which is synthesised in the liver and plays an essential role in the blood coagulation system (Ernst & Resch, 1993). It is especially involved in the last phase of coagulation as it is the main substrate of thrombin which cleaves off two peptides, fibrinopeptides A and B, from fibrinogen to form fibrin monomers which aggregate to form fibrin (Chandler, 1996). Owing to its central role in fibrin network formation, fibrinogen concentration therefore has the potential to affect the final str ucture of the resultant fibrin network (Weisel, 2007). It is also involved in the last common pathway of platelet aggregation by cross-linking platelets (Kamath & Lip, 2003) and is a major determinant of blood viscosity (Juhan-Vague & Vague, 1991). It is, in addition, a determinant of smooth muscle cell migration and proliferation (El-Sayed *et al.* 2004).

A meta-analysis of 31 prospective studies, which investigated the association between fibrinogen levels and CVD, showed that fibrinogen levels at baseline were associated with subsequent MI and stroke (Chiuve *et al.* 2011). Polymorphisms in fibrinogen are congenital abnormalities that have been associated with stroke, suggesting that there is a genetic component to raised fibrinogen levels and therefore, the risk of stroke (Kahn, 2003). Results from the Framingham study showed that for both men and women, each standard deviation increase in fibrinogen levels within the normal range is associated with a 20% age- and other risk factor-adjusted increase in the incidence of primary cardiovascular events (Kannel *et al.* 1987; Kannel, 1997). Fibrinogen is also associated with most other cardiovascular risk factors. Factors associated with high and low levels of fibrinogen are summarised in Table 2.5 (Ernst & Resch, 1993; Mertens & Gaal, 2002). Women generally have higher levels than men, and blacks tend to have higher levels than Caucasians (Fibrinogen Studies Collaboration *et al.* 2007; Mertens & Gaal, 2002). Fibrinogen levels also tend to increase with age and are higher in obese than non-obese subjects (Mertens & Gaal, 2002). Alcohol abstinence has also been associated with modestly higher fibrinogen levels (Fibrinogen Studies Collaboration *et al.* 2007). In a meta-analysis of 31 prospective studies, CRP was the strongest correlate of fibrinogen levels (Fibrinogen Studies Collaboration *et al.* 2007). These data are therefore consistent with previous suggestions that variability in fibrinogen levels is partly explained by low-grade inflammatory responses to environmental stimuli (Fibrinogen Studies Collaboration *et al.* 2007). Fibrinogen is therefore not only an integral

part of the haemostatic system, but is also considered as an acute-phase reactant associated with the inflammatory process (Mertens & Gaal, 2002). It is however not clear whether these associations are relationships of cause or effect.

Table 2.5 Factors associated with high and low fibrinogen levels (Adapted from Ernst & Resch, 1993 & Mertens & Gaal, 2002)

High fibrinogen	Low fibrinogen
Hypertension	High HDL cholesterol
Diabetes	Caucasian ethnic group
Smoking	Male gender
Obesity	(Moderate) alcohol intake
High white blood cell count	Physical activity
Increased LDL cholesterol	Hormone replacement therapy
Advanced age	
Black ethnic group	
Female gender	
Use of oral contraceptives	
Menopause	
Low socio-economic status	
Physical inactivity	

HDL: High-density lipoprotein; LDL: Low-density lipoprotein

When examining fibrinogen levels in healthy populations, a meta-analysis of 31 prospective studies (countries all in Northern hemisphere) found fibrinogen levels to be approximately 0.12g/L higher in blacks than in Caucasians, as well as higher in persons without employment and persons with a lower education (Fibrinogen Studies Collaboration *et al.* 2007). In healthy black South African volunteers, fibrinogen concentration increased with urbanisation to levels that are higher than 2.8 to 3.0g/L, which is already thought to be associated with an increased CVD risk (Vorster, 2002). This was observed in the THUSA study in the North West Province of South Africa, where 1854 “apparently healthy” black adult volunteers were recruited from 37 randomly selected sites (Vorster, 2002). When looking at different groups of black South Africans from different study groups, Pieters and Vorster (2008) found that mean values for men and women were above 2.5g/L, the value usually associated with CVD risk, indicating relatively high levels of fibrinogen for black South Africans. They also found that the women in all levels of urbanisation had higher fibrinogen levels than the men (Pieters & Vorster, 2008).

On the other hand, the fibrinolytic system is responsible for the degradation of fibrin in the blood vessels, with the enzyme plasmin playing a central role. Plasmin circulates in the blood as its inactive proenzyme, plasminogen. Two activators of plasminogen have been identified: tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Inhibition of the fibrinolytic system can occur at the level of the plasminogen activators (such as PAI-1) or at the level of plasmin, mainly by α-2 antiplasmin. Most of the research on fibrinolysis has focused on the role of PAI-1. Plasminogen activator inhibitor-1 is secreted by different cell types, including the endothelium, vascular smooth muscle cells, hepatocytes, platelets and the adipocytes (Lijnen & Collen, 1995). The estimated hepatic clearance half-life of free PAI-1 in humans is approximately ten minutes (Brommer *et al.* 1988). Plasma PAI-1 concentrations in healthy subjects are approximately 200 to 350pmol/L of total PAI-1, of which 125 to 150pmol/L is active and 30 to 120pmol/L inactive or latent. The concentration of PAI-1 that is in complex with t-PA_{ag} (antigen) is, on average, 50 to 80pmol/L (Chandler, 1996).

Numerous factors are known to influence plasma PAI-1. As far as genetic determinants are concerned, the 4G/5G polymorphism is the most extensively studied of the nine different polymorphisms that have been detected in the PAI-1-gene (Hoekstra *et al.* 2004; Nordt *et al.* 2001). As far as metabolic determinants are concerned, central obesity and insulin resistance appear to be the most important factors (Hoekstra *et al.* 2004). Other determinants include TG levels, smoking, diabetes (Wu & Zhao, 2002), exercise (Szymanski *et al.* 1994) and dietary factors (omega 3 fatty acids, antioxidants (Vorster *et al.* 1997), and alcohol (Ajjan & Grant, 2006). The effect of age on PAI-1 is not very clear, owing to conflicting results. Women at younger ages seem to have lower PAI-1 levels compared with men (De Pergola *et al.* 1997b).

As mentioned earlier, obese subjects have higher levels of PAI-1 than non-obese control subjects (De Pergola *et al.* 1997a; De Pergola *et al.* 1997b). Body fat distribution is an additional factor that can differentiate between subjects with low and high PAI-1 levels, as PAI-1 is associated with visceral obesity and is an important feature of the insulin resistance syndrome (Mertens & Gaal, 2002). Visceral obesity is characterised by high circulating levels of insulin, TGs, FFA, and a series of hormones and cytokines such as TNF-α. These could activate endothelial cells, liver cells and/or adipocytes to secrete PAI-1, explaining the association between hypofibrinolysis and obesity. However, the exact contribution of adipose tissue secretion of PAI-1 to total plasma levels needs to be further elucidated (Mertens & Gaal, 2002). Stromal cells, which make up a large part (20-40%) of adipose tissue (Hauner, 2005), and not adipose tissue itself, are considered to be the most important source of PAI-1 within adipose tissue (Bastelica *et al.* 2002). Visceral fat contains a higher

number of stromal cells than does subcutaneous fat, which might explain regional differences in PAI-1 production (Bastelica *et al.* 2002). Greyling *et al.* (2007), however, found a lower association of PAI-1_{act} levels with markers of the metabolic syndrome in black South African women than in the Caucasian group.

Levels of PAI-1 in African Americans tend to be lower than in Hispanics and Caucasians (Festa *et al.* 2003). Similar trends are seen in South Africa, where PAI-1_{act} levels are generally lower in black Africans when compared with levels observed in Caucasians (Appel, 2009; Greyling *et al.* 2007; Jerling *et al.* 1994). Even with urbanisation, PAI-1_{act} levels, although higher than in rural groups, are still within the normal ranges, including in obese and African women diagnosed with metabolic syndrome. This indicates a possible genetic influence on levels of PAI-1_{act} (Pieters & Vorster, 2008).

Smith *et al*. (2005), investigated whether haemostatic markers contribute to risk of CHD and ischaemic stroke independently of conventional risk factors. They found that fibrinogen, d-dimer, PAI-1_{act} and factor VIIc, each has the potential to increase the prediction of CHD / ischaemic stroke in middle-aged men, in addition to conventional risk factors. In the black South African population, it seems that fibrinogen could play a role in the development of CVD, because of the fact that fibrinogen levels are increased in the apparently healthy black population. PAI-1 seemingly plays a less important role in this population, with levels tending to be lower than those of Caucasians and less associated with visceral obesity and obesity. However, with urbanisation there are increases in the levels, and although these are still within accepted ranges, this may play a role as a risk factor in the future.

2.2.9. INFLAMMATION

Atherosclerosis is now widely accepted as a chronic inflammatory disorder that is induced by factors such as oxidised LDL, reactive oxygen species, diabetes and infection (Ross, 1999). All the stages of atherosclerosis, i.e. initiation, growth and complication of the atherosclerotic plaque, could be considered an inflammatory response to injury (Pearson *et al.* 2003). The role of inflammation in atherosclerosis will be discussed at a later stage in this chapter (section 2.4.2). There are numerous potential markers for measurement of inflammation. These are listed in Table 2.6 (Pearson *et al.* 2003). C-reactive protein has been shown in multiple prospective epidemiological studies to predict incidence of MI, stroke, peripheral artery disease and sudden cardiac death (Ridker, 2003), and is therefore the marker most commonly used to measure inflammation.

Table 2.6 Inflammatory markers for consideration as predictors of cardiovascular risk (Adapted from Pearson *et al.* 2003)

Adhesion Molecules
Intercellular adhesion molecule-1
Selectins
Cytokines
Interleukin 1
Tumour necrosis factor α
Acute-phase reactants:
Fibrinogen
Serum amyloid A
C-reactive protein
White blood cell count
Other (e.g. erythrocyte sedimentation rate)

C-reactive protein is an acute-phase protein produced primarily by the liver in response to inflammatory cytokines such as IL-6 (Libby, 2002). In terms of clinical application, according to Ridker *et al*. (2002), CRP seems to be a stronger predictor of CVD events than LDL-C and it adds prognostic information at all levels of calculated Framingham risk and at all levels of the metabolic syndrome (Ridker, 2003). The strong predictive value of CRP may be explained by its long-term stability during storage, its long half-life, its lack of diurnal variation, and its lack of age and sex dependence (Ridker, 2003). The high-sensitivity assays used to measure CRP are widely available, and levels of <1, 1 to 3, and >3mg/L correspond to low-, moderate- and high-risk groups for future cardiovascular events (Ridker, 2003). Because the evidence is not entirely consistent across published studies, the writing group for the AHA and Centres for Disease Control (CDC) Scientific Statement recommended against screening of entire adult populations for high sensitivity-CRP (hs-CRP) as a public health measure. Additional prospective studies are required to define risk more precisely at various strata and to assure consistency in other age, sex and race-ethnicity groups. They did conclude, however, that it is reasonable to measure hs-CRP as an adjunct to the major risk factors in order to further assess absolute risk for primary prevention of coronary disease (Pearson *et al.* 2003). Therefore, it is recommended that CRP levels must be interpreted in conjunction with the lipid profile (Yeh & Willerson, 2003).

This issue of whether CRP has a causal role in the development of CVD is still a topic of debate. A meta-analysis by the Emerging Risk Factors Collaboration (ERFC) group, looking at CRP concentrations and risk of CHD, stroke and mortality, found that CRP concentration has continuous associations with the risk of CHD and other outcomes. They also found that

the association between CRP and various CVD outcomes was attenuated when adjusting for known risk factors, and this was interpreted by the authors as an argument against causality (Emerging Risk Factors Collaboration *et al.* 2010). Some sceptics argue that CRP *per se* does not cause CVD; however, inflammation *per se* possibly contributes to CVD. Elevated CRP levels are therefore more likely a marker for the extent of atherosclerosis or inflammation, and CRP is more likely to be an innocent bystander (Nordestgaard & Zacho, 2009).

On the other hand, there is emerging evidence that CRP is not only a marker of inflammation, but that it has atherogenic properties itself (Ridker, 2003). An example of this is that CRP activates endothelial cells to express adhesion molecules, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), selectins and the chemokine, monocyte chemotactic protein-1 (Pasceri *et al.* 2000; Pasceri *et al.* 2001). It has also been seen that CRP amplifies the proinflammatory effects of several other mediators, including endotoxin (Yeh & Willerson, 2003). The concentration of CRP which causes these proinflammatory responses in *in vitro* experiments is in excess of 5mg/L, this being higher than the 1 to 3mg/L that is associated with CVD risk (Yeh & Willerson, 2003). Therefore, patients with CRP levels of 1 to 3mg/L are at intermediate risk and those with levels above 3mg/L are at high risk of CVD. However, when CRP levels are above 10mg/L, the test should be repeated to exclude other acute inflammatory processes (Yeh & Willerson, 2003).

The ERFC meta-analysis also showed that the adjusted risk ratio for CHD with high levels of CRP was 1.37, which was higher than non-HDL-C concentration and SBP (Emerging Risk Factors Collaboration *et al.* 2010). In addition, the JUPITER trial found that in intermediate risk subjects with elevated hsCRP levels who did not qualify for statin treatment because of LDL-levels below 3.37mmol/L, rosuvastatin of 20mg per day reduced risk for first MI by 55%, the risk for thromboembolic stroke by 52% and the need for coronary artery bypass grafting or percutaneous coronary intervention by 47% and total mortality by 20% (Ridker *et al.* 2008a). According to the investigators, the results of this trial have given a clear answer to whether CRP is seen as a risk factor for heart disease.

When looking at CRP levels in population groups, the ERFC meta-analysis found that CRP concentration can differ substantially in different ethnic groups. For example, the concentrations were 26% higher in black individuals and 16% lower in East Asian individuals than in Caucasians (ERFC *et al.* 2010). This confirmed results found in other studies (Kelley-Hedgepeth *et al.* 2008). However, there is not much available data on inflammation markers in the South African population. Schutte *et al.* (2006) found levels of three inflammatory markers (hsCRP, fibrinogen and leptin) in a group of black women to be

significantly higher than in the Caucasian group, although this appeared to be related to obesity. It is thought that although there may not be a difference between blacks and Caucasians in the role that inflammation plays in development of CVD, higher levels of sub-clinical inflammation are expected to be seen in black South Africans. The reasoning behind this is that in poor socio-economic conditions, there are possibly unhealthy lifestyles (e.g. low fruit and vegetable intake (Schneider *et al.* 2007); in addition to this, access to efficient health care is often limited and patients would seek health care at a later stage in the progression of their disease (Steyn *et al.* 2005).

While the debate as to whether CRP itself has a causal role in CVD is on-going one, there appears to be consensus that it is considered a risk factor. It provides a valuable tool in primary prevention for identifying patients at risk of cardiovascular events, in conjunction with lowering LDL-C (Yeh & Willerson, 2003). Consequently, it seems that there would be benefit in including CRP in risk-prediction scores, specifically for black populations in Africa. Although not much is known about the CRP levels of the South African population, it is expected that including CRP in risk prediction of CVD would improve prediction accuracy in this population.

2.2.10. PHYSICAL INACTIVITY

Outcomes of diseases such as CVD, thromboembolic stroke, hypertension, T2DM, osteoporosis, obesity, colon cancer, breast cancer, anxiety and depression, have all been shown to be inversely related to regular physical activity in prospective observational studies (Kesaniemi *et al.* 2001). Globally, physical inactivity contributed to 5.5% of total deaths (fourth ranked risk factor cause of death), in 2004, while for low-income countries it contributed to 3.8% of deaths (ranked eighth) (WHO, 2009). In South Africa, physical inactivity ranked ninth when compared with other risk factor causes of death and was estimated to have caused 3.3% of all deaths and 1.1% of all DALYs in 2000 (Joubert *et al.* 2007b). Compared with the global average of 17% and Africa's average of 10%, South Africa stands out as having a particularly high prevalence of physical inactivity, with 49% of all adult women and 43% of all adult men reportedly insufficiently active to achieve health benefits (Joubert *et al.* 2007b). The Eastern European sub-region showed the highest level of physical inactivity among the WHO sub-regions, estimated at 25% (Bull *et al.* 2004). South Africa's level of inactivity is far higher than that.

This high level of inactivity in South Africans has also been reported in smaller studies of sub-population groups (black and coloured), which consistently report approximately half of the adults as having insufficient levels of physical activity during leisure and occupational

time (Kruger *et al.* 2003; Levitt *et al.* 1993; Steyn *et al.* 2004). South African females are less active than males and older adults less active than younger adults, following global trends (Joubert *et al.* 2007b).

It is not clear why black and coloured South Africans are particularly inactive. Evidence exists of associations between urbanisation, increased availability of motorised transport, mechanisation of labour, television viewing, obesity and inactivity in adults and children (Kruger *et al.* 2002; McVeigh *et al.* 2004). The THUSA study found high levels of physical inactivity among the black women in this study (Kruger *et al.* 2002). One would expect this, with the high prevalence of obesity among black South Africa women. Two studies on South Africans of black and mixed ancestry, as well as the INTERHEART study, all found substantial evidence of the protective role of physical activity against chronic diseases of lifestyle, even in communities and populations undergoing the epidemiological transition (Kruger *et al.* 2003; Steyn *et al.* 2004; Steyn *et al.* 2005).

The question has arisen as to whether being physically active outweighs the effects of obesity on the development of CVD. In the Nurses' Health Study from 1980 to 2000, physical activity and adiposity independently predicted risk of CHD in women. Being physically active moderately attenuated but did not eliminate the adverse effect of obesity on CHD health, and being lean did not counteract the increased risk associated with physical inactivity (Li *et al.* 2006).

2.2.11. DAILY FRUIT AND VEGETABLE CONSUMPTION

Fruit and vegetables contain many nutrients such as minerals, vitamins, phytochemicals and fibre, which may individually or in combination be protective against CVDs and certain cancers (Willett *et al.* 2006). It has been estimated globally that 4.9% of deaths and 1.8% of DALYs per year in 2000 were attributable to low fruit and vegetable intake, and also that this caused about 31% of IHD and 19% of ischaemic stroke (Lock *et al.* 2004). In South Africa in 2000, it was shown that low fruit and vegetable intake made a significant contribution to the number of deaths and DALYs from IHD and ischaemic stroke, accounting for 35% and 22% respectively. Low fruit and vegetable intake was ranked eleventh on the list of 17 selected risk factors for South Africa in 2000, accounting for 1.1% of the 16.2 million DALYs (Schneider *et al.* 2007).

Unfortunately, nationally representative data on fruit and vegetable intake by population group are not available in South Africa. Smaller studies have given indications that eating patterns vary within the country. Fruit and vegetable intake is higher in urban areas than

rural areas (168g/day versus 137g/day), probably as a result of greater access to and availability of fruit and vegetables in urban areas, but is still less than the recommended intake of 400g/day (as discussed below) (Nel & Steyn, 2002; Steyn *et al.* 2003). One of the few studies undertaken among Caucasians showed a far higher fruit and vegetable intake of 391g/day (Wolmarans *et al.* 1989). In the African INTERHEART study, 37% of the black Africans consumed fruit and vegetables daily, compared with 50% of the European or other Africans (Steyn *et al.* 2005).

The WHO has recognised that there is convincing evidence of decreased risk of CHD, stroke, hypertension and obesity associated with an increased fruit and vegetable intake (WHO & FAO, 2003). To protect against CVDs and certain cancers, the WHO recommends an intake of 400g/day; this is equivalent to five portions of 80g each of fruit and vegetables per day (WHO & FAO, 2003). The intake of fruit and vegetables in South Africa is estimated to be similar to that in the United Kingdom, with an average per capita of 200g/day (less than three servings per day). Although this is greater than that of India (120-140g/day), the South African intake is considerably less than that of China (369g/day) and Spain (600g/day) (Willett *et al.* 2006).

Irregular consumption of fruit and vegetables was identified as one of the nine risk factors that accounted for ≈97.4% of the AMI observed in African populations (Steyn *et al.* 2005). The Women's Health Study (n=39876 female health professionals) found that a higher fruit and vegetable intake was associated with a lower risk of AMI. The data suggested that higher intake of fruit and vegetables may be protective against CVD and supported current dietary guidelines to increase fruit and vegetable intake (Liu *et al.* 2000a). Several studies relating the constituents of fruit and vegetable intake to CVD risk found that higher intakes of dietary fibre, folate and/or antioxidants are associated with lower risk. It is possible, however, that the combined effect of these and other constituents of fruit and vegetables are best assessed by examining the association between fruit and vegetable intake and CVD risk directly (Liu *et al.* 2000a).

Black South Africans eat well below the recommended five servings of fruit and vegetables per day. This is of concern, particularly considering that low fruit and vegetable intake has been shown to contribute to the burden of diseases such as IHD and ischaemic stroke. A non-profit, non-governmental organisation, the 5-a-day for Better Health TRUST, was founded to promote the education of South Africans as to the importance and benefits of including a minimum of five servings of fruit and vegetables in their daily diet (5 a day for better Health TRUST). This initiative is helping to further promote the food-based dietary

guideline “Eat plenty of vegetables and fruit”. Irregular or low fruit and vegetable intake is therefore an important risk factor for CVD in the South African population.

2.2.12. STRESS

Popular opinion holds that stress is an important risk factor for CHD (Rosengren *et al.* 2004). Low socio-economic status, lack of social support and social isolation, stress at work and in family life, depression, anxiety, hostility and anger, as well as a type D (distressed) personality, have all been identified as psychosocial risk factors in CHD (Albus, 2010). The problem is that, compared with other major risk factors, psychosocial variables such as stress are difficult to define and measure objectively, as stress consists of several different (and interrelated) elements (Rosengren *et al.* 2004). Another pattern of thought is that both the abatement in the decline of CVD in developing countries and also the fact that many CVD patients continue to experience cardiac events despite optimal treatment of traditional risk factors, suggest that other non-traditional risk factors must be responsible. Psychosocial stress is a newly recognised (non-traditional) risk factor that appears to contribute to all the recognised mechanisms underlying cardiac events, specifically, clustering of traditional cardiovascular risk factors, endothelial dysfunction, myocardial ischaemia, plaque rupture, thrombosis, and malignant arrhythmias (Merz *et al.* 2002). Studies investigating this concept have adjusted for traditional risk factors and still found correlations between psychosocial stress variables and these mechanisms, suggesting that they do provide additional, independent risk (Merz *et al.* 2002).

There is a complex interaction of individual differences and environmental influences that impact on stress reactivity patterns, contributing to various mechanisms that might lead to the development of pre-clinical disease states, and ultimately, CVD (Hamer & Malan, 2009). The mechanisms leading to pre-clinical risk may not act independently of one another, but may to some extent be caused or influenced by each other (Hamer & Malan, 2009).

The INTERHEART study also investigated the association of psychosocial risk factors with the risk of AMI in 52 countries (Rosengren *et al.* 2004). They found that people with myocardial infarction (MI) reported higher prevalence of all four measured stress factors than the controls. Stressful life events in the past year, as well as depression, were more frequent in cases than in controls. The excess risk of AMI associated with high levels of stress was still significant after adjusting for other cardiovascular risk factors. These differences were found to be consistent across regions, in different ethnic groups, and in both men and women (Rosengren *et al.* 2004). Permanent stress was also found to be one of the

strongest risk factors in the African sample of the INTERHEART study, particularly the black African group (Steyn *et al.* 2005).

It is known that, in comparison with Caucasians, African Americans respond with heightened vascular reactivity and peripheral resistance responses at rest when exposed to stressful situations (Hinderliter *et al.* 2004). South African black volunteers have been reported to respond in the same way as African Americans when exposed to stress (Malan *et al.* 2008; Van Rooyen *et al.* 2000; van Rooyen *et al.* 2002). The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study aimed to investigate the association between cardiovascular function and psychological distress in urbanised black South African volunteers (n=200). The results showed that psychological distress was associated with higher BP in hypertensive men and also with the development of left ventricular hypertrophy in hypertensive black South African men and women (Mashele *et al.* 2010). Although it seems that there are differences in response to stress between blacks and Caucasians, and that psychological stress appears to play a role in the development of CVD in the black population, there is not enough evidence available to decide whether including stress as a risk factor will improve the determination of risk of CVD in black South Africans.

2.2.13. CONCLUSION

Although it was previously thought that the black South African population was immune to CHD, it is becoming evident that there is an increase in the incidence of CVD within this population (Akinboboye *et al.* 2003; Stewart *et al.* 2011). The risk factor profile of the black South African population appears to be changing with urbanisation. Table 2.7 provides a summary of the risk factor profile of the black as compared to the Caucasian South African. Lipid levels, always thought to be favourable, as well as haemostatic markers, are starting to rise with urbanisation. This population has a very high prevalence of hypertension, DM and obesity (particularly among the women), which are all known major risk factors for CVD. Possible compounding factors are the low levels of physical activity and fruit and vegetable intake, inflammation and the heightened vascular reactivity to stress. Although the incidence of CHD is still not as high as one would expect, it would seem that, as the presence of these risk factors continues to increase, especially within the urbanised black South African population, so will CHD.

Table 2.7 Risk factor profile of the black South African compared with Caucasian South African

RISK FACTOR	Black South Africans
Hypertension	Higher prevalence Increasing with urbanisation
Diabetes mellitus	Inadequate data available, but lower than other ethnic groups (self-reported prevalence) DM in black South Africans characterised by a high rate of acute and long-term complications
Dyslipidaemia	Tends to be lower than in Caucasians, but increasing with urbanisation
Obesity	Black women highest prevalence, black men have the lowest, compared with Caucasian men with the highest prevalence Black African American women have less visceral fat than Caucasian women for the same waist circumference In black African American women, obesity and fat distribution associated with lower odds of hypertension than in Caucasian women
Smoking	Black South Africans have the lowest level of smoking prevalence
Haemostatic variables	**PAI-1** lower than in Caucasians Increasing with urbanisation, but still lower than Caucasians **Fibrinogen** Higher than in Caucasians Higher than 2.8-3.0g/L (levels associated with CHD risk)
Inflammation	Not much data available on this population One study found inflammatory markers (hsCRP, fibrinogen and leptin) to be significantly higher in black women than in Caucasian
Physical inactivity	Black South Africans have a high level of inactivity, are less active than Caucasians
Fruit and vegetable intake	Lower than Caucasians Urban slightly higher than rural but still lower than recommended 400g/day
Stress	African Americans respond to exposure to stress with a heightened vascular reactivity, compared with Caucasians; black South Africans reported to react in same way Black South Africans with an active coping style have higher risk

2.3. RISK SCORES FOR PREDICTION OF CARDIOVASCULAR DISEASE AND CORONARY HEART DISEASE RISK

One of the main goals of this thesis is to investigate how diet quality influences the negative effects of dietary fat intake in the Prospective Urban and Rural Epidemiological (PURE) study population, by comparing those individuals at high risk with those at low risk of developing CVD. The PURE study is a large-scale cohort study that tracks changing lifestyles, risk factors and chronic disease, using periodic standardised data collection in urban and rural areas of seventeen countries in transition. The present study used baseline data from just over 2000 randomly selected subjects of the South African arm of the PURE study. We are specifically looking at individuals at risk for developing CVD, since known CVD was an exclusion criterion for baseline inclusion in the PURE study. Because cardiovascular disease event data from the follow-up data collection are also not yet available for the PURE study population, the risk of developing CVD will have to be estimated. There are two routes one can use to estimate the risk of CVD for a population. One route is to estimate risk by focusing on the individual risk factors present in the study population, and the other is to do a total risk estimation using a risk score. The term "total risk estimation" is perhaps a misnomer, as no risk estimation system accommodates all the known risk factors. However, it refers to the fact that CVD, in most people, is the product of several risk factors that may interact to greatly increase risk, while an approach that focuses on single risk factors may result in inappropriate management decisions (Cooney *et al.* 2009).

Despite the fact that there are numerous guidelines and abundant evidence regarding the efficacy of interventions to prevent CVD, the majority of people with CVD risk factors do not have them under adequate control (Sheridan & Crespo, 2008). According to a systematic review by Sheridan and Crespo (2008), a contributing factor to this problem is that many physicians do not accurately estimate a patient's risk of CVD. Several of the major guidelines, such as NCEP (NCEP, 2002), AHA (Pearson *et al.* 2002), European Society of Cardiology (Graham *et al.* 2007), as well as the National Institute for Health and Clinical Excellence (NICE) (NICE Clinical Guideline, 2008), now advocate routine assessment of cardiovascular risk, using various CHD risk estimation systems or scores as a means to aid clinicians in decision-making. The South African Heart Association (SA Heart) and Lipid and Atherosclerosis Society of Southern Africa (LASSA), for example, recommend that absolute risk of MI be calculated using the updated Framingham risk score in their Dyslipidaemia Guideline Consensus document (Klug *et al.* 2012). For this reason, the decision was taken to use a risk score or risk estimation system to classify the PURE study population according to their risk of developing CVD.

Risk scores can be used or applied in various settings, ranging from an individual or patient level, to an epidemiological level. On the individual or patient level, risk scores are used for determining CVD risk in individuals (by their physicians), so as to aid in appropriate management of risk factors. On an epidemiological level, risk scores can be used firstly to determine absolute risk of individuals and secondly to stratify a population for CVD risk, thereby dividing the population into groups with, for example, low (<5%), low to medium (5–10%), medium to high (10–20%) and high risk (>20%). The best known is the Framingham risk score, which was developed for the American Caucasian population.

Framingham Function Equation (Marrugat *et al.* 2003; Wilson *et al.* 1998):

Ten-year risk estimate = $1 - S(t)^{\exp(\sum \beta i Xi - \sum \beta i \dot{X}i)}$

S(t): 10-year average rate free of hard CHD (survival rate)

βi: Cox regression coefficients for each level of each risk factor

Xi: The values of the risk factors for a given patient

$\dot{X}$**i:** The means of the risk factors

The formula contains information specific to the population it was developed in, S(t) being the survival rate and $\dot{X}$**i** the population mean for each risk factor. Also, the β-coefficients (βi) were obtained by including the risk factors in a Cox proportional hazards model, to determine their contribution to the outcome (CVD risk). Therefore, to use a risk score in a new population, it should, ideally, first be calibrated and validated in this new population. To calibrate the formula, the population-specific variables in the equation are replaced with those of the population in question, and to validate it, the predicted risk is compared with actual risk in the population.

In order to know the actual risk of the population, outcome measures are required. Therefore, longitudinal data should be available for the study population, with baseline risk factor data available, as well as outcome data (CVD events) after a specific time period, such as 5 or 10 years. Should the existing risk score after calibration and validation be found unsuitable for a new population, a new risk score can be developed by incorporating risk factors specific to that population in the Cox proportional hazards model and ultimately in the risk score. This process, however, requires representative epidemiological baseline risk factor data and CVD end points at follow-up (5 to 10 years). It is currently not clear whether existing risk scores, after calibration and validation, can be successfully used in African

populations. From the previous section it is clear that many of the traditional risk factors in Caucasian populations also apply to the black population. There are indications, however, that cut-off levels defining "increased risk" may differ from those used in the Caucasian population and also that additional risk factors may emerge. An example of this is the increased fibrinogen that is present in apparently healthy black Africans, which is not typically observed in healthy Caucasians.

Unfortunately, at this stage we are not yet able to determine whether an existing risk score can be used in the PURE study population or whether a new one should be developed, as we do not yet have outcome measurements available for this population. At best we can currently only calibrate an existing risk score by incorporating the population-specific means into the risk score, but since the follow-up data for the PURE study are not yet available, they cannot be validated. It should be kept in mind, however, that the purpose of this thesis is not to determine absolute CVD risk, but to stratify the participants according to CVD risk in order to compare diet quality, fat intake and the interaction of these with each other. For this purpose, a known risk score can be used since any error in risk prediction will be incorporated systematically without reducing the validity of the data. This approach has been used by several groups (Cappuccio *et al.* 2002; Steyn *et al.* 2004) who want to report on CVD risk for different populations but who do not yet have CVD event or outcome data available.

Returning to the development of a reliable CHD risk score or estimation for a specific population, should this be required, it is important to have a longitudinal study, standardised measurements at baseline and adjudicated outcomes that are consistent over the follow-up interval. A prospective design is necessary because critical risk factors may change after the occurrence of CHD, and such a design allows the inclusion of fatal events as outcomes. An example of this is smoking: after experiencing an MI, a person may stop smoking or underreport the amount of smoking that occurred before the event. This would lead to analyses that would show a smaller effect of smoking on MI risk than is actually present (Wilson, 2009). It is also important to standardise measurements when assessing the role of factors that might increase risk for vascular disease outcomes, such as, for example, blood pressure and lipid measurements (Wilson, 2009). The criteria for clinically useful risk estimation systems are outlined in Table 2.8 (Cooney *et al.* 2009).

Table 2.8 Criteria for a clinically useful risk estimation system (Cooney *et al.* 2009)

Appropriate statistical methods for derivation of the function

- Representative sample from the population from which the system is to be applied
- Sufficient power (large enough sample size)
- Accepted statistical methods
- The end point predicted by the function should be defined in such a way that it is easily standardised across populations and relevant to the outcomes of randomised controlled trials of preventative measures

Performance of the function – internal and external validity

- Discrimination: the ability of the function to separate those who will develop the end point from those who will not. Often assessed using:
    - Area under the receiver operating characteristic curve (AUROC) – a means for expressing the maximum achievable sensitivity and specificity. An AUROC of 1 indicates perfect discrimination; 0.5 equates to chance. Values in the region of 0.9 are often achieved for diagnostic tests. Values rarely exceed 0.8 for risk estimation. Harrell's C statistic gives the same information but can be used with variable follow-up.
- Sensitivity/specificity/ positive predictive value/ negative predictive value
- Calibration – a measure of how closely predicted outcomes agree with actual outcomes. Often assessed using either:
    - Hosmer-Lemeshow goodness of fit testing – lower values indicate better fit, values <20 generally considered good fit. Significant p values indicate lack of fit
    - Predicted to observed ratios – the closer the value to 1, the better the fit. Values >1 indicate overestimation and vice versa.
- Reclassification
    - Net reclassification index – a measure of the net percentage of those who do and who not develop the end point within the time period that are correctly reclassified to a different risk category when a new risk factor is added to the risk estimation system

Usability of the system

- The format affects the ease of use of the system. This will also impact on the uptake of the system by users.

Inclusion of appropriate risk factors

- Most risk estimation systems include age, sex and conventional risk factors, including lipid levels, smoking and blood pressure
- Inclusion of other factors may be important, especially if they have been shown to be powerful risk determinations and prevalent in the population to which the system is to be applied (e.g. social deprivation)
- Some advocate the use of only risk factors that are potentially modifiable, although most agree that risk factors to be included should be chosen based on whether they improve risk estimation because those identified as high-risk can still modify their risk by favourably altering their other risk factors.
- Systems using only easily measured non-laboratory measures have been developed recently

Has the use of the system been shown to result in measurable health gains?

An important aspect of risk assessment is the actual risk factors incorporated into the risk score. A risk factor may be defined as a characteristic that is associated with an increased risk of developing a specific disease such as atherosclerotic CVD. To be clinically relevant, it should be accepted as causal and modifiable and benefit should result from such modification. Most risk estimation systems include age, sex, smoking, blood lipids and BP. In this context, age is a measure of exposure to time and not a risk factor as such (Cooney *et al.* 2009). From the previous section pertaining to risk factors in the black South African population, it is evident that there are several risk factors which potentially play a role in the development of CVD in this population, particularly the urbanised black population. These are hypertension, DM, dyslipidaemia, obesity, inflammation (hs-CRP), fibrinogen, physical inactivity, stress and a low fruit and vegetable intake. It is also evident that there are some differences between blacks and Caucasians in the prevalence and roles that these risk factors play (Table 2.7).

Another important issue to be aware of is that the various risk scores use different end points. Table 2.9 provides a summary of the estimation systems that are recommended by various guidelines on CVD prevention. Early systems usually estimated CHD risk, which included MI, CHD death as well as "soft" end points such as angina pectoris and coronary insufficiency (Wilson *et al.* 1998). More recent systems tend rather to use total CVD as an end point, which is defined as a composite of CHD (coronary death, MI, coronary insufficiency, angina pectoris), cerebrovascular events (including ischaemic stroke, haemorrhagic stroke and transient ischaemic attack), peripheral artery disease (intermittent claudication) and heart failure. The reason for this is that atherosclerosis may manifest elsewhere in the body apart from the heart, for example, as stroke or peripheral vascular disease (Conroy *et al.* 2003; D'Agostino *et al.* 2008). It is helpful, however, to retain the capacity to estimate risk of cause-specific events, because stroke, for example, may be proportionately more common in certain populations such as in older persons and the black South African population (Seedat, 1999). The end point must be as clearly defined as possible to prevent coding difficulties when the function is applied to other populations. Initial versions of the Framingham incorporated "softer" end points, including onset of angina of effort and silent MIs based on electrocardiographic re-examinations (Anderson *et al.* 1991). Another risk estimation system, the SCORE system, estimates the risk of fatal CVD events only, whereas all the other systems in Table 2.9 estimate risk of all CVD events (Conroy *et al.* 2003). The investigators' reasoning for this is that they hope to give a better estimate of risk to the person, and also a better reflection of the health-service implications of cardiovascular risk factors (Conroy *et al.* 2003).

Table 2.9 Characteristics of current risk estimation systems (Adapted from Cooney *et al.* 2009)

	FRAMINGHAM[1]	QRISK 1[2] and QRISK2[3]	SCORE[4]	ASSIGN[5]	PROCAM[6]	WHO/ISH[7]	REYNOLD'S RISK SCORE[89]
Data	Prospective studies: Framingham Heart Study and Framingham Offspring study.	QRESEARCH database	12 pooled prospective studies from 11 European countries	Scottish Heart Health Extended Cohort (SHHEC)	Prospective study	Methods differ from other risk estimation functions – not based on prospective data	Randomised controlled trials Women: Women's Health Study Men: Physicians' Health Study II
Population and sample type	General population, Framingham, Mass, U.S. volunteers.	Health records of general practice attendees – not random QRISK 2: White/ not recorded, Indian, Pakistani, Bangladeshi, other Asian, black African, black Caribbean, Chinese, other including mixed	Mostly random samples from general population, some occupational cohorts Ethnicity: not stated, would assume mostly Caucasian	Random sample from general population in Scotland Ethnicity: not stated, would assume mostly Caucasian	Healthy employees Volunteers – not random Ethnicity: not stated, would assume mostly Caucasian	NA	Women: Health Service employees (largely Caucasian) Men: Physicians Volunteers – not random
Sample size	3969 men 4522 women	1.28 million(QRISK1) 2.29 million(QRISK2)	80080 women 117098 men	6540 men 6757 women	18460 men 8515 women	NA	24558 women 10724 men
Statistical methods	Cox proportional-hazards regressions	Cox proportional-hazards model. Imputation of substantial missing data	Weibull proportional-hazards model	Cox proportional-hazards model	Cox and Weibull Exploratory analyses with neural networks also	Relative risks associated with risk factors were taken from comparative risk-assessment project; these were combined with estimated absolute risks for each WHO sub-region based on global burden of disease study.	Cox proportional-hazards model

	FRAMINGHAM	QRISK 1 and QRISK2	SCORE	ASSIGN	PROCAM	WHO/ISH	REYNOLD'S RISK SCORE
Calculates	10-yr risk of CHD events originally Latest version: 10-yr risk of CVD events Risk age	10-yr risk of CVD events	10-yr risk of CVD mortality	10-yr risk of CVD events	2 separate scores calculate 10-yr risks of major coronary events and cerebral ischaemic events	10-yr risk of CVD events	10yr risk of incident MI, stroke, coronary revascularization or cardiovascular death
Age range	30 – 74 years	35 – 74 years	40 – 65 yrs	30 – 74 years	20 – 75 yrs	40 – 79 yrs	45 – 80 years
Variables	Sex, age, TC, HDL-C, SBP, smoking status, diabetes, hypertensive treatment	QRISK1: sex, age, TC to HDL ratio, SBP, smoking status, diabetes, area-based index of deprivation, family history, BMI, antihypertensive treatment. QRISK2: also includes ethnicity and chronic diseases	Sex, age, TC or TC/HDL-C ratio, SBP, smoking status Versions for use in high- and low-risk countries	Sex, age, TC, HDL-C, SBP, smoking – no. of cigarettes, diabetes, area-based index of deprivation, family history of CHD	Age, sex, LDL-C, HDL-C, diabetes, smoking, SBP.	Sex, age, SBP, smoking status, diabetes ± TC; different charts available for different worldwide regions	Sex, age, SBP, smoking, hsCRP, TC, HDL-C, family history of premature MI, HbA1C if diabetic.
Recommended by guidelines	NCEP[10] guidelines and other national guidelines recommend adapted versions including New Zealand Also recommended by SA Heart and LASSA	NICE guidelines on lipid modification	European guidelines on CVD prevention	Recommended by SIGN (Scottish Intercollegiate Guidelines Network)	International Task Force for prevention of Coronary Disease guidelines	WHO guidelines on CVD prevention	No

[1](D'Agostino *et al.* 2008); [2](Hippisley-Cox *et al.* 2007; Hippisley-Cox *et al.* 2008); [4](Conroy *et al.* 2003); [5](Woodward *et al.* 2007); [6](Assmann *et al.* 2002); [7] WHO/ISH; [8](Ridker *et al.* 2008b) [9] (Ridker *et al.* 2007) [10] (NCEP, 2002)

SCORE: Systematic COronary Risk Evaluation; **ASSIGN:** ASSessing cardiovascular risk, using SIGN guidelines; **PROCAM:** PROspective CArdiovascular Münster study; **WHO/ISH**: World Health Organisation/International Society of Hypertension

Since it was decided to use an existing risk estimation system for the purpose of this thesis, the immediate question which now arises is which of the known risk estimation systems are truly appropriate for predicting risk of CVD in the black South African population, particularly as the study populations used in the development of the available risk estimation systems were mostly Caucasian. Risk estimation systems using the Framingham equations have been widely tested in North America (D'Agostino *et al.* 2001) and in European populations of European origin (Brindle *et al.* 2003; Ferrario *et al.* 2005; Marrugat *et al.* 2003) and have also been validated in the Chinese population (Liu *et al.* 2004). For American black and Caucasian men and women, the Framingham functions performed reasonably well for prediction of CHD events within five years of follow-up (D'Agostino *et al.* 2001).

The vast majority of the evidence on the benefit and potential harm of interventions to reduce CVD risk, however, comes from high-income countries. The limited observational epidemiological data from low- and middle-income countries, recently extended by the INTERHEART case-control study (Steyn *et al.* 2005), support the view that existing cardiovascular risk factors are seemingly equally predictive of CVD events in a wide range of low-, middle- and high-income countries (WHO, 2007). However, as discussed in section 2.1, potential risk factors are emerging that are not part of known risk estimation systems, such as haemostatic variables (fibrinogen), waist-hip ratio and stress. The INTERHEART study also identified low fruit and vegetable intake and physical activity as playing a role in the African study populations (Steyn *et al.* 2005).

Most scores use the same risk factors that form part of the Framingham risk score, namely age, sex, smoking, blood lipids and BP. Several scores, however, include additional risk factors. The European guidelines on CVD prevention use a model for total risk estimation based on the SCORE system (Conroy *et al.* 2003). SCORE has different charts for populations at high and at low risk of CVD (Conroy *et al.* 2003). The latest European Guidelines on CVD prevention recommend the continued use of the SCORE risk assessment charts in European countries (Authors/Task Force Members: *et al.* 2012). The QRISK1 and QRISK2 scoring systems include an area-based index of deprivation. This was determined using the Townsend score, which is a good area measure of material deprivation based on four variables (unemployment, overcrowding, non-car ownership and non-home ownership) (Hippisley-Cox *et al.* 2007; Hippisley-Cox *et al.* 2008). The ASSIGN system also addresses the issues of social deprivation, by using an index based on the postcode of residence at recruitment, called the Scottish Index of Multiple Deprivation (SIMD) (Woodward *et al.* 2007). The other scores did not take social deprivation or socio-economic status into account.

All the risk scores, apart from SCORE, include DM as a risk factor. Diabetes is not included in SCORE simply because data on DM had not been collected uniformly in the SCORE study cohorts (Conroy *et al.* 2003). The Reynolds Risk Score includes haemoglobin A_{1c} if diabetic. The β-coefficient has as yet been published for women only and not yet for men, because the study population consisted of non-diabetic men (Ridker *et al.* 2007; Ridker *et al.* 2008b). However, the authors provided us with the B-coefficient for haemoglobin A_{1c} for men, which will be published soon (personal communication with Paul Ridker and Nancy Paynter, July 2010). The Reynolds Risk Score is also the only risk estimation system that acknowledges the importance of inflammation in CVD, by including CRP in their model.

It seems that for the purpose of our study, therefore, the most suitable risk estimation system is the Reynolds Risk Score. As discussed in the previous section, inflammation (hs-CRP) has been shown to be an important potential risk factor in the black South African population. The REasons for Geographical and Racial Differences in Stroke (REGARDS) study (n=19080) found that women and blacks had a higher prevalence of elevated CRP, and also that lower socio-economic status was associated with elevated CRP (Cushman *et al.* 2009). This is also what is expected to be seen in our study population. Using the Women's Health Study participants, the Reynolds Risk Score reclassified 40 to 50% of women, currently predicted to be at intermediate risk, into higher- or lower-risk categories, and did so with greatly improved accuracy when compared with models based on the current ATP-III prediction scores (Ridker *et al.* 2007). The literature also suggests that hs-CRP is a potential extra risk factor to measure those at intermediate risk to further define risk in this group (Cook *et al.* 2006; Wilson *et al.* 2008).

There are two models of the Reynolds Risk Score: computational formulas for 10-year risk using best-fitting Model A, and a clinically simplified Model B. These are shown below:

Model A:

10-year CVD risk(%) = $[1 - 0.98756^{(\exp [A-19.848])}] \times 100\%$ where

A = 0.0785 x age + 3.271 x natural logarithm (SBP) + 0.202 x natural logarithm (hs-CRP) + 0.00820 x apolipoprotein B-100 - 0.00769 x apolipoprotein A-1 + 0.134 x haemoglobin A_{1c} (%) (if diabetic) + 0.825 (if current smoker) + 0.427 (if family history of premature MI) + 0.00742 x (lipoprotein(a)-10) (if lipoprotein(a) >10 and apolipoproteinB-100 ≥ 100)

Model B, the Reynolds Risk Score for women:

10-year CVD risk(%) = $[1 - 0.98634^{(\exp[B-22.325])}] \times 100\%$ where

B = 0.0799 x age + 3.137 x natural logarithm (SBP) + 0.180 x natural logarithm (hs-CRP) + 1.382 x natural logarithm (TC) – 1.172 x natural logarithm (HDL) + 0.134 x haemoglobin A_{1c} (%) (if diabetic) + 0.818 (if current smoker) + 0.438 (if family history of premature MI)

Model B, the Reynolds Risk Score for men:

10-year CVD risk(%) = $[1 - 0.8990^{(\exp[B-33.097])}] \times 100\%$ where

B = 4.385 x natural logarithm (age) + 2.607 x natural logarithm (SBP) + 0.963 x natural logarithm (TC) – 0.772 x natural logarithm (HDL) + 0.405 (if current smoker) + 0.102 x natural logarithm (hs-CRP) + 0.541 (if family history of premature MI)

For the purpose of our study, we will be using the clinically simplified model B for women and men, as most of the variables required for this model were measured in our study population. The model we will be using for men is one that includes the β-coefficient for HbA_{1c} (%) (not yet published) provided to us by the authors of the Reynolds Risk Score (personal communication with Paul Ridker and Nancy Paynter, July 2010). This is listed below:

10-year CVD risk(%) = $[1 - 0.908^{(\exp[B-30.651])}] \times 100\%$ where

B = 4.034 x natural logarithm (age) + 2.562 x natural logarithm (SBP) + 0.800 x natural logarithm (TC) – 0.760 x natural logarithm (HDL) + 0.352 (if current smoker) + 0.108 x natural logarithm (hs-CRP) + 0.487 (if family history of premature MI) + 0.098 x haemoglobin A_{1c} (%) (if diabetic)

Family history of premature MI is not available for our study population. According to the authors, however, this has a minor effect on other risk factors.

There is a variety of risk scores available for assessing CVD risk, incorporating traditional as well as emerging additional risk factors, and also calibrated for specific populations. The ideal situation is to develop, or at least recalibrate, a risk score for the specific population that is being studied, in this case the black South African population. This will only be possible, however, once there are outcome data from a longitudinal study with an adequate follow-up interval. Bearing in mind that the goal of this thesis is not to determine absolute risk, but rather to stratify the population according to the risk categories, the use of an existing risk score is appropriate. Any errors that may occur because the study population's own data have not been used will be consistent across the strata and should therefore not significantly affect conclusions drawn from the data.

2.4. THE ROLE OF DIET IN CVD

2.4.1. INTRODUCTION

As has been shown earlier in this chapter, there are a myriad of factors that play a role in the development of CVD, and specifically atherosclerosis. Irregular fruit and vegetable consumption was identified as one of the nine risk factors that accounted for ≈90% of the AMI observed in African populations (Steyn *et al.* 2005). This highlights the fact that diet plays an important role in atherosclerosis in the African population. This role is a dual one: on the one hand, there are nutrients that, when consumed in excess, play an active role in the progression of atherosclerosis, and on the other hand, there are nutrients that play an important role in the protection against or the prevention of atherosclerosis, and a deficiency of these nutrients has deleterious effects. Therefore, in breaking down the enormous topic of "the role of diet in atherosclerosis", it appears to be advisable to view it from both sides of the atherosclerosis process, in other words, the excess of potentially harmful nutrients as against the deficiency of protective nutrients.

Another issue that needs to be addressed when discussing the role of diet in CVD, or in any disease for that matter, is: how does one assess this? In nutritional epidemiology, the focus has long been on the impact of single dietary components, such as nutrients, on health and health outcomes. It is fairly obvious, however, that the human diet is complex, and conclusions about the effect of the consumption levels of a single nutrient, food or dietary constituent on a specific health outcome may be misleading. There is a growing body of evidence showing that diets must be investigated in a more holistic way; in other words, it is preferable to study diet patterns and quality rather than single nutrients. Investigating dietary patterns and quality will ensure that issues such as nutrient interactions are taken into account.

In this section of the literature chapter, the issues surrounding the dual role of diet in the prevention or progression of atherosclerosis, as well as the role of nutrients and foods in the atherosclerotic process, will be discussed. First, there will be a brief discussion of the process of atherosclerosis, followed by one on the role of nutrients and foods in atherosclerosis. This will be followed by a look at how to assess diet quality by exploring the various diet quality scores that are available.

2.4.2. ATHEROSCLEROSIS

To be able to understand fully the role of diet in the development of atherosclerosis, one first needs to understand the basic process of atherosclerosis, which will be discussed briefly.

This will be followed by a more detailed analysis of the role that specific nutrients play in atherosclerosis.

According to Packard and Libby (2008), atherosclerosis was traditionally seen as a "plumbing problem". In other words, the degree of stenosis on an angiogram and symptoms and signs of ischaemia, which indicated impaired perfusion of target tissues, provided the main tools for assessing atherosclerosis. In more recent times, various observations, such as the fact that many MIs occur in individuals without previous ischaemic symptoms, have shifted the understanding of this disease (Packard & Libby, 2008). Understanding of the pathophysiology of atherosclerosis traditionally rested on the cholesterol hypothesis. Although cholesterol plays an important role in atherosclerosis, many individuals who experience MIs have cholesterol concentrations at or below the NCEP threshold of 5.2mmol/L TC and 3.4mmol/L LDL cholesterol (Castelli, 1996). A better understanding of vascular biology led to the concept of the atheroma as a graveyard of cellular lipid debris enrobed by a capsule of proliferated smooth muscle cells (SMCs). In this section, the pathophysiology of atherosclerosis will be briefly discussed according to the current understanding of the atherosclerosis process (Libby & Theroux, 2005). This will take the form of a schematic representation (Figure 2.3).

In the schematic representation (Figure 2.3) it can be seen that atherosclerosis is initiated when the endothelium is exposed to bacterial products or various other risk factors (as explained in figure 2.3). This exposure results in the endothelium expressing adhesion molecules such as P-selectin and VCAM-1. This results in the sticking of blood leucocytes to the inner surface of the arterial wall and then the transmigration of these leucocytes into the arterial intima. From here the blood leucocytes (mainly mononuclear phagocytes and T lymphocytes) communicate with endothelial cells and SMCs, resulting in the SMCs migrating from the tunica media into the intima, where these cells proliferate to form a rich and complex extracellular matrix. Together with the endothelial cells and monocytes, they secrete matrix metalloproteinase (MMPs) in response to various oxidative, haemodynamic, inflammatory and autoimmune signals. Certain constituents of the extracellular matrix, (notably proteoglycans) bind lipoproteins, prolonging their residence in the intima and rendering them more susceptible to oxidative modification and glycation. The products of lipoprotein modification then sustain and propagate the inflammatory response. With progression of the lesion, calcification may occur. The extracellular lipid matrix that accumulates in the intima can coalesce, forming the classic lipid-rich "necrotic" core of the atherosclerotic plaque (Libby & Theroux, 2005).

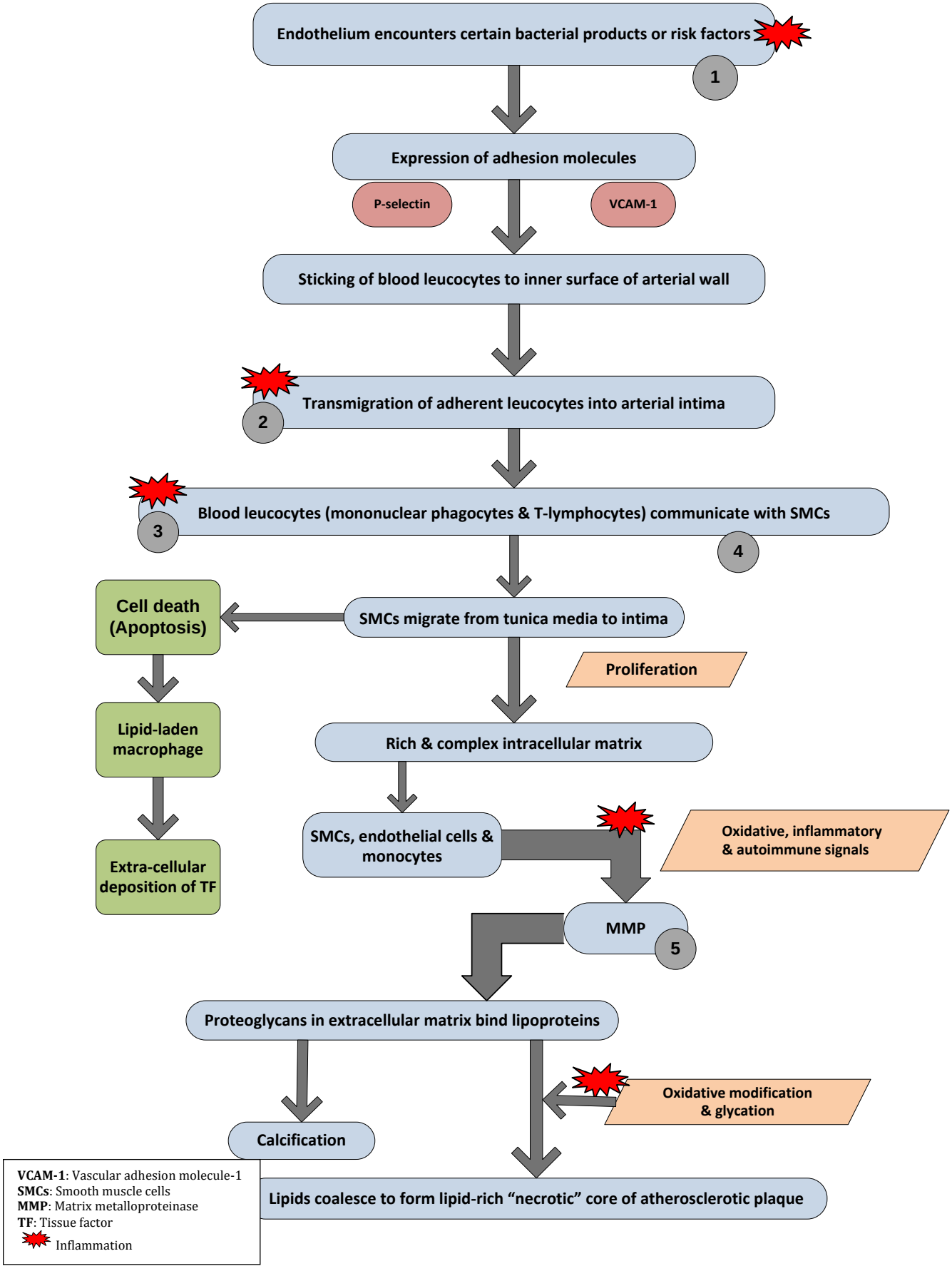


Figure 2.3 Simplified schematic representation of atherosclerosis process

Explanatory notes for Figure 2.3.

1. The risk factors include diet high in saturated fat, dyslipidaemia, vasoconstrictor hormones (hypertension), products of glycoxidation associated with hyperglycaemia (DM) or pro-inflammatory cytokines derived from adipose tissue (obesity), insulin resistance and smoking (Libby & Theroux, 2005; Packard & Libby, 2008).

2. Transmigration depends in large part on the expression of chemoattractant cytokines regulated by signals associated with traditional and emerging risk factors for atherosclerosis (Packard & Libby, 2008).

3. Mediators for this include small molecules that include lipid mediators such as prostanoids and other derivatives of arachidonic acid e.g. leucotrienes. Also of importance are protein mediators of inflammation and immunity, including cytokines and complement components.

4. This communication process is dependent on inflammatory and immunity mediators.

5. Matrix metalloproteinases in balance with their endogenous tissue inhibitors modulate numerous functions of vascular cells, including activation, proliferation, migration of smooth muscle cells and cell death, as well as new vessel formation, geometric remodelling, healing or destruction of extracellular matrix of arteries and the myocardium.

As mentioned earlier, the traditional viewpoint that risk of events was dependent on the degree of stenosis and which envisioned atherosclerosis as a segmental or focal disease has undergone radical revisions (Libby & Theroux, 2005). It is now recognised that for much of its life history, the atherosclerotic lesion grows outward, or abluminally, rather than inward. Therefore, a substantial burden of atherosclerosis can exist without producing a stenosis. By the time lesions have progressed to the point of causing stenoses, intimal atherosclerosis usually abounds in widespread distribution (Libby & Theroux, 2005). This is depicted in Figure 2.4, which is a schematic representation of the life history of an atheroma.

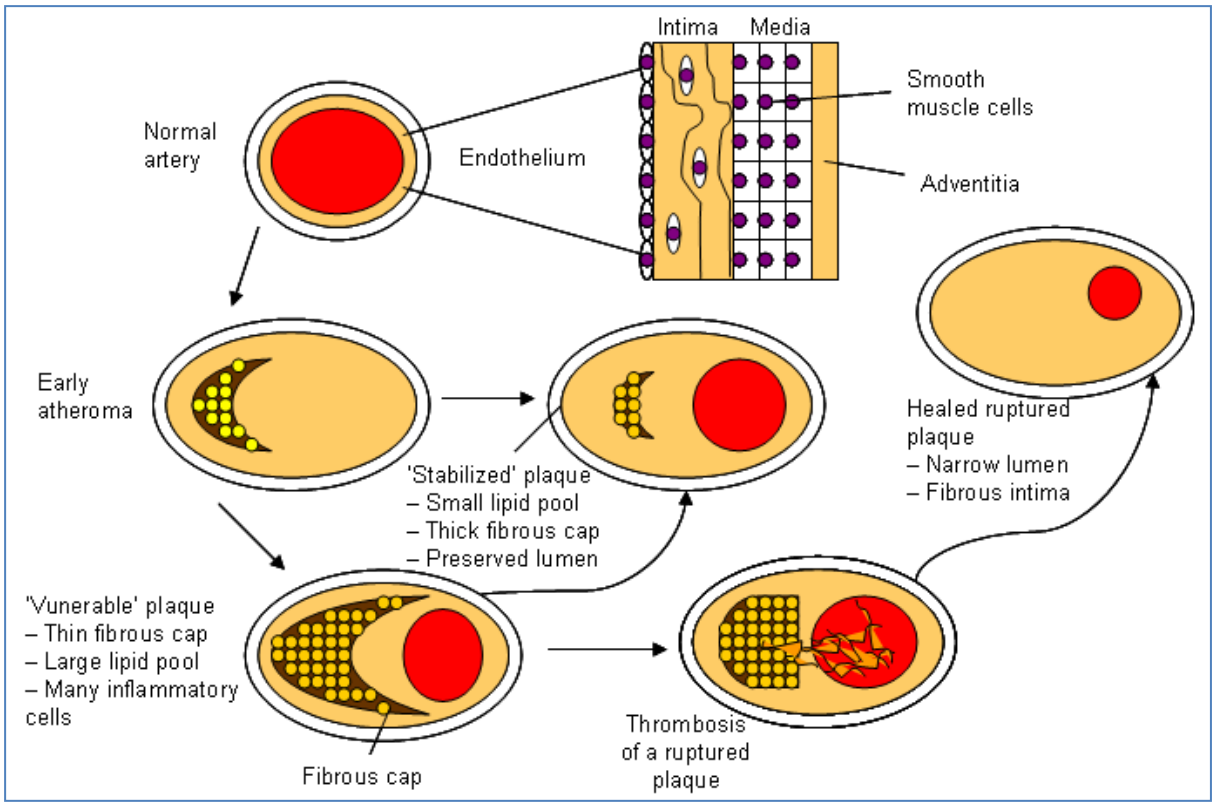


Figure 2.4 Schematic representation of the life history of an atheroma (Adapted from Libby, 2002)

As Figure 2.4 illustrates, the most common cause of all lethal coronary thrombosis appears to be the physical rupture of the plaque's protective fibrous cap. Disrupted plaques provoke thrombosis in numerous ways. Contact of blood with collagen in the plaque's extracellular matrix can trigger platelet activation or tissue factor produced by macrophages, and SMCs then activate the coagulation cascade. The disrupted plaque thereby represents a "solid-state" stimulus to both thrombosis and coagulation; these pathways reinforce each other, as thrombin generation amplifies the activation of platelets and other cells in the lesion. Conversion of fibrinogen to fibrin and release of von Willebrand factor from activated platelets can provide the cross-linking molecular bridges between platelets that yield the dense, three-dimensional network of platelets entrapped in fibrin, characteristic of the "white" arterial thrombus (Libby & Theroux, 2005).

In addition to the solid state of the disrupted plaque, the "fluid phase" of the blood can further predispose toward coronary thrombosis, through increased levels of haemostatic proteins. Plasminogen activator inhibitor-1, for example, extinguishes the body's natural fibrinolytic mechanism that combats persistence and accumulation of thrombi by inhibiting urokinase-like and tissue-type plasminogen activators. Elevated levels of circulating PAI-1 and fibrinogen are observed in disease states such as diabetes and obesity, and mediators of hypertension such as angiotensin II can augment PAI-1 expression by various cell types.

Furthermore, disrupted plaques can elaborate particulate TF, which can heighten the thrombogenicity of the blood (Libby & Theroux, 2005). Understanding the fluid-phase changes gives a better appreciation of the concept of the "vulnerable patient". This is defined as a patient who is susceptible to an acute coronary syndrome or sudden cardiac death, based on plaque (solid-phase), blood (fluid-phase) or myocardial vulnerability (Naghavi *et al.* 2003).

This summary, based on the work of Libby and Theroux, provides us with a better understanding of the atherosclerosis process. Instead of atherosclerosis being seen as a localised disease, this highlights the fact that inflammation, which is a more diffuse problem, is the underlying factor. Previously, it was thought that the role statins played in the treatment of CVD was mainly due to the cholesterol-lowering property of the drug. However, retrospectively, it has been observed that statins also decrease the inflammatory response (decrease hs-CRP level) (Sadowitz *et al.* 2010), thus showing that improvement in the lipid profile as well as the inflammatory status probably explains the effectiveness of statins in treating CVD. In the JUPITER (Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial, statins were also beneficial in individuals with normal to low LDL-C but with an elevated marker of inflammation (Ridker *et al.* 2008a). Treatment and prevention strategies have therefore had to be re-evaluated. Treatment would have to be more comprehensive than the local therapies of, for example, bypass surgery or percutaneous revascularisation. Prevention would also have to address the issue of inflammation and ways to combat this.

From the schematic representation of the atherosclerosis process (Figure 2.3), it is clear that numerous factors can influence this process, such as hypertension and smoking. Dietary intake, specifically, can also influence or contribute to this process in numerous ways. A diet high in saturated fat and cholesterol has been shown to raise LDL-C and also to stimulate the endothelial cells to express VCAM-1 (Li *et al.* 1993; Packard & Libby, 2008). Antioxidants play a role in lowering oxidative stress, which in turn plays a role in inflammation. A generally poor or unhealthy diet contributes to the development of conditions such as obesity and insulin resistance, which in turn play a role in atherosclerosis. In the next section, the contribution of diet to atherosclerosis will be discussed in detail, in the context of highlighting where specific nutrients can play a role in this process.

2.4.3. NUTRIENTS, FOODS, DIETARY QUALITY AND CVD

There is a large body of evidence linking diet to either the development of, or protection against CVD. Methods for assessing this link between diet and CVD as well as for assessing whether a population is adhering to healthy dietary guidelines have evolved over the last few decades. The dietary adequacy of a population can be assessed using either a quantitative or a qualitative approach. Both evaluation methods can be used at an individual as well as at a population level. With the quantitative approach, nutrient adequacy is based on the comparison of nutrient intakes per day with tables of nutrient reference intakes, while the qualitative approach is based on foods rather than nutrients, and provides guidelines for healthy food choices (Gibson, 2005). In the quantitative approach, there are numerous issues that need to be taken into account with regard to which nutrient-based reference levels are best to use. The definitions and applications pertaining to Dietary Reference Intakes (DRIs) are summarised in Tables 2.10 and 2.11, in order to give clarity as to why specific nutrient reference intakes are used and which will have to be used for the purpose of this study. From these tables it can be concluded that for the quantitative approach, that is, nutrient adequacy, one must not use recommended dietary allowance (RDA) but rather estimated average requirement (EAR) when assessing nutrient intake for a group. By definition, the RDA is set at an intake level that exceeds the requirements of 97 to 98% of all individuals; therefore, the use of the RDA as a cut-off to calculate the proportion of individuals in the group with inadequate intakes will result in a serious overestimation of the proportion at risk (Gibson, 2005).

The qualitative approach uses food-based dietary guidelines. Food-based guidelines give advice on the consumption of types of foods or food components for which there are diet-related public health issues specific to a country. These guidelines usually relate to the total diet and are expressed in terms that are less technical and easier to understand than nutrient recommendations. Food-based dietary guidelines, however, lack precision and are often interpreted differently by different individuals. Several indices based on food-based dietary guidelines have been developed to evaluate the overall quality of the diet (Gibson, 2005). These are discussed in more detail in section 2.4.3.2.

Table 2.10 Dietary Reference Intakes (DRIs) definitions (Gibson, 2005)

Dietary Reference Intakes (DRI)	The term is a collective one and refers to a set of at least four nutrient-based reference values.
Estimated average requirement (EAR)	The intake that meets the estimated needs of a nutrient of 50% of individuals in a specified gender group, at the given life-stage. It is a dietary intake value and includes an adjustment for an assumed bioavailability of the respective nutrient. The EAR is used as the basis for setting the RDA; therefore, if sufficient scientific evidence is not available to set an ERA, no RDA is set.
Recommended Dietary Allowance (RDA)	The intake that meets the nutrient needs of almost all (97–98%) individuals in that gender group, at the given life-stage. It is important to recognise that the RDA applies to individuals and not to groups and is the goal for dietary intake by the individual.
Adequate Intake (AI)	This is used in cases where the scientific evidence is inadequate to set an EAR. In such cases, the AI is used instead of the RDA. The AI is based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group of healthy people, who have normal circulating nutrient blood concentrations, growth or other functional indicators of health.
Tolerable Upper Intake Level (UL)	The maximum nutrient intake by an individual, which is unlikely to pose risks of adverse health effects in almost all (97–98%) individuals in a specified group.

Table 2.11 Application of the DRIs (Gibson, 2005)

Type of Use	For the Individual	For a Group
Assessment	**EAR*:** Use to examine probability of inadequacy of usual intake.	**EAR****: Use to estimate the prevalence of inadequate intake within a group.
	RDA: Usual intakes at or below this level have a low probability of inadequacy.	**RDA**: Do not use to assess intakes of groups.
	AI: intakes at or below this level have a low probability of inadequacy.	**AI**: Mean intake at this level implies a low prevalence of inadequate intake.
	TUIL: An intake above this level may increase the potential risk of adverse effects.	**TUIL**: Use to estimate the percentage of a population that may be at risk of adverse effects.
Planning	**RDA**: Aim for this intake.	**EAR:** Use to plan an intake with a low prevalence of inadequate intake.
	AI: Aim for this intake.	**AI**: Use to plan mean intakes.
	TUIL: An intake above this level may increase the potential risk of adverse effects.	**TUIL**: Use to plan an intake with a low prevalence of potential risk of adverse effects.

*Accurate measure of usual intake is required; can only be used as one component of a more comprehensive assessment of nutritional status.

**Statistically valid approximation of usual intake is necessary.

2.4.3.1. THE HARMFUL OR PROTECTIVE ROLE OF SPECIFIC NUTRIENTS ON ATHEROSCLEROSIS

In this section, the role that specific nutrients play in the development or prevention of atherosclerosis will be discussed.

FATTY ACIDS

It is the position of the American Dietetic Association (ADA) that dietary fat for the adult population should provide 20% to 35% of total energy, while emphasising a reduction in saturated fatty acids (SFAs) and trans fatty acids (TFAs) and an increase in n-3 polyunsaturated fatty acids (PUFAs) (ADA & Dietitians of Canada, 2007). Despite lack of evidence that high-fat diets *per se* promote CVD, dietary recommendations emphasised low-fat diets more than dietary fat quality up until the late 1990s. From recent literature it can be seen that dietary fat quality clearly plays a greater role than quantity with respect to cardiovascular and metabolic risk factors such as HDL-C and LDL-C, TG, abdominal obesity, insulin resistance and the metabolic syndrome, as well as T2DM (Erkkilä *et al.* 2008; Willett, 2002). A brief discussion follows on the different fatty acids and their role, if any, in the development of atherosclerosis.

- **Saturated fatty acids**

Saturated fatty acids contain no double bonds. They include lauric acid (C12:0, commonly found in coconut oil), myristic acid (C14:0, commonly found in butter fat and coconut oil), palmitic acid (C16:0, found in most fats and oils) and stearic acid (C18:0, found in most fats and oils, cocoa butter and fully hydrogenated vegetable oils) (ADA & Dietitians of Canada, 2007). Epidemiological studies have shown a link between SFAs and incident CHD (ADA & Dietitians of Canada, 2007; Erkkilä *et al.* 2008) and clinical studies have shown that SFAs raise TC and LDL-C, which play a prominent role in plaque formation (Schaefer, 2002). For every 1% increase in energy from SFA, LDL-C levels increase by 0.034 to 0.044mmol/L, while HDL-C also increases by 0.01 to 0.013mmol/L (ADA & Dietitians of Canada, 2007). There are differences, however, in how individual fatty acids affect serum lipids. Lauric acid and myristic acid have a greater effect on increasing TC than palmitic acid (Mensink *et al.* 2003), while stearic acid has a neutral effect on TC, LDL-C and HDL-C, postulated to be due to its rapid conversion to oleic acid (cis18:1n-9) (Grundy & Denke, 1990). Lauric acid, and not myristic or palmitic acid, lowers TC:HDL ratio because of an increase in HDL-C. It is important to remember, however, that foods contain a mixture of SFAs, and therefore trying to select foods based on individual SFA content is not recommended (ADA & Dietitians of Canada, 2007). It is therefore evident that the type of SFA plays an important role in atherosclerosis, mainly through its role in increasing LDL-C.

- **Monounsaturated fatty acids**

Monounsaturated fatty acids (MUFAs) contain one double bond. The most abundant MUFA in the diet is oleic acid (C18:1n-9). Oleic acid is found in most fats and oils, as well as nuts, seeds and avocados (ADA & Dietitians of Canada, 2007). Earlier studies have shown that, when substituting SFAs with MUFAs, the MUFAs do have a hypocholesterolaemic effect (Dreon *et al.* 1990; Mensink & Katan, 1992). Overall data however, indicate that MUFAs do not lower LDL or HDL cholesterol relative to SFAs as much as PUFAs do (Mensink & Katan, 1992). A slightly protective effect against CHD was observed by replacing 5% of energy from carbohydrates with equivalent energy from MUFAs. However, many prospective studies did not find any association between MUFA and the risk of CHD. Studies have shown that MUFAs in serum lipids have either not been associated with CHD risk or suggested a small increase in risk of CHD (Erkkilä *et al.* 2008). When compared with carbohydrates, MUFAs decrease TG, increase HDL-C and are inversely related to TC:HDL-C ratio. A meta-analysis of studies on individuals with DM showed that high-fat diets with 22 to 33% energy from MUFAs resulted in lower plasma TC, VLDL and TG levels than did low-fat high-carbohydrate diets (49–60% energy) (ADA & Dietitians of Canada, 2007; Garg, 1998). Although the majority of evidence shows MUFAs to have a neutral effect on CHD, they may have a slightly protective effect in diabetic patients in that decreases in TC, VLDL and TG have been documented.

- **Trans Fatty Acids**

Trans fatty acids are so named because the carbon atoms adjacent to their double bonds are on opposite sides, resulting in a straight configuration. The amount of TFA in the diet became a major concern, particularly after the Nurses' Health Study in 1993, which reported that an escalating intake of TFA was associated with an increased risk for CVD (Willett *et al.* 1993). Naturally occurring unsaturated fatty acids are generally in the *cis* configuration, which has a characteristic U-shaped bend. The *cis* configuration means that unsaturated fatty acids are less tightly packed than SFAs, and *cis* fatty acids tend to be liquids/oils at room temperature, in contrast with SFAs, which are solid at room temperature (Remig *et al.* 2010). The double bonds in TFA produce a more rigid configuration that requires less space than the *cis* double bond, resulting in a melting point around room temperature (between that of SFAs and unsaturated fatty acids) (Remig *et al.* 2010). This intermediate melting point is ideal for food manufacturers as it provides favourable characteristics such as texture and mouth feel, as well as enhancing shelf life (Remig *et al.* 2010).

There are two primary sources of TFA, the first being formed naturally by bacteria present in rumens of ruminant animals (Khanal & Dhiman, 2004). Milk and meat products from these animals contain small amounts of TFA; it is therefore impossible to eliminate TFA completely from a balanced diet (Remig *et al.* 2010). The second source is from partial hydrogenation of liquid vegetable oils. This commercial process is used to convert liquid oils to solids and to improve the oxidative stability of these fats (Remig *et al.* 2010).

The Nurses' Health Study showed that, after adjusting for age and total energy intake, the relative risk of CHD for those in the highest quintile of TFA intake was 1.5 times higher than for those in the lowest quintile (Willett *et al.* 1993). A meta-analysis by Mozaffarian *et al*. (2006) of four prospective cohort studies involving nearly 140 000 subjects revealed that a 2% increase in energy intake from TFA was associated with a 23% increase in the incidence of CHD. Potential mechanisms for this include effects on serum lipids and inflammatory markers. The effects on lipid levels are thought to be due to an increased activity of cholesteryl-ester transfer protein. These effects include increases in LDL-C, TG, Lp(a) and ratio of TC:HDL, as well as decreased HDL-C and smaller particle size of LDL-C (Ascherio *et al.* 1999; Mensink *et al.* 2003; Smit *et al.* 2009). When it comes to inflammation, TFAs have been shown to increase inflammatory markers, including CRP, IL-6 and TNFα, possibly through modulation of monocyte and macrophage activity (De Roos *et al.* 2001). Elevated levels of circulating soluble adhesion molecules, intercellular adhesion molecule-1 and VCAM-1, as well as NO-mediated endothelial cell dysfunction, have also been observed in individuals consuming large quantities of TFA (Lopez-Garcia *et al.* 2005; Mozaffarian *et al.* 2003). Other possible adverse effects include the inhibition of incorporation of other fatty acids into cell membranes, interference with elongation and desaturation of essential fatty acids, increased platelet aggregation and increased body weight (Simopoulos, 2008).

Although TFAs are found naturally in certain products, the majority of TFA consumed in the diet comes from foods containing industrially derived TFA. From the evidence, the effect this has on the development of CHD is clear, and efforts are being made, and should continue being made, to reduce the amount of TFA in commercially available foods. The AHA therefore recommends limiting TFA to less than 1% of energy intake (American Heart Association Nutrition Committee *et al.* 2006), and the ADA and NCEP all recommend limiting dietary trans fat intake from industrial sources as much as possible (NCEP, 2002). The South African Department of Health has stipulated in its regulations that oils and fats used in the retail trade and food industry must contain less than 2 grams TFA per 100 grams of oil or fat. For a foodstuff to have a label of "Trans-fat free", the TFA content must be less than 1 gram per 100 grams of fat or oil in the final product (South Africa, 2011).

- **Polyunsaturated fatty acids**

Polyunsaturated fatty acids have more than one double bond and are further classified, based on the position of the first double bond from the methyl terminus of the fatty acid, as n-6 or n-3. Linoleic acid (LA) is the major n-6 fatty acid in the diet, found mostly in vegetables and vegetable oils (corn, soybean, safflower and sunflower), with the exception of coconut and palm oils. Linoleic acid is an essential fatty acid and is a precursor for arachidonic acid (AA). The other major essential fatty acid in the body is α linolenic acid (ALA), an n-3 fatty acid, found in flaxseed, canola oil, soybean oil and walnuts. This fatty acid can be rapidly converted in the body to eicosapentaenoic acid (EPA), which can further be elongated, desaturated and β-oxidised to docosahexaenoic acid (DHA), both of which are found in fish oil (ADA & Dietitians of Canada, 2007; Schaefer, 2002). The elongation and desaturation of n-6 and n-3 PUFAs are summarised in Figure 2.5.

There are numerous prospective cohort studies in which dietary PUFA intake was associated with a lower incidence of outcomes for CVD. One such study was the Kuopia Ischaemic Heart Disease (KIHD) Risk Factor study. This was a Finnish prospective population-based study which showed that men (n=1551) with energy-adjusted dietary intake of PUFA and LA intake in the upper third were 62% less likely to die of CVD than men in the lower third, after adjustment for age. CVD mortality was also lower (58–75%) for men with proportions of serum esterified LA and PUFA in the upper versus the lower third. Serum, and to a lesser extent, dietary LA and PUFA were also inversely associated with overall mortality. This showed that dietary PUFA and, more specifically, LA intake may have a substantial cardioprotective benefit (Laaksonen *et al.* 2005). The Nurses' Health Study is the largest cohort study to provide evidence that increased PUFA intake and, more specifically, LA intake may protect against CHD, probably due to the effect on blood lipids (Hu *et al.* 1997).

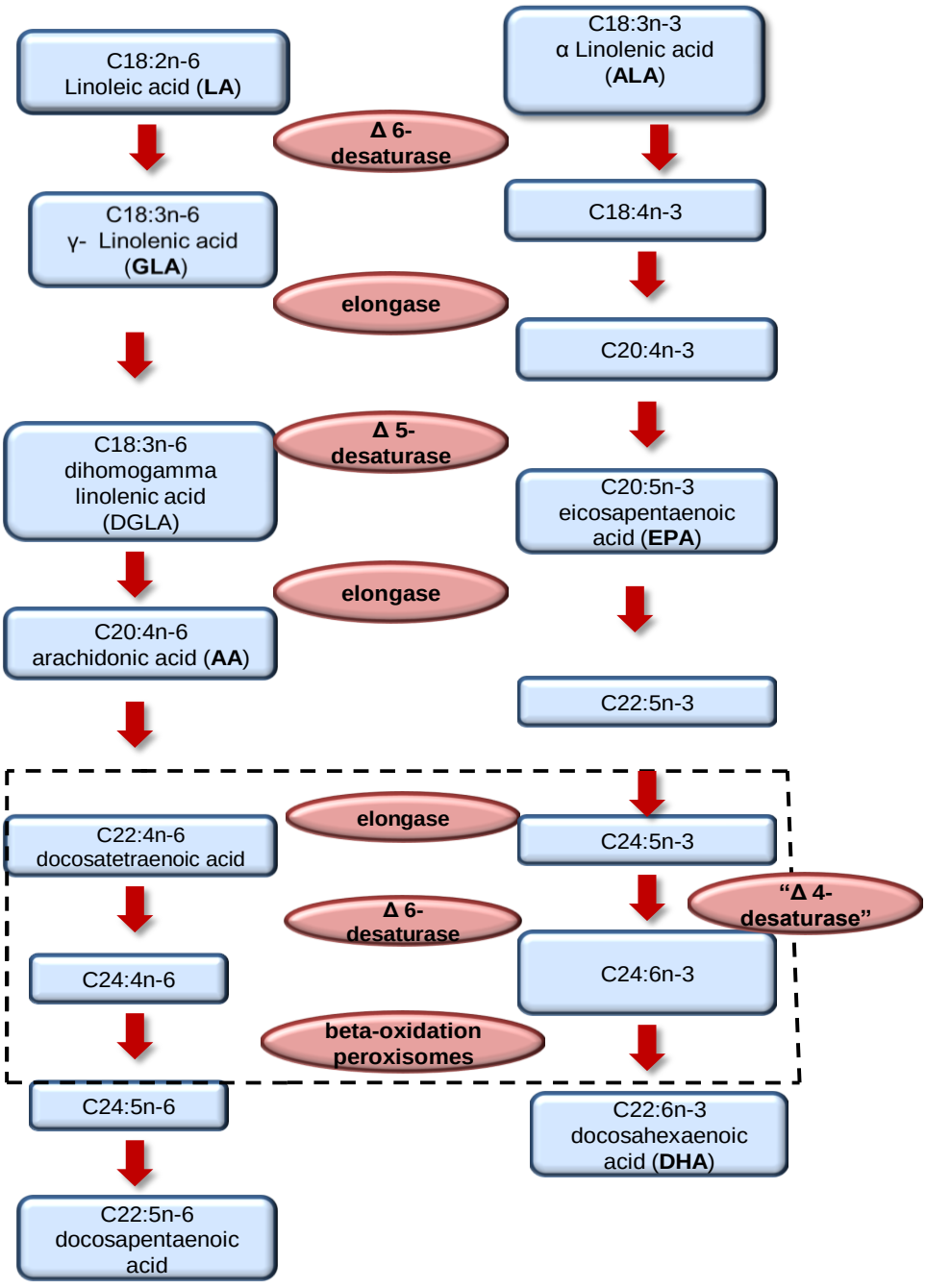


Figure 2.5 The elongation and desaturation of n-6 and n-3 polyunsaturated fatty acids (Simopoulos, 2008)

- **n-6 Fatty acids**

Linoleic acid (C18:2n-6), the predominant PUFA in the Western diet, is derived from plant sources. Linoleic acid and other n-6 fatty acids were shown to be proinflammatory (Calder, 2001), prothrombotic (Lahoz *et al.* 1997) and to promote insulin resistance (Storlien *et al.* 1997) in some early studies. This was based mainly on experimental or *in vitro* studies; however, a review of the literature showed that linoleic acid also appears to have anti-thrombotic properties, and may decrease arrhythmias and improve insulin sensitivity (Erkkilä *et al.* 2008). In 2009, the AHA Science Advisory came to the conclusion that an n-6 PUFA intake of at least 5% to 10% of energy, in the context of other AHA lifestyle and dietary recommendations, is beneficial. Lowering the n-6 PUFA intakes from their current levels would be more likely to increase than decrease the risk for CHD (Harris *et al.* 2009). This conclusion was based on evidence from numerous types of investigations, including randomised control trials (RCTs) of morbidity/mortality outcomes, case-control and cohort observational studies, short-term RCTs of surrogate risk factors as well as long-term animal feeding experiments (Harris *et al.* 2009; Kris-Etherton *et al.* 2010). Blood/tissue LA has also been shown to be inversely associated with CHD risk (Harris *et al.* 2007).

There is another point of view that a lower ratio of n-6/n-3 PUFA intake is more desirable in reducing the risk of CHD. This has been summarised in a review by Simopolous (2008). There is competition between n-6 and n-3 for desaturation enzymes, although the Δ-4 and Δ-6 desaturases prefer n-3 to n-6 fatty acids (Figure 2.5). According to the review, because of increased amounts of n-6 in Western diet, eicosanoid metabolic products from AA, specifically prostaglandins, thromboxanes, leucotrienes, hydroxy fatty acids and lipoxins, are formed in larger quantities than those formed from n-3, especially EPA. Eicosanoids, when formed in large amounts, contribute to the formation of thrombi and atheromas as well as to allergic and inflammatory disorders, particularly in susceptible people. The mechanisms for this include the fact that LA increases LDL oxidation because diets enriched in LA increase the LA content of LDL and thereby also its susceptibility to oxidation. Linoleic acid-enriched diets especially affect oxidation of small, dense LDL and inhibit EPA incorporation from dietary fish oil supplements (Simopoulos, 2008). According to a commentary by Kris-Etherton *et al*. (2010) on behalf of the ADA, there are several misunderstandings, extrapolations and assumptions that need to be considered. In summary, it is important to note that variations in LA intake in the diets of humans do not substantially affect tissue AA levels, as only about 0.2% of dietary LA is converted to AA (Hussein *et al.* 2005). The role of eicosanoids *per se* in the inflammatory component of CHD is not that clear, especially when one considers that AA (and LA itself) can be converted to a variety of both anti-inflammatory as well as proinflammatory mediators (Kris-Etherton *et al.* 2010).

According to Erkkilä *et al*., warnings that "excessive" intake of n-6 fatty acids at the levels commonly consumed in most Western countries may increase the risk of chronic diseases such as T2DM and CVD are not supported by scientific literature (Erkkilä *et al.* 2008). A systematic review and meta-analysis looking at effects on CHD of increasing total or n-6 PUFAs in place of SFAs showed a reduction in CHD events by 19% and a decrease in CHD risk by 10% for every 5% energy increase in consumption of PUFA. This meta-analysis also found no evidence for increased risk in trials with PUFA consumption at very high levels (mean 14.9%E, range 8.0%E to 20.7%E) (Mozaffarian *et al.* 2010). This raises questions about dietary recommendations having an upper limit of 10%E, suggesting that these guidelines should be revisited.

- **n-3 Fatty acids**

Alpha-Linolenic acid (C18:n-3) is an essential fatty acid found mostly in canola and soybean oil as well as in some nuts, while the longer-chain n-3 fatty acids EPA and DHA are mostly derived from fatty fish. A meta-analysis of five cohort studies looking at the association between ALA and CHD risk suggested that there was a non-significant inverse relationship between ALA intake and CHD risk in men and women (Brouwer *et al.* 2004). More recent follow-up studies from two of these cohort studies revealed a modestly lower risk of CHD in men with higher median ALA intake (Mozaffarian *et al.* 2005), as well as a reduced risk of sudden cardiac death, but no association with other fatal or non-fatal events (Albert *et al.* 2005). The Health Professionals' study reported that a 1% energy increase in ALA intake was associated with a 40% lower risk of MI after adjustment for total fat intake (Albert *et al.* 2005). Recent epidemiological studies continue to support a beneficial effect of dietary ALA, particularly in the presence of a low fish intake. According to Erkkilä *et al.* (2008), overall data from prospective observational studies on association of dietary ALA with cardiovascular outcomes are less consistent and weaker than those for linoleic acid. Findings nevertheless suggest an inverse association of ALA with CVD risk.

Alpha-Linolenic acid can be elongated and desaturated to EPA and DHA, although this conversion of ALA to EPA and especially to DHA in humans is very low. As previously mentioned, they are derived mostly from fatty fish. Consumption of fish or fish oils has favourable effects on several cardiovascular risk factors, such as being antiarrhythmic and antithrombotic, and also lowering serum-TG, heart rate and blood pressure (Mozaffarian & Rimm, 2006). The beneficial effects may result from altered cell membrane fluidity and receptor responses following incorporation of n-3 PUFA into cell membrane phospholipids (Clandinin *et al.* 1991; Feller & Gawrisch, 2005) as well as direct binding of n-3 PUFA to cytostolic receptors that regulate gene transcription (Mozaffarian, 2009; Van den Heuvel,

2004). A meta-analysis of 11 cohort studies which examined fish consumption and CHD concluded that moderate consumption of fish (at least one meal per week) is associated with lower CHD mortality than a fish intake of less than one meal per month (He *et al.* 2004). Results from case-control studies, prospective cohort studies and RCT all indicate that modest consumption of fish or fish oil lowers risk of CHD mortality, specifically from CHD death and sudden cardiac death (Mozaffarian & Rimm, 2006). The effect appears to be non-linear in that, compared with little or no intake, modest consumption (~250mg/day) of the marine n-3 PUFA EPA and DHA significantly lowers risk of cardiac mortality, whereas higher intakes do not substantially further lower risk, suggesting a threshold of effect (Mozaffarian & Rimm, 2006). It does seem that the beneficial effect of fish intake is related to the n-3 PUFA content, as observational studies of fish consumption and randomised controlled trials of n-3 PUFA supplementation have shown very similar results (Mozaffarian, 2009). Also relevant are the type and amount of fish consumed. There is definitely lower risk associated with fatty (oily or dark meat) fish, compared with lean (white meat) fish (Mozaffarian *et al.* 2003; Oomen *et al.* 2000). The quantity of fish needed to consume an average 250mg/day of EPA + DHA varies depending on the particular species of fish, but for fatty fish (e.g. anchovies, herring, salmon, sardines, trout, white tuna) it is ~1–2 servings per week (Mozaffarian & Rimm, 2006). There is, of course, also the concern and controversy regarding the potential effects of exposure to the mercury found in some fish. According to Mozaffarain (2009), based on current evidence, the health risks for adults of not consuming fish outweigh potential risks from mercury or other contaminants. The other issue is balancing increasing fish intake with concerns of sustainability of fish stocks. The Marine Stewardship Council (MSC) is a global organisation working with fisheries, seafood companies, scientists, conservation groups and the public to promote the best environmental choice in seafood (www.MSC.org). The South African Sustainable Seafood Initiative (SASSI) is a collaborative initiative that aims to promote voluntary compliance with the law through education and awareness, to shift consumer demands from overexploited species to more sustainable options and to create awareness around marine conservation issues (www.wwfsassi.co.za). These initiatives are aimed at addressing the dilemma of increasing demand for fish with the problem of diminishing fish stocks.

Another possible benefit of n-3 fatty acids, particularly supplementation thereof, is a decrease in homocysteine levels (homocysteine is discussed in section 4.1.3.5). Potential mechanisms for an observed homocysteine-lowering effect by n-3 PUFAs as well as the implication of this effect have not yet been fully investigated or understood (Huang *et al.* 2011).

Summary statement on the Health Significance of Fat Quality

The International Expert Meeting: Health Significance of Fat Quality of the diet, held in Spain in 2009, published the following summary statement (Diekman *et al.* 2009):

Dietary guidelines:

- The quantity of fat is an important factor determining energy intake, which should be balanced with energy expenditure to achieve and maintain healthy weight.
- The quality of fat in the diet is important for normal growth and development, and has a marked impact on blood cholesterol and the occurrence of CHD and stroke.
- In line with authoritative international health bodies and current evidence, the following recommendations on the quality of fat in the diet are made for optimal health across the life course worldwide, from an age of two years onwards:
- Fat may provide up to 30–35% of the daily energy intake;
- Saturated fat should provide no more than 10% of the daily energy intake;
- Essential polyunsaturated (n-6 and n-3) fats should contribute 6–10% of the daily energy intake;
- The intake of trans fats should be less than 1% of the daily energy intake;
- The remainder of the energy from fat can be provided by MUFAs.

GLYCAEMIC RESPONSE TO CARBOHYDRATES

Intake and digestion of dietary carbohydrates ultimately results in glucose uptake in the bloodstream. Post-prandial hyperglycaemia has been seen to play a major role in all-cause and cardiovascular mortality in normal populations (The DECODE study group on behalf of the European Diabetes Epidemiology Group, 1999). The blood glucose concentration 2 hours after a standard glucose tolerance test is taken as a surrogate measure of this mealtime-induced hyperglycaemia (Brand-Miller, 2003). The Hoorn study, in particular, revealed that there was a significant association between 8-year risk of cardiovascular death and 2-hour post-load blood glucose in subjects with normal fasting glucose concentration, even after adjustment for known risk factors (De Vegt *et al.* 1999). Hyperglycaemia appears to cause damage to the epithelium through a number of mechanisms. It is associated with increased IMT in non-diabetic individuals and it also interferes with vasodilation by inhibiting nitric oxide synthase and thereby reducing the production of nitric oxide. Excessive post-prandial hyperglycaemia is also directly toxic to the endothelium, increasing protein glycation, generating oxidative stress and causing impaired endothelial function (Ceriello *et al.* 1999).

Another consequence of hyperglycaemia is hyperinsulinaemia. Insulin resistance (IR) and compensatory hyperinsulinaemia are also implicated in the development of dyslipidaemia (high VLDL-C, high TG and low HDL-C), hypertension and impaired fibrinolysis, as well as other abnormalities that contribute to risk of CHD (Facchini *et al.* 2001; Steiner & Lewis, 1996).

It is important that both the amount and nature of carbohydrate be considered because both contribute equally to the variation in postprandial glycaemia (Brand-Miller, 2003). The type of dietary carbohydrate, as measured by glycaemic index (GI) or glycaemic load (GL), has also been associated with CHD risk independently of known risk factors. Glycaemic index is a ranking of carbohydrates according to their effect on blood glucose concentration, while GL is calculated, using GI, carbohydrate content and actual or estimated intake of food items. In the Nurses' Health Study, low GI and/or low GL diets were independently associated with reduced risk of heart disease (Liu *et al.* 2000b). An association between high GL from refined carbohydrates and increased risk of CHD, on the other hand, was seen in a large prospective cohort study (>15000 middle-aged women) (Beulens *et al.* 2007). However, a recent meta-analysis showed only borderline significant associations of increased GI with heart disease (Barclay *et al.* 2008) and more recent studies have failed to show that a low GI/GL diet significantly lowers risk of CHD in men and women (Grau *et al.* 2011; Levitan *et al.* 2010). However, in a Swedish study, it was seen that women in the top quartile of GI had a non-significant 12% higher rate of MI than those in the bottom quartile, and those in the top quartile of dietary GL had a non-significant 22% higher rate of MI than those in the bottom quartile (Levitan *et al.* 2010). This supports a role for GI/GL in heart disease. Although there is evidence that preventing post-prandial hyperglycaemia lowers the risk, based on the mechanisms mentioned earlier, further research is needed in this area, particularly in men, as most of the available studies were done on women.

FIBRE

According to the position statement of the ADA on health implications of dietary fibre, the recommended intake of dietary fibre should be 20 to 35g/day for adults (Marlett *et al.* 2002). Over the past few years, confusion has been generated regarding the definition of dietary fibre, due to conflicting definitions being available (Mann & Cummings, 2009). In 2008, the Codex Committee on Nutrition and Foods for Special Dietary Uses came to a consensus regarding the definition of dietary fibre (Codex alimentarius commission, 2009):

Dietary fibre means carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:

- Edible carbohydrate polymers naturally occurring in the food as consumed
- Carbohydrate polymers which have to be obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities
- Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Numerous studies have shown that dietary fibre, particularly water-soluble fibre, lowers blood TC and LDL-C levels. These fibres include β-glucan, (found in cereals, particularly oats and barley as well as in yeast, bacteria, algae and mushrooms) as well as psyllium, pectin (found mainly in citrus fruit and apples) and guar gum (leguminous plant originating from India and Pakistan) (Theuwissen & Mensink, 2008). A meta-analysis of studies investigating the cholesterol-lowering effects of these fibres found that these various fibres reduce total and LDL cholesterol by similar amounts (Brown *et al.* 1999). The exact mechanism by which these water-soluble fibres lower serum LDL-C levels is not known. Evidence suggests that water-soluble fibres may interfere with lipid and/or bile acid metabolism. There are other suggested mechanisms, such as inhibition of hepatic cholesterol synthesis by fermentation products (short-chain fatty acids: acetate, propionate and butyrate) and delayed absorption of macronutrients leading to increased insulin sensitivity (Theuwissen & Mensink, 2008). The lowering effect is small within a practical range of intake, for example, 3g soluble fibre from oats (three servings of oatmeal, 28g each) can decrease TC and LDL-C by ≈0.13mmol/L. Brown *et al.* (1999) concluded that increasing water-soluble fibre makes only a small contribution to dietary therapy to lower cholesterol. The ADA, in its position statement, recommends that, except in certain therapeutic situations, dietary fibre should always be obtained through the consumption of foods. In addition to fibre, minimally processed foods such as fruit, vegetables, legumes and whole-grain and high-fibre grain products provide micronutrients and non-nutritive ingredients that are essential components of healthful diets. A fibre-rich diet tends to be lower in energy density, have a lower fat content, is larger in volume and richer in micronutrients, all of which may have an impact on obesity, T2DM and CVD (Marlett *et al.* 2002).

It therefore makes sense to look at the effect of foods, in particular whole-grain foods, on CVD. The term "whole grain" means the intact grain or dehulled, ground, milled, cracked or flaked grain, where the constituents – endosperm, germ and bran – are present in proportions that represent the typical ratio of those fractions occurring in whole cereal, and includes whole meal. Refined grains are those where the bran and germ have been removed, having a reduced nutrient content, because the milling process results, in varying degrees, in the loss of dietary fibre, vitamins, minerals, lignans, phytoestrogens, phenolic compounds and phytic acid (Slavin, 2004).

According to a review by Flight and Clifton (2006), whole-grain consumption has been shown to be linked to improvements in BMI, insulin sensitivity and DM. These are all known risk factors for heart disease. A meta-analysis of five large prospective studies revealed that persons in the highest quintile for whole-grain intake had a 28% reduction in their risk for CHD, compared with those in the lowest quintile (Anderson *et al.* 2000; Anderson, 2003). This provided strong support for the hypothesis that generous intakes of whole grains are cardioprotective. These studies had been controlled for other dietary and lifestyle factors, including BMI, and the associations behaved in a dose-response manner. This fact led the authors, Pereira and Liu (2003), to conclude that "…whole-grain foods, through their fibre, antioxidants and other components, reduce the risk of CHD in a causal manner". Flight and Clifton (2006) ascertained that findings from studies were consistently positive for whole grains; this occurred using a variety of different data collection methodologies. They, together with other reviewers, concluded that there is good evidence that whole-grain foods substantially reduce the risk of CHD. Whether all grains are equal in their effectiveness, or the effectiveness of different parts of the grain, could not be concluded from these studies (Flight & Clifton, 2006; Jacobs *et al.* 2000).

Anderson *et al*. (2000) explored the relationship between whole grains and whole-wheat bread, cereal fibre, total dietary fibre, fruit and vegetables and risk of CHD by doing a pooled analysis. After adjusting for confounders, they found the strongest inverse association was between whole-grain and whole-wheat bread intake and risk of CHD. Cereal fibre by itself had the least influence on CHD risk (Anderson *et al.* 2000). This analysis and other studies (Burr *et al.* 2003; Jacobs *et al.* 2000; Truswell, 2002) highlight the thinking that health benefits stem from more than just one type of fibre; whole grain is nutritionally more complete because it delivers a whole package of nutrients and phytoprotective substances that may work synergistically to promote health (Flight & Clifton, 2006). Therefore, in terms of CHD prevention, it appears that fibre is probably best obtained from whole-grain sources (Flight & Clifton, 2006).

SODIUM AND POTASSIUM

As discussed in section 2.2.2., hypertension is the leading cause of CVD worldwide. Habitual salt intake has been shown to have a causal role in hypertension through epidemiological, experimental, intervention and migration studies (He *et al.* 1991; Intersalt Cooperative Research Group., 1988; Poulter *et al.* 1990; Sacks *et al.* 2001). Potassium intake also plays a role in BP, and has been shown to be cardioprotective and is associated with a lower BP (Dickinson *et al.* 2006; Whelton *et al.* 1997). Globally, most adult populations consume more than 6 grams of salt per day, and in many Eastern European and Asian countries, salt intake exceeds 12 grams per day, compared with the International recommendations of 5-6g/day (Brown *et al.* 2009). In South Africa, a cross-sectional study in Cape Town found that the salt intake of the black participants was 7.8g/day and that their potassium intake was low (Charlton *et al.* 2005).

The actual physiology of the role of salt intake in the development of hypertension is complex. Sodium is a major cation in the extracellular fluid, with a key role in maintaining fluid balance in the body. The lack of the ability of the kidney to fully excrete excess salt is one of the major mechanisms in the association between salt intake and BP (Meneton *et al.* 2005). With aging, this excretory capability declines and even a small increase in salt intake may increase BP. Sodium retention decreases the synthesis of NO, an arteriolar vasodilator produced by the endothelial cells, and increases the plasma level of asymmetric dimethyl 1-arginine, an endogenous inhibitor of NO production (Fujiwara *et al.* 2000). The homeostasis between sodium and potassium plays a vital role in endothelium-dependent vasodilatation (Fujiwara *et al.* 2000). A diet rich in potassium and increases in serum potassium cause endothelium-dependent vasodilatation by hyperpolarising the endothelial cell through stimulation of the sodium pump and opening of potassium channels. Endothelial hyperpolarisation is transmitted to the vascular smooth cells, resulting in decreased cytosolic calcium, which, in turn, promotes vasodilatation (Amberg *et al.* 2003; Haddy *et al.* 2006). There are numerous other proposed mechanisms by which potassium can influence BP. Therefore, both excess sodium and potassium deficiency have an impact on vascular SMCs and diets high in sodium or low in potassium can consequently increase BP (Adrogué & Madias, 2007).

The response of BP to dietary sodium and potassium intake differs between groups of people. In general, sodium has a greater effect on BP in black individuals as well as in middle-aged and elderly individuals (Luft *et al.* 1979; Morris *et al.* 1999; Vollmer *et al.* 2001). African Americans experience a greater reduction in BP from sodium reduction, increased potassium intake and the Dietary Approaches to Stop Hypertension (DASH) diet when

compared with non-African Americans (Appel, 2009; Sacks *et al.* 2001; Whelton *et al.* 1997). According to the American Society of Hypertension (ASH), there are no effective means to identify exactly which individuals are more or less salt-sensitive, and there is considerable overlap between the above-mentioned subgroups. So the concept of salt sensitivity has no clear clinical or public health application (Appel, 2009).

The report of the joint WHO/FAO Expert Consultation on 'Diet, Nutrition and the Prevention of Chronic Diseases' recommends that sodium intake of adults should be less than 2g/day (WHO & FAO, 2003). Specific recommendations are an upper limit of 2300mg/day in the general population and an upper limit of 1500mg/day in blacks, middle-aged and older persons, as well as for individuals with hypertension, diabetes or chronic kidney disease (WHO & FAO, 2003). The recommendation for potassium intake by ASH is 4.7g/day, which is the level provided by the DASH diet (Appel, 2009). The DASH diet emphasises fruit, vegetables and low-fat dairy products as well as whole grains, poultry, fish and nuts, and a reduction in saturated and total fat, red meat, sweets and sugar-containing beverages. The diet is, therefore, rich in potassium, magnesium and calcium and reduced in total and saturated fat and cholesterol, as well as being slightly high in protein (Karanja *et al.* 1999). The DASH diet was shown to lower BP substantially (Appel *et al.* 1997). The 2003 WHO/ISH statement recommends a diet high in fruit and vegetables, reduction of sodium intake and an increased potassium intake to reduce the incidence of hypertension (Whitworth *et al.* 2003). The South African Food-Based Dietary Guidelines (FBDGs) state "Eat salt sparingly" (Charlton & Jooste, 2001).

B-VITAMINS

Homocysteine is a nonessential sulphur-containing amino acid produced during the catabolism of an essential amino acid, methionine. Homocysteine can be metabolised via two major pathways. When methionine is in excess, homocysteine is directed to the transsulphuration pathway, where it is irreversibly sulfoconjugated to serine by cystathionine β-synthase in a process requiring vitamin B6 as a cofactor. However, under conditions of negative methionine balance, homocysteine is primarily metabolised through a methionine-conserving remethylation pathway. In most tissues, homocysteine is remethylated in a process that requires methionine synthase, vitamin B12 as a cofactor, and methyltetrahydrofolate as a cosubstrate. This pathway requires an adequate supply of folic acid and the enzyme methylene tetrahydrofolate reductase (MTHFR). Genetic and/or acquired abnormalities in the function of these enzymes or deficiencies in folic acid, vitamin

B6 or vitamin B12 cofactors can lead to elevated homocysteine levels (Eikelboom *et al.* 1999).

Blood homocysteine levels have been directly associated with CVD risk in numerous observational studies (Homocysteine Studies Collaboration, 2002). Epidemiological studies have also shown an association between elevated total homocysteine concentration and cardiovascular risk. A meta-analysis of observational studies showed that lowering homocysteine concentrations by three μmol/l from current levels (achievable by increasing folic acid intake) is associated with reducing the risk of IHD by 16%, deep vein thrombosis by 25% and stroke by 24% (Wald *et al.* 2002). Humphrey *et al.* (2008) conducted a meta-analysis to determine whether an elevated homocysteine level is an independent risk factor for the development of CVD in persons without known CHD. They found that each five μmol/l increase in the homocysteine level increases the risk of CHD events by approximately 20%, independently of traditional CHD risk factors. A critical issue is that the kidneys play an important role in homocysteine metabolism - as renal function declines, homocysteine concentrations increase. Renal dysfunction is a recognised risk factor for CVD (Friedman *et al.* 2001), suggesting that homocysteine can be viewed as a risk marker rather than a conventional risk factor (Milani & Lavie, 2008).

Several possible mechanisms may underlie the positive association between homocysteine and risk of CHD. Studies have suggested that homocysteine promotes endothelial damage and thrombus formation (Miller *et al.* 2010) by mechanisms including oxidation of LDL-C, toxic effects on endothelial cells, impaired platelet activity, and increased smooth muscle proliferation (Eikelboom *et al.* 1999).

Daily supplementation with folic acid, vitamin B6, Vitamin B12 or a combination of these have been shown to decrease homocysteine levels in varying degrees in intervention studies (Homocysteine Lowering Trialists' Collaboration, 1998). However, when investigating the effect of supplementation with folic acid, B6 and B12, various randomised control trials revealed that, despite lowering homocysteine levels, the supplementation brought about no reduction in total cardiovascular events (Albert *et al.* 2008; Clarke *et al.* 2010). A 2009 Cochrane review provided evidence that homocysteine-lowering supplementation does not prevent cardiovascular events. This was based on eight randomised controlled studies (24 210 participants), which assessed vitamin B6 (pyridoxine), vitamin B9 (folic acid) and vitamin B12 (cyanocobalamine), given alone or on combination, at any dosage compared with placebo or standard care or different regimes (Marti-Carvajal *et al.* 2009). A more recent meta-analysis of folic acid-supplementation trials on the risk of CVD confirmed that supplementation with folic acid reduced homocysteine levels, but had no overall effect on

CVD, mortality or stroke. This meta-analysis, however, also looked at risk interaction with baseline homocysteine levels, which showed a higher risk of CVD events with folic acid supplementation in participants who had high levels of homocysteine at baseline, and lower risk for those who had low levels at baseline (Miller *et al.* 2010). Folic acid supplementation appeared to increase CVD risk in patients with high homocysteine levels at baseline levels, suggesting that folic acid may affect atherosclerotic disease through pathways that are independent of the lowering of homocysteine (Miller *et al.* 2010). This is possibly because high-dose supplementation has been seen to promote the formation of asymmetrical dimethyl arginine (a nitric oxide synthase inhibitor) as well as DNA hypermethylation, which are two potentially atherogenic processes (Böger *et al.* 2000; Zaina *et al.* 2005). Folic acid supplementation may have further atherogenic effects by promoting cellular proliferation (Glynn & Albanes, 1994; Lange *et al.* 2004).

Another possible explanation for why homocysteine-lowering supplementation does not appear to prevent cardiovascular events, is that lowering homocysteine may not add to the benefit of aspirin and other anti-platelet drugs used in preventing IHD. Aspirin counteracts the negative effects of high homocysteine levels by irreversibly blocking the formation of thromboxane A2 in platelets, producing an inhibitory effect on platelet activation and aggregation and thus preventing thrombosis (Vane & Botting, 2003). Most trials include patients with pre-existing CVD who are already receiving aspirin or anti-platelet therapy as secondary prevention. Therefore, there is the possibility that folic acid has a useful role in primary prevention of IHD, where aspirin is not generally used, but not in secondary prevention, where aspirin is routinely used, as no additional benefit is seen (Wald *et al.* 2011).

There is, therefore, still some controversy over homocysteine's role in CVD and the possibility that supplementation with B-vitamins contributes to the prevention of CVD, although more studies are being done, particularly on identifying specific populations that may benefit from supplementation with B-vitamins. Until then, however, supplementation with B-vitamins for the prevention of CVD is not justified (Marti-Carvajal *et al.* 2009; Ntaios *et al.* 2009).

ANTIOXIDANTS

As discussed in section 2.4.2, oxidation of LDL is one of the key initial steps in the atherosclerosis process and the development and progression of CHD, and oxidative stress is considered a central factor in endothelial dysfunction and plaque disruption. *In vitro*

studies have shown that antioxidants such as vitamins E and C, beta-carotene and selenium reduce lipid peroxidation and free radical damage, which are important intermediaries in the pathogenesis of atherosclerosis. For this reason, the use of vitamin and mineral supplementation was seen as a potential strategy to prevent atherosclerosis (Morris & Carson, 2003; Tribble, 1999). Numerous randomised control trials were conducted with various doses and combinations of antioxidant vitamins, which, surprisingly, showed no real reduction in mortality. In a meta-analysis on the use of antioxidant vitamins and CVD, Vivekananthan *et al*. (2003) concluded that the use of vitamin supplements containing beta carotene and vitamin A should be actively discouraged, as this family of agents was associated with a small but significant excess of all-cause mortality and cardiovascular death. Vitamin E, when used as secondary prevention, also did not reduce the risk of CVD end points (Vivekananthan *et al.* 2003).

A 2008 Cochrane review on antioxidant supplements for prevention of mortality in healthy participants with various diseases assessed the effect of supplementation of β-carotene, vitamin A, vitamin C and selenium on overall mortality in primary or secondary prevention trials. The researchers found no convincing evidence that antioxidant supplements decrease mortality; in fact, beta-carotene, vitamin A and vitamin E seemed to increase mortality (Bjelakovic *et al.* 2008). A meta-analysis looking specifically at selenium and CHD found that, although selenium concentrations were inversely associated with CHD risk in observational studies, past experience has revealed that evidence regarding observational studies and antioxidants can be misleading and the validity of this association is therefore uncertain. This, together with inconclusive evidence from randomised control trials, means that, as a result, selenium supplementation can still not be routinely recommended for the prevention of CVD (Flores-Mateo *et al.* 2006).

Several studies have reported that vitamin C can improve endothelial dysfunction. This has been seen in smokers, where impaired endothelium vasomotor dysfunction was markedly improved (Antoniades *et al.* 2003; Heitzer *et al.* 1996), as well as in hypertensive patients (Solzbach *et al.* 1997) and in patients with CAD (Levine *et al.* 1996). Studies have also found an inverse relationship between vitamin C status, fruit and vegetable intake and markers of inflammation and haemostasis (Wannamethee *et al.* 2006). It was found that fruit intakes, in particular, have significant inverse associations with CRP, blood viscosity and t-PA (Lowe *et al.* 2003; Wannamethee *et al.* 2006). It is also important to remember, however, that there are other nutrients present in vitamin C-rich foods, including folate, potassium and phytochemicals, which might confound the effects of vitamin C.

The negative results from several large-scale studies and numerous meta-analyses of the evidence that studied various combinations and dosages of antioxidant nutrients were perhaps unexpected and definitely surprising. This does not mean, however, that more investigations in this area are pointless. There are numerous issues that need to be addressed in more depth, such as, for example, the identification of types of patients that would benefit most, and when to start with supplementation and for how long, to see benefit (Steinberg & Witztum, 2002). Another major issue is that initial studies seemed to "lump" all antioxidants into one class and see them all as sharing certain common properties that are functionally more or less interchangeable. This is not necessarily the case. For example, β-carotene is an excellent trapper of singlet oxygen but is much less effective at terminating free radical chain reactions, while the opposite is true of vitamin E (Steinberg & Witztum, 2002). There is also the issue of the complex biological system involved, such as the rate of absorption and transportation, as well as the fact that we do not have a clear understanding of how and where LDL is oxidatively modified *in vivo*, which would help to predict which antioxidant, at what dose and administered by which route, would have benefit (Steinberg & Witztum, 2002).

Addressing oxidative stress remains an attractive target for cardiovascular prevention and therapy. A deeper understanding of its source and role in vascular pathology is needed before new trials are attempted (Munzel *et al.* 2010). In the meantime, it seems prudent to recommend an adequate intake of fruit and vegetables as the evidence does suggest that increased dietary intake of antioxidant vitamins has encouraging prospects for possible CVD prevention (Ye & Song, 2008).

Apart from vitamins, polyphenols have also been shown to have a wide range of biochemical properties such as antioxidant, anti-inflammatory, vasodilatory and anti-thrombotic effects, which may explain their beneficial effects on CVD (Kris-Etherton *et al.* 2004; Scalbert *et al.* 2005). Flavonoids are a class of polyphenols and can be subdivided into flavonols, flavones, flavon-3-ols, flavonones, anthocyanins and isoflavones (Erdman *et al.* 2007). Flavonoids occur naturally in fruit and vegetables, tea and red wine (Hollman & Katan, 1999). A detailed discussion on the role of polyphenols in CVD prevention, however, falls outside the scope of this dissertation and can be obtained from in-depth reviews by Cano *et al*. (2010), Heiss *et al*. (2010) and Schroeter *et al*. (2010). Several population studies have found an inverse association between flavonoid intake and risk of CHD. A meta-analysis of RCT looking at the effect of flavonoids on CVD risk showed that some flavonoid-rich products such as chocolate or cocoa and black tea have a beneficial effect on some intermediate markers for CVD, such as endothelial function, BP and LDL-C (Hooper *et al.* 2008).

VITAMIN D

Vitamin D, a fat-soluble vitamin, is known predominantly for its role in calcium and bone homeostasis as well as for the regulation of parathyroid hormone (PTH) secretion. Although there are five known forms of vitamin D, vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol) are the most studied forms (Vanga *et al.* 2010). The main circulating form of vitamin D in the body is 25(OH)D, and this is representative of total vitamin D stores (Holick, 2007). In recent years, it has come to light that vitamin D also plays an important role in cardiovascular disease. Cross-sectional studies have reported a consistent association between lower serum 25(OH)D concentration or vitamin D intake and prevalent cardiometabolic outcomes (Nemerovski *et al.* 2009; Pittas *et al.* 2007). Cardiometabolic outcomes are defined as incident hypertension, incident cardiovascular disease and change in BP (Pittas *et al.* 2010). In longitudinal observational studies, lower blood 25(OH)D concentration or vitamin D intake was associated with an increased risk of incident hypertension and possibly CVD, but the strengths of these studies were weaker when compared with those from cross-sectional studies (Pittas *et al.* 2010). Vitamin D supplementation has had no statistically significant effect on DBP or SBP or on glycaemic or cardiovascular outcomes in trials. Although it did reduce SBP by a statistically non-significant 2mmHg (Pittas *et al.* 2010), it is evident that more research needs to be done in this area.

According to a systematic review by Pittas *et al*. (2010), there are several possible or plausible mechanisms which explain how vitamin D can modify the risk of cardiometabolic outcomes. Vitamin D regulates the renin-angiotensin system, suppresses proliferation of vascular smooth muscle cells, and improves insulin resistance and endothelial-dependent vasodilation. It also inhibits anticoagulant activity and myocardial cell hypertrophy. Vitamin D may also modulate macrophage activity and cytokine generation. As mentioned earlier, despite the evidence from cross-sectional and observational studies, trials have not thus far shown a consistent and statistically significant effect of vitamin D supplementation on cardiovascular outcomes. There are numerous reasons that may explain this, such as that additional components in foods rich in vitamin D (like fish or fortified dairy products) may directly affect cardiometabolic disease, or that foods rich in vitamin D may replace other foods that increase risk for cardiometabolic disease (for example, fortified milk may replace sweetened drinks) (Jacobs & Steffen, 2003; Pittas *et al.* 2010).

A low vitamin D status does appear to be associated with an increased risk of hypertension and CVD. At this stage there is not yet enough evidence to support the recommendation of supplementation to improve actual clinical outcomes.

ALCOHOL

According to the WHO, alcohol, or ethanol, is one of the most-consumed beverages in Africa (WHO, 2004). In apparently healthy people, moderate, regular alcohol consumption is associated with lower cardiovascular mortality and morbidity than in abstainers from alcohol (Corrao *et al.* 2004; Di Castelnuovo *et al.* 2002; Ronksley *et al.* 2011). This is also true for patients who have CVD, as there is reasonable evidence that regular and moderate alcohol intake is significantly associated with a reduction in incidence of secondary cardiovascular events and all-cause mortality (Costanzo *et al.* 2010). Possible mechanisms for the beneficial effects of moderate alcohol consumption include the beneficial regulation of lipids and fibrinolysis, a decrease in platelet aggregation and coagulation factors, as well as beneficial effects on endothelial function and inflammation (Estruch *et al.* 2004; Rimm *et al.* 1999; Villegas *et al.* 2004). A recent systematic review and meta-analysis showed that moderate alcohol consumption had favourable effects on levels of HDL-C, apo-A1, adiponectin and fibrinogen (Brien *et al.* 2011). In the South African segment of the cross-sectional epidemiological PURE study (n=2010), Pisa *et al* (2010) found that increased alcohol consumption (mean intakes of 29.9 and 23.3 grams per day respectively for men and women) was associated with higher HDL-C levels but also with increased BP values (this relationship becoming even stronger after adjusting for BMI and smoking). This study shows that the cardioprotective effect of alcohol on CVD probably disappears at higher dosages because the increase in BP outweighs the benefits of the increase in HDL-C with increased alcohol consumption.

It is, of course, important to mention that too much, or abuse of, alcohol is harmful. Besides the commonly known detrimental effects on the liver and gastro-intestinal system, alcohol abuse is also associated with an increase in stroke incidence and hypertension, among numerous other negative effects. Excessive alcohol intake increases oxidative stress and often displaces nutrients like folate, thiamine and other vitamins (Cahill *et al.* 2002; Lieber, 2004).

The relationship between alcohol and ischaemic cardiovascular events or all-cause mortality in healthy people is depicted by a J-shaped curve attributed to a dose-related combination of beneficial and harmful effects. A significant association with reduced risk is found up until 25g/day of alcohol (Di Castelnuovo *et al.* 2002). Numerous studies have looked at the type of alcohol, quantity and pattern of alcohol intake. It appears that the ethanol itself rather than a specific component of the wine, beer or spirits, appears to be the major factor in conferring the health benefits, and most studies show equal protection from all types of alcohol (O'Keefe *et al.* 2007). A meta-analysis (including only six studies), which investigated

whether drinking pattern modifies the effect of moderate alcohol consumption on risk of CHD, suggested that binge and heavy irregular drinking modify the favourable effects of alcohol on CHD risk (Bagnardi *et al.* 2008).

The AHA has recommended that "If you consume alcohol, do so in moderation (equivalent of no more than 1 drink for women and 2 drinks for men per day)" (Lichtenstein *et al.* 2006). This is similar to the South African Food-Based Dietary Guidelines: "If you drink alcohol, drink sensibly" (Van Heerden & Parry, 2001). Table 2.12 gives the definition of what a standard drink is. Patients with cardiovascular disease should be informed that low-to-moderate alcohol consumption is considered not to be harmful to their health; however, those who do not regularly consume alcohol should not be encouraged to do so (Brouwer *et al.* 2010). Alcohol can therefore be seen as having a dual role in the development of atherosclerosis, in that it has beneficial protective effects in moderate dosages, but when abused, can play a role in the actual development of atherosclerosis.

Table 2.12 Definition of a standard drink (Van Heerden & Parry 2001)

Drink	Average alcohol content (% volume)	One drink	Alcohol content (g)
Beer, malt	5	340 ml	12
Beer, sorghum	3	500 ml	12
Stout	6	375 ml	17
Cider	6	340 ml	16
Cooler/flavoured grape liquor	5-10	340ml	8
Liqueur	30	25ml glass	6
Sherry	17	50ml glass	7
Brandy, whisky	43	25ml tot	11
Gin, cane, vodka	43	25ml tot	11
Wine	12	120ml glass	11

In a discussion of the effect of alcohol consumption on health, the social aspects of alcohol misuse or abuse should also be considered, especially within the South African context, where alcohol abuse is widespread. The socio-economic effects associated with alcohol abuse include unemployment, violence, crime and sexually risky behaviour, as well as disruptions to work performance and family life. Addressing alcohol abuse and its consequences in communities struggling with this problem has become an important health

issue in this country (Setlalentoa *et al.* 2010) and therefore the encouragement of moderate consumption of alcohol should be approached with caution.

2.4.3.2. FOODS AND DIET QUALITY AND CVD

In the early part of this century, public health nutritional problems were mostly related to deficiency of nutrients, in comparison with problems faced now, which are a result of excesses or imbalances in dietary intake (Arvaniti & Panagiotakos, 2008). In earlier years, the majority of investigations used quantitative research, focusing on nutrients and health outcomes. However, the failure of single-nutrient supplementation to protect against CVD (Bjelakovic *et al.* 2008) and cancers (Omenn *et al.* 1996; The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994) highlighted the fact that it was important to develop a more holistic view of food intake. There has been a definite shift, with more qualitative research being done and with more investigations including dietary patterns, dietary quality and variety (Trichopoulos & Lagiou, 2001). This ensures that the complexity of dietary behaviours is taken into account. This shift has also been seen in the approach to prevention programmes for CVD and other chronic diseases in numerous countries, with culturally sensitive food-based dietary guidelines being developed and implemented.

The investigation of dietary patterns is therefore a more holistic approach to determining dietary adequacy. There are two approaches to investigating dietary patterns, namely theoretically defined dietary patterns and empirically derived dietary patterns. Theoretically derived patterns are derived *a priori*, based on the current nutritional knowledge. They consist of nutritional variables, generally foods and/or nutrients which are considered to be important to health, which are quantified and summed to provide an overall measure of diet quality (Waijers *et al.* 2007). On the other hand, empirically derived dietary patterns consist of patterns statistically derived *a posteriori* from collected food consumption data based on correlations in intakes of various dietary components, usually using factor or cluster analysis (Waijers *et al.* 2007).

To determine empirical dietary patterns, the health outcomes for the specific population being studied are required. As mentioned previously, this is not the case for the PURE study population being studied for this thesis. For this reason, a predefined index of diet quality will have to be used to assess the dietary intake of this population. The next section will therefore focus on predefined diet quality scores in order to determine which index will be most appropriate for the purpose of this thesis.

ASSESSING DIET QUALITY

According to a review (Kant, 1996), there are three major approaches to constructing diet quality indices. There are indices derived from nutrients only, indices based on foods or food groups and also indices based on a combination of nutrients and foods. Table 2.13 provides a summary of some of the better-known diet quality scores. As to the validity of diet quality scores (DQS), some scores tend to have higher validity, although it must be remembered that composing an index remains a complex matter with a large degree of subjectivity (Waijers *et al.* 2007). The ultimate validation of any measure of total diet quality may be to examine its association with longevity (health outcomes). Outcome measures have been included in Table 2.13. When examining the associations of diet quality with biomarkers and health outcomes, it was found that lower DQS were consistently associated with higher rates of all-cause mortality and selected disease-specific rates or mortality. The associations were attenuated when adjusted for common confounding variables but still remain significant and appear to be stronger in men and for all-cause CVD mortality. When an index is selected, its limitations and the specific context in which it is used need to be considered in the interpretation of the results and comparison of studies. Because there are numerous DQSs and variations of each method, it is recommended that researchers model scores based on existing tools and select more than one when testing associations with health outcomes (Wirt & Collins, 2009), which will be the approach for this thesis.

In composing a DQS there are numerous decisions that need to be made. These include the choice of the index components for inclusion in the score and assigning foods to food groups. The choice of cut-off values must be decided on, as well as the exact quantification of the index components judged against cut-off values. A decision must be made on the relative contribution of the individual components to the total score. Another issue that must be considered is whether or not to adjust for energy intake (Waijers *et al.* 2007). The index components of DQSs are nutrients and foods or food groups that are assumed to be healthy or unhealthy. Table 2.13 provides an indication of which scores are based on nutrients and which on foods. The nutrients most commonly used in DQS are total fat, SFA or ratio of MUFA:SFA, cholesterol, alcohol, sodium, (complex) carbohydrate, dietary fibre and protein. With regard to units of measurement, total fat and SFA are usually expressed in energy percent (energy %), while other units are used for other nutrients. Micronutrients are usually expressed in micrograms or in percentage of RDA (Waijers *et al.* 2007).

Table 2.13: Summary of Diet Quality Indices (Adapted from Waijers *et al.* 2007 and Wirt & Collins, 2009)

OBJECTIVE	METHODS	OUTCOME MEASURE	REFERENCE
Healthy Eating Index (HEI) Based on foods and nutrients. Assesses adherence to US Food Guide Pyramid and Dietary Guidelines for Americans	Ten components based on aspects of a healthy diet. Components 1 – 5 based on conforming to serving recommendations for five major groups including vegetables, fruit, meat, milk. Others based on overall fat % E, saturated fat % E, cholesterol, Na, variety in diet.	Biomarkers for CVD: not significantly associated with any biomarkers. Biomarkers: Correlation.	(Fung *et al.* 2005; Hann *et al.* 2001; McCullough *et al.* 2002)
Alternative Healthy Eating Index (AHEI) Acknowledges the benefits of unsaturated oils, distinguishes quality within food groups, and excludes potato and its products from vegetable group	. Nine components including vegetables; fruit; nuts and soya; ratio of white to red meat; cereal fibre; trans fat % energy; ratio PUFA to SFA; alcohol servings daily; duration of multivitamin use.	. Biomarkers for CVD: significantly inversely associated with most biomarkers.	. . (Fung *et al.* 2005; McCullough *et al.* 2002; McCullough & Willett, 2006)
Diet quality Index (DQI) Based on nutrients	Based on eight National Research Council Diet and Health recommendations. Includes six nutrient intakes: total fat; SFA; cholesterol; protein; calcium, Na; servings from 2 food groups: fruit and vegetables, grains.	CVD mortality, cancer mortality, all mortality: limited ability to predict mortality.	(Seymour *et al.* 2003)
Diet Quality Index Revised (DQI-R)	Ten components instead of eight. In contrast to DQI, higher scores indicate adherence to dietary guidelines. Fruit and vegetables are separated according to the Pyramid recommendations, therefore includes Fe intake; excludes protein intake; and scores dietary moderation and diversity.	Dietary biomarkers (Plasma carotenoids, tocopherols, retinol, cholesterol, triacylglycerol). Positive correlation from FFQ with α-carotene, β-carotene, lutein, α – tocopherol. Inverse correlation with TC.	(Fung *et al.* 2006; Newby *et al.* 2003)

OBJECTIVE	METHODS	OUTCOME MEASURE	REFERENCE
Mediterranean Diet Score (MDS) Assesses overall diet pattern based on traditional Mediterranean diet	Eight desirable components including: high MUFA to SFA ratio; high legume consumption; high fruit consumption; high cereal consumption; moderate ethanol consumption; low milk/dairy consumption; low meat/meat product consumption. Cut-off points were used for each component based on median values for each sex. Higher scores indicate a better diet.	All mortality 17% reduction in mortality for 1-unit increase in 8-point score.	(Trichopoulou *et al.,* 1995)
Mediterranean Diet Score (MDS-f)	Similar to previous MDS; however, fish added as a component, making a total of nine.	CHD, cancer and all mortality 25% reduction in all mortality, 33% in CHD mortality for 2-unit increase in the 9-point score.	(Trichopoulou *et al.* 1995)
Mediterranean Diet Score (MDS-f)	Similar to original MDS but has nine components. Legumes group replaced with legumes/nuts/seeds; and vegetable group replaced with vegetables and potatoes; meat and meat products replaced with meat and poultry. Fish was also added as a component.	All-cause and cause-specific mortality: In low-risk group (MDS≥4): there was a reduction in all mortality 23% and CHD mortality of 39%.	(Trichopoulou *et al.* 2003)
Alternative Mediterranean Diet Score (MDS-a)	Variation of original MDS. Components have been modified: potato products excluded from vegetable group; fruit and nuts separated into two groups; dairy group eliminated; includes whole-grain products only; includes only red and processed meats for meat group; and assigning 1 point for 5 and 15g/d alcohol intake.	Biomarkers for CVD. MDS-a significantly inversely associated with most biomarkers.	(Fung *et al.* 2005; Knoops *et al.* 2004)

OBJECTIVE	METHODS	OUTCOME MEASURE	REFERENCE
Healthy Diet Indicator (HDI) Based on WHO dietary recommendations for preventing chronic disease.	Dichotomous variables used, with 1 indicating being within the recommendations and 0 being outside recommendations. Food groups include SFA; PUFA; protein; complex carbohydrates; dietary fibre; legumes/ nuts/seeds; fruit/vegetable; mono- and disaccharides; cholesterol.	All mortality: 13% reduction in mortality (in study in three European countries).	(Huijbregts *et al.* 1997)
Food-based Indices **Healthy Food Index (HFI)** Based on previous diet quality indices and current recommend-ations for a healthy diet. Assesses food patterns defined *a priori* on basis of food recommendations and *a posteriori* by factor analysis, using mortality as an outcome**.**	Four components, each receiving 1 point if met daily: not consuming margarine, butter or lard; consumption of boiled or raw vegetables at least once; consumption of coarse rye or white bread at least once; consumption of fruit at least once.	CHD and all mortality: No significant association	(Osler *et al.* 2001; Osler *et al.* 2002)

% E: percentage energy; Na: sodium; CVD: cardiovascular disease; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; Fe: iron; MUFA: monounsaturated fatty acids; CHD: coronary heart disease.

In a critical review of predefined diet quality scores, Waijers *et al*, (2007) made some recommendations regarding the choices that need to be made when constructing a DQS. An index should contain two macronutrients (fat, carbohydrate or protein) to ensure overall balance. It is also desirable to let the score be proportional to intake, instead of using simple cut-off values, or else to design a scoring range. To avoid confounding by energy intake, scores should depend on or be adjusted for energy intake. Another important issue to be taken into account is that diet is culturally determined and therefore general dietary habits within the population being studied need to be considered when the index items and their cut-offs are chosen. Dietary variety should also be considered, but not necessarily included as an index item. The index should also be constructed in such a way that that dietary variety is ensured of obtaining a high score.

As mentioned earlier, the best-known DQSs have been summarised in Table 2.13. Most DQSs are derived from one of these scores. Thiele *et al*, (2004) have, however, adopted a slightly different approach. They constructed two indices, which separate excessive intake and deficient intake. Their score strives to identify whether a specific diet quality is the result of over-consumption (e.g. of fat and cholesterol) or under-consumption (e.g. of vitamins, minerals and trace elements). The score of these indices is determined by the ratio of nutrient intake relative to the respective reference intakes, which, in this case, are the guidelines of the German Nutrition Society. The two indices were constructed by adding up scores of nutrients. The first index (deficient index) includes 13 vitamins, 12 minerals and trace elements, protein, carbohydrate, two essential fatty acids and dietary fibre. The second index (excess index) consists of fat, cholesterol, ratio of saturated to unsaturated fatty acids, sugar, alcohol and sodium (Thiele *et al.* 2004).

These indices were applied to the participants of the German Nutrition Survey of 1998 and the impact of lifestyle and sociodemographic characteristics on the diet quality indices was analysed. The results showed that there is a considerable variation of diet quality between different groups of individuals. High diet quality was positively associated with income, education level, age, energy intake, food diversity, sport activity and vegetarianism. This was, of course, for the German population, but it would be of great interest to assess these associations in the black South African population. The disadvantages of these indices are that they consider information neither on the quantities of food consumed nor on the heterogeneity of different foods (Thiele *et al.* 2004).

The decision needs to be made as to which DQS should be used to assess the diet quality of the black South African population in this study. Although food diversity has been studied in this population (Oldewage-Theron & Kruger, 2011; Steyn *et al.* 2006), a DQS as such has

not been constructed and validated. To be in a position to draw valuable conclusions, it would be advisable to use both a score that uses nutrients as well as one that uses foods. In the review by Wirt and Collins (2009), it was also recommended that more than one score should be used when testing associations with health outcomes. In keeping with the focus of this thesis on investigating the dual role of diet in CVD, the indices by Thiele *et al*. (2004) that measure excess as well as deficiency will be used. A benefit of the score by Thiele *et al.* (2004) is that South African guidelines can be used as the reference intake, remembering to use EAR and RDAs. An important issue to address, however, is the choice of nutrients to be included in the indices. This will depend on the availability of data on the specific nutrients in the South African Food Composition database.

As we have decided to use more than one index, and specifically to include one that incorporates foods, as the Thiele indices use nutrients, we would have to select an existing index from Table 2.13. The Alternate Mediterranean Diet Score (MDSa) (Table 2.14) seems to be a possible option, chiefly because the standard or reference used in this score is the median intake for the population, which will help to make the score more specific to our population. The score was originally constructed by Trichopolou *et al*., in 1995 (Trichopoulou *et al.* 1995) and then later revised to include fish intake (Trichopoulou *et al.* 2003). The scoring criteria are shown below:

Table 2.14 Alternate Mediterranean Diet Score (Fung *et al.* 2005; Trichopoulou *et al.* 2003)

Food group	Foods included	Criteria for 1 point[1]
Vegetables	All vegetables except potatoes	Greater than median intake (servings/day)
Legumes	Tofu, string beans, peas, beans	Greater than median intake (servings/day)
Fruit	All fruit and juices	Greater than median intake (servings/day)
Nuts	Nuts, peanut butter	Greater than median intake (servings/day)
Whole grains	Whole-grain ready-to-eat cereals, cooked cereals, crackers, dark breads, brown rice, other grains, wheat	Greater than median intake (servings/day)
Red and processed meats	Hot dogs, deli meat, bacon, hamburger, beef	Less than median intake (servings/day)
Fish	Fish and shrimp, breaded fish	Greater than median intake (servings/day)
Ratio of MUFA to SFA		Greater than median intake (servings/day)
Ethanol	Wine, beer, "light" beer, liquor	5 – 25g/day

[1]0 points if these criteria not met.

The benefits of the Mediterranean diet in preventing CVD are well known and have been studied and documented extensively (Menotti *et al.* 1999; Trichopoulou *et al.* 1994). The traditional Mediterranean diet is characterised by a high intake of fruit, vegetables, legumes, nuts and cereals, a high intake of olive oil but a low intake of SFAs, a moderately high intake of fish (depending on proximity of the sea), a low-to-moderate intake of dairy products (and then mostly in the form of cheese or yoghurt), a low intake of meat and poultry and a regular but moderate intake of ethanol (mostly in the form of wine and generally during meals) (Willett *et al.* 1995). The diet emphasises food groups rather than specific nutrients, and this traditional plant-based diet may provide benefit through the intake of antioxidants and fibre, or indirectly, by lowering blood pressure. Other population groups have successfully used the Mediterranean diet as a benchmark for assessing diet quality (Rubba *et al.* 2007; Woo *et al.* 2001).

Another possible option is the Healthy Diet Indicator (HDI). This DQS includes both foods and nutrients, and uses the WHO guidelines for the prevention of chronic diseases as cut-off points. In a cohort study with 20 years of follow-up, the HDI was inversely related to all-cause mortality. After adjusting for confounders, the group with the highest HDI score had an 18% lower risk of death from CVD than the group with the lowest HDI score (Huijbregts *et al.* 1997). The criteria for the HDI score are as follows:

Table 2.15 Criteria for Healthy Diet Indicator (Huijbregts *et al.* 1997)*

Nutrient or food group (daily intake)	1	0
Saturated fatty acids (% of TE)	0-10	>10
Polyunsaturated fatty acids (% of TE)	6-10	<6 or >10
Protein (% of TE)	10-15	<10 or>15
Complex carbohydrates (% of TE)	50-70	<50 or >70
Dietary fibre (g)	>25	<25
Fruit and vegetables (g)	>400	<400
Pulses, nuts and seeds (g)	>30	<30
Monosaccharides and disaccharides (% of TE)	0-10	>10
Cholesterol (mg)	0-300	>300

* Based on WHO dietary guidelines for the prevention of chronic diseases (WHO & FAO, 2003).

The rationale for electing to use these specific DQSs over the other known scores is, as previously mentioned, that the median intake of the population being studied is used as reference, that the focus is not only on nutrients but also on food groups, and that diet quality is assessed in relation to known and proven dietary guidelines for the prevention of CVD. It will be relatively simple to fit South African foods into the food groups used in the scores.

2.5 SUMMARY AND CONCLUSIONS

The prevalence of CVD appears to be on the rise in developing countries such as South Africa. It was previously thought that the black South African population was immune to CHD; however, there is evidence to show that the incidence of CHD in this population is on the rise. With this literature study, firstly an effort has been made to determine the role that the various risk factors for CVD play in the black South African population and whether this role differs from that in Caucasian South Africans. Then assessment of risk of CVD was discussed, with the aim of finding an existing risk score that would be suitable for use in a black South African population. The focus then moved to understanding the dual role that diet plays in atherosclerosis, and how to assess this role by looking at both nutrients and foods as well as by determining overall diet quality.

With regard to understanding the risk factor profile of the black South African population compared with that of Caucasians, the summary in Table 2.13 shows that the risk factor profile of the black South African population does appear to be changing with urbanisation. It was seen that known risk factors such as hypertension, DM and obesity (particularly in women) have a very high prevalence in this population. Risk factors such as dyslipidaemia and haemostatic markers, previously thought not to play a significant role in this population, are on the increase. With reference to modifiable risk factors, physical activity and fruit and vegetable intake were shown to be low, probably aggravating the problem. Inflammation and heightened vascular reactivity to stress also appear to play a role. Risk assessment for CVD was discussed with the purpose of selecting the most appropriate risk score for the purpose of this thesis. Because the Reynolds Risk score incorporates inflammation (a potential risk factor for the black South African population) in the score by means of CRP, it was decided that this score should be used.

In order to determine the role of the diet in CVD, it is important to consider not only nutrients but also foods and overall diet quality. Before it could be decided which DQS to use to assess diet quality, it was first necessary to explain the role that nutrients play in the development of or protection against CVD. It was also necessary to understand the role of nutrients and the role of foods in diet quality assessment. Dietary intake can influence or contribute to atherosclerosis in numerous ways, and plays a dual role by either protecting against or contributing to the cause of atherosclerosis. It is evident that, in the case of dietary fat for instance, the focus should be on the quality of the fat and not on the quantity in the diet. Diets high in SFA, TFA and cholesterol, for example, have been shown to increase LDL-C and also stimulate endothelial cells to express VCAM-1. Polyunsaturated fatty acids, on the other hand, have a more protective effect, with an inverse relationship being seen between ALA and CVD risk. Eicosapentaenoic acid and DHA in fish have also been shown

to have numerous favourable effects on atherosclerosis. Antioxidants play a protective role in lowering oxidative stress, which plays a role in inflammation, but they can also have pro-oxidative effects when consumed in excess. It was decided to use two DQSs for the purpose of our study: one that assesses adequacy and excess (examining the dual role of diet) as well as one that incorporates foods in the assessment. In this way, the dietary intake will be assessed in a holistic manner, making it possible to answer the research questions.

The population of South Africa is in different phases of the nutrition transition, while poverty remains a major issue; the result is a country that is facing the growing problem of noncommunicable diseases such as CVD, while still battling traditional risks and infectious diseases such as malnutrition and HIV/AIDS. As mentioned earlier, although the incidence of CHD is on the rise, it is still not as high as one would expect; nevertheless, it seems that, as the presence of these risk factors continues to rise in the black South African population, so will CHD.