Introduction and a brief review of literature

1.1 Risk factors for CVD

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in the Western world and is increasing in developing countries (1). In 2003, an estimated 16.7 million or 29.2% of total global deaths resulted from the various forms of CVD, with coronary heart disease (CHD) as the biggest contributor (1). CHD is caused by atherosclerotic narrowing of the coronary arteries leading to a reduced blood supply to the heart muscle, which can result in angina and/or myocardial infarction.

Hypercholesterolemia is one of the classic risk factors for CHD and the positive association between serum cholesterol and CHD risk has been shown and confirmed repeatedly over the last five to six decades (2-5). Cholesterol is transported in different lipoprotein fractions, including low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. Elevated serum concentrations of LDL are positively associated with the risk of CHD (6-8), and lowering of total and LDL cholesterol, with dietary (or drug) therapy, reduces the risk of CHD (9, 10).

An inverse association exists between serum concentrations of HDL cholesterol and CHD risk (11-13). One of the mechanisms by which HDL protects against CHD is through the reverse transport of cholesterol, whereby HDL promotes the efflux of cholesterol from cells, bringing it to the liver to be metabolized into bile acids and excreted (14). Although a number of treatments (especially drugs) that reduce total and LDL cholesterol cause an increase in serum HDL cholesterol, whether this raised HDL
independently reduces the risk of CHD is not known (15). Epidemiological and experimental research has also shown that the ratio of total to HDL cholesterol is a good predictor of lipid related CHD risk (16).

Apart from total, LDL and HDL cholesterol, there are many other CHD risk factors (17) and new factors are added to this list regularly. These factors individually and/or jointly impair a number of metabolic and vascular functions, increasing the risk of CVD. The following section (Figure 1.1) of this literature review briefly outlines the interrelationship between some of the risk factors for CVD relevant to this thesis.
Figure 1.1 Interrelationship between some of the CVD risk factors
Many of these factors are affected by genetics and lifestyle; CVD cardiovascular disease; LDL low density lipoprotein; SNS sympathetic nervous system
1.1.1 Obesity

Obesity is one of the risk factors for CHD (18). The World Health Organization (WHO) defines a body mass index (BMI) (calculated as weight/height$^2$) of 25-29.9 kg/m$^2$ as overweight, and of $\geq$ 30 kg/m$^2$ as obese. Observational studies have shown a positive association between BMI and CHD mortality (19, 20) with a stronger association in non-smokers than smokers (20), and in white populations compared to black (21). A BMI greater than 28 kg/m$^2$ is associated with a three to four times higher risk of morbidity from coronary artery disease or stroke than a BMI of less than 28 kg/m$^2$ (22).

In addition to the total body weight, total body fat and the pattern of fat distribution is a critical factor in the relationship between obesity and metabolic abnormalities, with a positive association between abdominal/visceral fat and CHD risk (23, 24) and type 2 diabetes (25). Waist circumference is an indicator used to determine abdominal fat/obesity (26). BMI and visceral fat have also been found to be associated with impaired endothelial function (27-29). Obesity substantially increases the occurrence and risk of various other CHD risk factors including: insulin resistance, hyperglycemia, hyperinsulinemia, hypertension, and dyslipidemia (30) and the combination of these pathologies is frequently referred to as the metabolic syndrome or syndrome X (26, 31).

Although the close link between obesity and its related complications has been well documented, the exact mechanism linking one to another is still not clearly understood. It has been hypothesized that in obesity (especially abdominal obesity), insulin resistance occurs due to the increased concentrations of circulating free fatty acids, which inhibit efficient glucose uptake by cells (32). Moreover, a number of hormones produced by adipose tissue are increased in obese people. Leptin, the hormone responsible for regulation of food intake and energy expenditure, is also a marker for body fat content and metabolic activity (33). Other hormones including tumour necrosis
factor alpha (TNF-α), plasminogen activator inhibitor-1 (PAI-1), and resistin have been shown to induce obesity related insulin resistance, impaired lipid profiles and diabetes under experimental conditions (34, 35). Adiponectin, another protein secreted by adipose tissue, is reduced in obesity and is closely related to the degree of insulin resistance and hyperinsulinemia (36, 37). Animal studies have shown delayed clearance of circulating free fatty acids, increased concentrations of TNF-α, severe high fat diet-induced insulin resistance and reduced activity of insulin receptors in adiponectin knock-out rats (38). Conversely, data from human studies indicate that weight loss, through diet, drug/surgical means or physical activity, increases adiponectin (39), improves insulin sensitivity (40, 41), lipid profile (42, 43) and decreases the incidence of diabetes (44).

1.1.2 Insulin resistance

Insulin resistance is an associated factor for obesity and CHD risk. Insulin resistance is defined as the reduced ability of the whole-body to take up insulin-mediated glucose, as measured by the glucose clamp technique (45). This decreased ability is usually due to reduced insulin receptor activity (46). Obesity is thought to be a major contributing factor for the development and progression of insulin resistance (47). Insulin resistance is likely to be genetically determined, but is also dependent on lifestyle factors (48).

It is often assumed that insulin resistance is a muscle tissue defect, probably because the reduced insulin-mediated uptake of glucose is due to reduced ability of skeletal muscle to take-up and store glucose; however, liver, adipose tissue and kidney may also be involved in the process of insulin resistance (48, 49). Although insulin resistance is more prominent in obese than lean people, it does exist in lean people and not all obese people are insulin resistant (50-52). Insulin resistance results in hyperglycemia,
hyperinsulinemia, dyslipidemia and endothelial dysfunction, some of the risk factors of CVD.

1.1.3 Hyperglycemia

With disturbances in insulin receptor activity, the ability of insulin to transfer glucose through cell membranes is reduced, resulting in hyperglycemia. A continuous graded relationship exists between blood glucose and risk of CVD (53-55) particularly in women (56).

Different mechanisms have been proposed describing the association between hyperglycemia and CVD. These include glycation of collagen and other vessel-wall proteins and lipoproteins; accelerated generation of reactive oxygen species; increased oxidative stress on glycated end products, LDL cholesterol and vascular endothelial cells, and alteration in haemorrheological characteristics or changes in vascular reactivity (57). Chronic hyperglycemia induces non-enzymatic glycosalation of molecules and irreversible formation of advanced glycation end products (AGEs) (58). AGEs accumulate in the vessel wall, and via free radicals, reduce nitric oxide (a vasodilator) activity and induce endothelial dysfunction. In addition, glycated collagen may induce smooth muscle proliferation, increase the arterial wall thickness and reduce the arterial elasticity. Glycation of LDL may affect its binding to LDL receptors, resulting in accumulation in the circulation, and later, uptake by macrophages, followed by oxidation (59). Similarly, glycation of HDL may promote the accumulation of cholesteryl esters in the arterial wall (59).

Impaired glucose tolerance, i.e. postprandial hyperglycemia with ‘normal’ fasting glucose is also an independent risk factor for atherosclerosis and CVD (56, 60-63).
Elevated glucose levels can alter the activity of protein kinase C (64), causing increased secretion of endothelin-1 (a vasoconstrictor) (65), and increased secretion of collagen IV and fibronectin (64). Postprandial hyperglycemia also produces oxidative stress (66) via the generation of free radicals, causing oxidation of LDL (67). It also impairs endothelium-dependent vasodilation (68, 69) possibly due to reduced nitric oxide (NO) production/activity, and causes hypertension in diabetic and healthy individuals (65). These atherogenic effects of postprandial hyperglycemia appear to be independent of other CVD risk factors, including hyperlipidemia (70).

Although there is a positive association between blood glucose concentrations and CVD risk, randomised controlled trials of intensive glycaemic control (especially with insulin therapy) have failed to demonstrate significant reductions in the CVD end points (71-74). It is possible that the beneficial effects of insulin on glycaemic control are counterbalanced by adverse effects on CVD (65), as insulin therapy may be associated with higher incidence of fatal CHD in Pima Indians (75).

### 1.1.4 Hyperinsulinemia

Insulin has excitatory actions which includes stimulating glucose uptake, lipid and protein synthesis and inhibitory actions which include inhibiting lipolysis, proteolysis, glycogenolysis, gluconeogenesis and ketogenesis (76). In addition, insulin demonstrates significant biological effects on the vasculature (77) by exerting vasodilator and vasoconstrictor effects, mediated through NO and endothelin-1 (ET-1), respectively.

Hyperinsulinemia is thought to be a compensatory response to decreased insulin sensitivity (78), as β cells in the pancreas secrete higher concentrations of insulin in response to the hyperglycaemia. The hyperinsulinemia may also be due to β cell
dysfunction. Insulin secretion is biphasic and reduced insulin secretion in the first phase (first 30 min), after glucose or mixed meal, in non-diabetic insulin resistant individuals results in impaired glucose tolerance, and excessive glucose excursions and hyperinsulinemia at two hours (79). High amounts of oxidative free radicals, produced due to high concentrations of fasting free fatty acids are the probable mechanism for impaired first-phase glucose-induced insulin secretion (80, 81).

Insulin resistance occurs not only in skeletal muscle but also in the liver and kidneys. The liver is the major site for insulin clearance, where approximately fifty percent of insulin is cleared during the first liver pass before reaching the peripheral circulation (82). However, in the obesity-induced insulin resistance state, the hepatic clearance of insulin is reduced. Experimental studies have shown similar hepatic extraction of insulin in lean and obese subjects at fasting state, but a reduced extraction in obese compared to lean during hyperglycaemic clamp tests (83, 84). This may be attributed to a reduced number of receptors due to prolonged increases in portal insulin levels and/or as a return effect of impaired free fatty acid metabolism, a result of hyperinsulinemia (85).

Hyperinsulinemia is associated with CHD (86) and a number of related risk factors, including: hypertension (87), high concentrations of PAI-1 (88-90), increased triglyceride and lower HDL concentrations (91), endothelial dysfunction (27) and elevated heart rate (92). The hypothesized mechanisms by which hyperinsulinemia may promote atherosclerosis include stimulating smooth muscle proliferation and binding of LDL to cell membranes in monocytes, increasing noradrenaline release through the activation of sympathetic nervous system (SNS) activity, and renal sodium and water retention (65, 78).
Acute hyperinsulinemia in healthy individuals dose dependently induces skeletal muscle vasodilation (93), reduces arterial pressure and stiffness (94-96) and increases coronary perfusion (97, 98) possibly by stimulating endothelial nitric oxide synthase (eNOS) and increased synthesis of NO (93, 99, 100). However, acute hyperinsulinemia in healthy individuals increases susceptibility of LDL to oxidation (101, 102). In hypercholesterolemic subjects acute hyperinsulinemia impairs endothelial dependent vasodilation (103). In insulin resistant individuals, the vascular action of insulin is blunted (95, 98, 104) and is closely associated with the degree of obesity and insulin’s action on glucose uptake (95, 98, 104). Additionally, during hyperglycemia and inhibition of NOS, insulin stimulates vasoconstriction (105), mediated by ET-1 (105-107).

Although there is support for hyperinsulinemia as an independent risk factor for cardiovascular disease, this relationship is weak, and it is probable that hyperinsulinemia reflects insulin resistance as a factor enhancing atherogenesis, by causing adverse changes in many CVD risk factors (108-113). Furthermore, hyperinsulinemia and CVD risk association is stronger in lean than in obese subjects (108) and more prominent in Caucasians than other ethnic populations (113).

1.1.5 Hypertriglyceridemia

In an insulin resistant state with hyperinsulinemia, the ability of insulin to suppress lipolysis is reduced, and hepatic synthesis and secretion of very-low-density-lipoprotein (VLDL) triglycerides is increased (114, 115). There is also a decrease in the lipoprotein lipase activity, which reduces VLDL and triglyceride clearance (116). In hypertriglyceridemia (Figure 1.2), the increased number of large VLDLs exhibit an increased acceptor activity for cholesterol ester transfer protein (CETP), which leads to
an increased transfer of cholesterol ester (CE) from HDL to triglyceride rich lipoproteins (TRL), and triglycerides from TRL to HDL and LDL (116-119). These triglyceride rich LDL and HDL are the substrates for hepatic lipase which hydrolyses phospholipids and triglycerides and generates small dense LDL and HDL particles (120, 121).

**Figure 1.2 Effect of cholesterol ester transfer protein (CETP) in normal triglyceride (TG) and hypertriglyceridemic state.**

There has been an ongoing debate about whether or not hypertriglyceridemia is an independent risk factor for CHD (122). Fasting serum triglyceride levels are negatively associated with HDL (123) and the positive association between triglycerides and CHD risk was lost when other lipid risk factors were taken into account (122). However, prospective cohort (11, 124-126) and meta-analysis (127, 128) studies have supported the inclusion of elevated fasting triglyceride concentrations in the list of independent risk factors for CHD (15). Additionally, experimental studies on postprandial
triglyceride concentrations and CVD risk have also supported this inclusion of triglycerides as an independent risk factor (129, 130).

Although the exact mechanism by which hypertriglyceridemia increases the risk of CHD is not known, it has been proposed that elevated triglyceride levels may induce endothelial dysfunction (131, 132) with oxidative stress as a mediator (70). High triglycerides levels (fasting and postprandial) are associated with increased susceptibility of LDL to oxidation in healthy subjects (101) and type 2 diabetics (133). In vitro (134) as well as in vivo (130-132) studies have shown impaired endothelium dependent vasodilation in the presence of both fasting and acute hypertriglyceridemia. Subjects with metabolic syndrome also show an increased serum ET-1 concentration in presence of acute hypertriglyceridemia (135). This impaired endothelial dysfunction may occur because the catabolites of triglycerides - free fatty acids play a role in the impairment of endothelium dependent vasodilation (131, 136) causing reduced NO production (137).

1.1.6 Low density lipoprotein

In addition to elevated levels of LDL, high amounts of small dense LDL increases the risk of CHD (138-141), especially in Caucasians (142). Small dense LDL are rich in apolipoprotein B (apoB), which facilitates the uptake of cholesterol in peripheral tissues (143). Small dense LDL have a poor affinity for binding to LDL receptors and hence stay in plasma for longer periods of time (144, 145). These small dense LDL interact with proteoglycans of the arterial wall matrix, leading to retention and accumulation in the arterial intima (145). Furthermore, small dense LDL rich in triglycerides have lower antioxidant concentration (146), reduced free cholesterol (147), and increased content of polyunsaturated fatty acids (148), all of which increase the susceptibility of LDL to
oxidation (133). Small dense LDL also causes endothelial dysfunction, resulting in reduced endothelium-dependent and endothelium-independent vasorelaxation, especially in type 2 diabetics (149-151).

1.1.7 Oxidation of low density lipoprotein

Substantial data is available to support the hypothesis that oxidation of LDL by free radicals is an important step in the development and progression of atherosclerosis (152). Oxidative modification of LDL is believed to occur in two steps: first when LDL is minimally modified, before monocyte recruitment and second full oxidation of LDL (Figure 1.3). Initially, native LDL are trapped in the subendothelial space of the arteries where they are modified by resident vascular cells, including: smooth muscle cells, subendothelial cells and macrophages (153-155). This modified LDL induces the release of local factors and increases adhesiveness towards mononuclear cells and leucocytes. It also inhibits the removal of monocytes from the vascular wall. This then leads to the stage where monocytes are differentiated into macrophages in the intima and further peroxidation of LDL and formation of foam cells. Oxidized LDL has additional atherogenic attributes (156). It also causes endothelial dysfunction and injury, resulting in changes that allow LDL to penetrate into subendothelial spaces, and making more LDL available for oxidative modification. Oxidized LDL interferes with the NO production pathway, as well as enhances the destruction of NO (157, 158), resulting in impaired vasorelaxation and increased local platelet aggregability (159, 160). These effects of oxidized LDL are possibly modulated through lysophosphatidylcholine and protein kinase C (161). Oxidized LDL also increases the ET-1 production in cultured cells and intact blood vessels (162)
1.1.8 High Density Lipoprotein

Small HDL have reduced amounts of apolipoprotein A-1 (apoA-1), the protein component of HDL (120) which is responsible for reverse cholesterol transport. This reduction in apoA-1 and the changed structure of HDL may alter some anti-atherogenic properties of HDL, including: antioxidative, anti-inflammatory, anti-thrombotic and endothelial stabilization (163, 164).

HDL protects LDL against oxidation, possibly through a combination of mechanisms. HDL acts as a reservoir for lipid peroxides generated by LDL, and thereby breaks down the chain of lipid peroxide and prevents LDL from oxidation (165). In addition, HDL contains an enzyme called paraoxonase-1, which may act at specific points in the lipid peroxidation cascade and help prevent LDL oxidation (166). HDL also maintains endothelial function, via the synthesis of vasodilators NO and prostacyclin (167, 168) and inhibition of vasoconstrictor ET-1 (169). Patients with low HDL and apoA-1
concentrations have been reported to have endothelial dysfunction (170) and low plasma HDL has been found to be an independent predictor of endothelial dysfunction in healthy subjects (29) and hyperlipidemic, diabetic and CHD patients (151, 171, 172). Furthermore, increased serum HDL concentrations by drug treatment e.g. nicotinic acid, or infusion of synthetic HDL improves the endothelial function (173) by increasing NO bioavailability (174).

1.1.9 Endothelial dysfunction

Endothelial dysfunction is an early factor in atherosclerosis (175) and precedes the development of clinically detectable atherosclerotic plaques in coronary arteries (176). The vascular endothelium maintains the vascular tone and structure, by balancing vasodilation and vasoconstriction, growth inhibition and growth promotion, antithrombosis and prothrombosis, anti-inflammation and pro-inflammation, and anti-oxidation and pro-oxidation (177). This vascular homeostasis is maintained through the release of a variety of substances including NO, prostacyclin, and ET-1.

NO is the major vasodilator and principal mediator for endothelial function. It is generated in endothelium by eNOS. The endothelium dependent production of NO can be stimulated by receptor-dependent pathways by substances such as: acetylcholine, bradykinin, and insulin and by receptor–independent mechanisms such as the mechanical force of flow (58). In response to stimuli or shear stress test, eNOS catalyses the production of NO from L-arginine (Figure 1.4). NO from endothelium diffuses to vascular smooth muscle and increases the conversion of guanosine triphosphate to cyclic guanosine monophosphate (178), thereby causing relaxation of smooth muscle and dilation of the artery. Reduced NO mediated vasodilatation would lead to increased vascular tone and reactivity, and might result in a stiffer vascular system, manifested by
wider pulse pressure, and inability to accommodate increases in intra vascular volume (179). NO also acts locally to prevent platelet and leukocyte aggregation and inhibits vascular proliferation and experimental studies have shown increased atherogenesis on inhibition of NO synthesis (179). In the case of reduced availability of L-arginine, eNOS may generate superoxide anions (free radicals) or hydrogen peroxide which can oxidize other molecules such as LDL, thereby increasing the risk of atherosclerosis (180).

**Figure 1.4 Effects of different stimuli (physical and chemical) on vascular endothelium.**

As opposed to NO, ET-1 is a potent vasoconstrictor which is released by stimuli including adrenaline, thrombin and hypoxia (182). ET-1 increases SNS activity and the vasoconstrictive effects of norepinephrine (182, 183). While acute hyperinsulinemia induces NO production and release, chronic hyperinsulinemia induces SNS activity and increases release of ET-1 (97, 105-107).
**Endothelial dysfunction – A cause or consequence of insulin resistance?**

Although considerable information is available on the functions and dysfunctions of endothelium, it still is not clear if endothelial dysfunction is the cause or the consequence of insulin resistance and related disorders. Children (9-11 years old) with low birth-weight show function impairment for flow-induced vasodilation in the brachial artery, without the presence of any dyslipidemia (184). Impaired endothelial dependent vasodilation of cutaneous microvasculation has also been observed in three day old (185) and three month old (186) babies of low birth weight. It is hypothesized that children born with low birth weight and insulin resistant adults at risk of type 2 diabetes and CVD are two phenotypes of the same insulin resistant genotype (187).

It is also possible that the risk factors of CHD including hyperglycemia, hypercholesterolemia, and hyperinsulinemia, damage the endothelial cells (58) causing imbalance between the endothelium derived relaxing agent (NO) and the vaso-constrictive agent (ET-1), and decreased vasodilation of the artery. As discussed previously, experimental and prospective studies have shown reduced endothelial vasodilation in individuals with hyperglycemia, dyslipidemia, hypertriglyceridemia and hyperinsulinemia.

It has been suggested that the potential mechanism linking endothelial dysfunction and insulin resistance (and related abnormalities such as: hyperinsulinemia, hyperglycemia, dyslipidemia) may be an intracellular (i.e. common cell signal transduction pathway abnormality), or extra-cellular (i.e. multiple receptor defects, e.g. altered cell membrane fluidity) or it may be the case of intracellular mechanism leading to the extra-cellular defects (177, 188).
1.1.10 Hypertension

Observational and some experimental studies have shown a positive association between insulin and blood pressure in both normotensive and hypertensive people (189-192) as well as in obese individuals with greater visceral fat deposition (193), while others have suggested that it is the insulin resistance and not hyperinsulinemia that is linked to hypertension (194). Equivocal data is also available for the association between essential hypertension and decreased insulin clearance (195, 196). The mechanism by which obesity and insulin resistance or hyperinsulinemia may cause hypertension is not clear. However, plasma free fatty acids, leptin, and aldosterone and reduced concentrations of ghrelin and adiponectin (all the factors that are known to be related to obesity and hyperinsulinemia) have also been found to be positively associated with hypertension (197-200). All these parameters (Figure 1.5) may be associated with increased arterial pressure either through the imbalance in vaso-relaxing and vasoconstrictive agents (NO and ET-1, respectively), or through increased sympathetic tone especially of kidneys (201).

Figure 1.5 Potential mechanisms linking obesity/insulin resistance and hypertension.

Reproduced from Rahmouni et al.(201).
In humans, SNS activity is positively associated with energy expenditure and negatively associated with energy intake (202). However, as discussed earlier increased SNS activity may be the cause of hypertension. In 1986, Lewis Landsberg hypothesised (Figure 1.6) that hyperinsulinemia is a compensatory mechanism for obesity and insulin resistance which limits further fuel storage, re-establish energy balance and stabilises weight by stimulating sympathetically mediated thermogenesis (203). The by-product of this increased SNS activity is the secondary effects on the kidney, vasculature and heart leading to hypertension (203).

**Figure 1.6 Landsberg Hypothesis – relation between obesity and hypertension**

Conversely, a number of studies have demonstrated that Pima Indians exhibit lower SNS activity, reduced β-adrenergic sensitivity, high obesity (central) but low rates of hypertension (204, 205). This may be the cause of a lower incidence of fatal CHD, despite a high prevalence of type 2 diabetes (75). However, the death rate in diabetics from heart disease, during the years 1965 to 1998, has increased about four-five fold (206). One possible reason for this may be the type of treatment e.g. insulin therapy (75), that may increase SNS activity and hence CHD risk.
1.1.11 Relation between SNS overactivity and metabolic diseases

Although acute hyperinsulinemia (e.g. meal-induced) increases the SNS activity which causes the secretion of catecholamines and stimulation of β-adrenergic receptors leading to homeostatic balance and increased energy expenditure in healthy individuals, the secreted catecholamines are also known to acutely increase serum free fatty acid concentrations and reduce insulin sensitivity, by stimulating hepatic gluconeogenesis and inhibiting insulin release from pancreatic β-cells and hence reducing the insulin-stimulated peripheral glucose uptake (207, 208). In adipocytes, the β-adrenergic stimulation induces a rapid down regulation of insulin receptors together with a decrease in insulin mediated glucose transport (209). This may start the vicious cycle of insulin resistance, hyperinsulinemia, dyslipidemia and endothelial dysfunction and an increased risk of CVD.

Figure 1.7 Metabolic changes due to elevated sympathetic nervous system activity

1.1.12 SNS overactivity: A cause of insulin resistance?

Another hypothesis suggests that high SNS activity precedes insulin resistance and may be responsible for causing obesity and insulin resistance. In the Tecumseh study, children aged seven with elevated heart rate and blood pressure were found to become
overweight and obese by the age of 20-22 years and insulin resistant by the age of 32 years (210). Similarly, in a study in Japanese young non-obese individuals with higher blood pressure were found to have high baseline plasma norepinephrine concentrations and normal insulin concentrations, which changed to high blood pressure, high norepinephrine and high insulin over the next ten years (211). In another five year longitudinal study Masuo et al. (212) reported that serum uric acid and plasma norepinephrine predict weight gain and blood pressure increase. The mechanism by which SNS overactivity may cause insulin resistance is thought to be related to sympathetic vasoconstriction, which shunts the nutritional blood flow away from metabolically active skeletal muscle cells, creating a state of insulin resistance (213) and has been confirmed by some experimental studies (214). This association may further feed the alliance between catecholamines and reduced insulin sensitivity and other metabolic changes (discussed earlier, Figure 1.7). In addition, despite the fact that increased SNS activity increases energy expenditure, chronic SNS activity and hypertension could also cause reduced responsiveness of β-adrenergic receptors, which may lose the effect on thermogenesis and possibly cause further weight gain (215).

1.2 Relation between diet and CHD risk factors

Dietary factors influence CHD risk in various ways, including: effects on blood lipids, blood pressure, blood clotting, oxidation of blood lipids, body weight and insulin. These different dietary factors include different types of fatty acids, carbohydrates, fruits and vegetables, whole grains and legumes.
1.2.1 Fats and carbohydrates

Studies in the 1950s suggested that the type of fat consumed may be more important in determining serum cholesterol than the total fat in the diet (216), and successive studies showed that when saturated fatty acids (SFA) were replaced by polyunsaturated fatty acids (PUFA), total plasma cholesterol was lowered (217) while monounsaturated fatty acids (MUFA) had either small or no effect (218). Subsequently came the results of the Seven Countries Study (219), which suggested that the regional differences in death from CHD were strongly correlated with the intake of SFA. Over the next three decades, numerous observational studies confirmed this association between SFA and risk of CHD (3, 217, 220-223). In addition, trials examining the effects of substituting PUFA for SFA reported a drop in CHD in the range of seven to 50 percent (224-229).

Fifteen year (230) and twenty five year (221) follow-ups of the Seven Countries study noted a negative association between MUFA intake and CHD mortality in the Greek populations. Even though no relationship was observed between CHD death rate and percent of energy from carbohydrates, CHD related deaths were lower in the Japanese population, for whom only 10 percent of total energy came from dietary fats (221, 230). Similarly, other epidemiological studies observed no association between the percentage of energy intake from carbohydrates and CHD risk (231-233). However, cross-sectional studies reported an inverse association between the glycaemic index (234), simple carbohydrates in the diet (235) and HDL cholesterol.

A number of small dietary intervention trials have since examined the effects of diets substituting SFA with other unsaturated fatty acids (PUFA, MUFA) or carbohydrates on different CHD risk factors, including: blood lipids, LDL oxidation, insulin resistance, obesity and diabetes. There is a considerable amount of data from intervention trials
suggesting that PUFA and MUFA reduces total cholesterol as well as LDL cholesterol, compared to SFA rich diets (236-240) and PUFA is slightly superior than MUFA (241). Meta-analysis studies have reported that both MUFA and PUFA elevate HDL, but the effect diminishes with the increased amounts and degree of un-saturation (241, 242). It has also been suggested that the PUFA content should be less than 8% of total energy or HDL levels may be reduced (243).

High carbohydrate low fat diets also reduce plasma total cholesterol and LDL cholesterol (244, 245). However there is a continuous debate about the efficacy of high carbohydrate diets, especially since the incidence of obesity and insulin resistance have increased in North America during the period since carbohydrate intakes have increased (246-248). High carbohydrate diets have been shown to reduce HDL cholesterol and increase triglyceride levels (244). Type of carbohydrate (simple vs. complex or high glycaemic index vs. low glycaemic index) may have an impact on the results. Moderately low fat, high complex carbohydrate diets do not show any deleterious effect on serum triglyceride and HDL levels (245, 249). Moreover it remains debatable whether carbohydrate rich diet-induced hypertriglycerideremia is potentially atherogenic, or not (250). Similarly, although mild-moderate hypertriglycerideremia is associated with endothelial dysfunction (132), in healthy individuals a high carbohydrate diet induced drop in HDL and increase in triglycerides shows similar flow-mediated vasodilation as to PUFA and MUFA rich diets (251, 252). Conversely, a study comparing the effects of saturated fat rich diet, low fat carbohydrate rich NECP-1 diet and MUFA rich Mediterranean diet in patients with hypercholesterolemia, showed an improved flow mediated vasodilatation with four weeks of MUFA rich Mediterranean diet compared to the saturated fat rich and NCEP-1 diet (253). It was suggested that the improved endothelial function was the result of antioxidant rich Mediterranean diet as the
triglyceride levels were similar after the three diets. And the changes in total cholesterol, LDL and LDL to HDL ratio were similar on the NCEP-1 and Mediterranean diet compared to the saturated fat rich diet.

High fat diets (≥ 40% of energy from fat) produce greater insulin resistance relative to high carbohydrate (20% of energy from fat) diets (254-257) particularly in obese individuals (254). However, in type 2 diabetics MUFA rich diets compared to carbohydrates result in better glycaemic control and insulin sensitivity (258, 259). Similarly, MUFA compared to SFA improves insulin sensitivity in overweight and obese subjects (260, 261) particularly with a total fat intake of less than 37% of total energy (261), but not in healthy lean subjects (260, 262-265).

Substituting carbohydrates for saturated fat in iso-energetic diets requires more insulin secretion to maintain glucose homeostasis (266). In insulin-sensitive individuals, this increased requirement for insulin is small, and maintains/lowers the concentrations of serum LDL. However, in the insulin resistant state, this increased insulin will add to the earlier compensatory hyperinsulinemia, and may worsen the condition by adversely affecting lipid metabolism (increased free fatty acids, triglycerides, small dense LDL etc.) and further increase the abnormalities of metabolic syndrome (266) and the risk of type 2 diabetes and CVD.

Additionally, MUFA prolongs the resistance of LDL oxidation compared to PUFA (236, 239, 267-269) and carbohydrate rich diets (270, 271). However it has been suggested, that it is the phenolic compounds, with their potent antioxidative capacity present in oleic acid rich oils, rather than oleic acid which resists LDL oxidation, because an extra virgin olive oil diet and not simply an olive oil diet (lacking in
phenolic compounds) has been shown to reduce LDL oxidation (272). In addition, some
dietary intervention trials have used other oleic acid rich oils e.g. oleic acid rich
sunflower oil, which are devoid of the phenolic compounds present in extra virgin olive
oil but are rich in vitamin E (270, 271). These dietary interventions and most other
vitamin E supplemental studies have been shown to reduce the susceptibility of LDL to
oxidation (273-276) in a dose dependent manner (277, 278). Conversely, reduced
susceptibility of LDL to oxidation has been shown with PUFA rich diet compared to
MUFA, probably because the vitamin E content of the PUFA diet was higher than the
MUFA diet (279). Moreover, high consumption and high plasma levels of vitamin E
have been negatively associated with mortality from ischaemic heart disease in case-
control studies (280, 281). However, long term trials of vitamin E supplementation have
mostly failed to show any effect on risk of atherosclerotic disease events and death
(282-289). It may be possible that the oxidation parameters including lag phase and rate
of oxidation tested in the feeding experiments may not translate into clinical benefit
such as a reduced risk of CHD.

1.2.2 Carotenoids

In the 1960s, the diets of the US, Greek and Japanese populations were not only
different in the type fat or amount of fat they contained, but also in the type and amount
of plant foods, and protein sources (meat, egg, fish and legumes) they contained (Table
1.1). Greek compared to US populations had higher intake of vegetables, fruits, cereals,
legumes, fish and lower intake of meat and eggs (290). Similarly Japanese populations
were eating higher amounts of vegetables, legumes, fish but lower amounts of meat and
egg than US populations (290).
Table 1.1 Dietary characteristics in the United States, Greece and Japan in the 1960s

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<th>Greece</th>
<th>Japan</th>
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<td>150</td>
</tr>
<tr>
<td>Eggs (gm/)</td>
<td>40</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Alcohol (gm/d)</td>
<td>6</td>
<td>23</td>
<td>22</td>
</tr>
</tbody>
</table>

A number of randomised prospective trials including: the Lyon diet heart (291), the diet and re-infarction trial (DART) (292) and the Indian diet heart study (293), for secondary prevention in myocardial infarct patients have shown the protective role of combined low saturated fat and high plant food diets consumption. These studies show a 30-70% reduced incidence of recurrent myocardial infarction in patients consuming reduced total/saturated fat diets rich in fish and/or plant foods compared to those on only low fat diets. Although a recent paper (294) has raised concerns about the validity of Singh’s data-the Indian diet heart study (293), the conclusions from the Singh’s paper appear consistent with those of some other investigators. Observational and prospective studies (295-299) have shown an association between fruits and vegetable intake and protection against CVD (297-302), while others have suggested an association between fruit and vegetable intake and life expectancy and not CVD risk (303, 304). Fruits and vegetables are good sources of antioxidant vitamins, carotenoids and fibre.
Carotenoids, such as β-carotene, lycopene, lutein and zeaxanthin, are a group of over 600 non-nitrogenous, fat-soluble pigments, widely distributed in nature. Carotenoids are responsible for the characteristic colour in leaves, fruit and vegetables, such as lycopene for tomatoes, and β-carotene for carrots. Epidemiological and case control studies have reported a negative association between the consumption of carotenoid rich foods and the relative risk of a coronary disease and events (280, 305-309). In addition, high serum/tissue concentrations of β-carotene and/or other carotenoids such as α-carotene, lycopene, lutein and zeaxanthin, have been found to be protective against CHD, angina or myocardial infarction (281, 310-317). The association between dietary carotenoids, serum carotenoids and CVD risk is stronger in current smokers than non-smokers (280, 311, 318).

1.2.2.1 Antioxidative properties

Experimental studies have suggested that these carotenoids have antioxidative properties. Carotenoids can inhibit the chain reactions of LDL oxidation, by reacting rapidly with the free radicals, and generating by-products that will not propagate auto-oxidation (319). The core of the LDL particles contains lipophilic antioxidants like α-tocopherol, carotenoids like β-carotene, lycopene and cryptoxanthin, ubiquinol-10 and phytofluene. These lipophilic antioxidants can quench singlet oxygen, neutralise thioyl radicals, combine with and stabilise peroxyl radicals and hence prevent free radical oxidation of other molecules (319). Although addition of β-carotene to LDL in vitro inhibits the susceptibility of LDL to oxidation (320, 321) the results for the dietary studies of β-carotene have not been that successful (274, 322). Similarly long term supplemental trials of β-carotene and CHD risk and events have been disappointing,
showing either no effect or sometimes even an increase in CHD mortality (285, 323, 324).

Similar to beta-carotene and vitamin E, lycopene reduces the susceptibility of LDL to oxidation. Dietary intervention studies with increased lycopene consumption have shown prolonged resistance in LDL oxidation in healthy subjects (325, 326), type 2 diabetics (327) and non-smokers, but not in current smokers (328) and renal transplant patients (329). Also, reduced lycopene intake increases the susceptibility of LDL to oxidation in healthy subjects (330).

1.2.2.2 Interaction and absorption

A number of factors are known to affect the absorption and serum concentrations of carotenoids. These factors include amount and type of carotenoid species, physical matrix, heat/ thermal treatment, amount and type of fat included in cooking of carotenoid rich foods (331-339), with higher serum carotenoid levels on increased consumption of pureed, heat-treated carotenoid rich food. Serum concentrations of lycopene increase with increased intake of lycopene-rich tomatoes and tomato products (325, 327, 329, 340-342) as well as lycopene supplements (343, 344). Conversely, serum concentrations of lycopene reduce with reduced consumption of lycopene rich foods (330, 345-347).

Increased consumption of one carotenoid may also improve or hamper the absorption of other carotenoids. For example, the same amounts β-carotene and lycopene taken in a supplement, increases serum lycopene concentrations without affecting β-carotene levels (348), whereas β-carotene and lutein taken together, increase serum β-carotene concentrations but reduces serum lutein (349, 350).
The amount of dietary fat present with the carotenoids may also affect the absorption of carotenoids, with higher absorption accompanying high fat intake (336, 339, 351, 352). This is probably because dietary fat stimulates bile flow from the gall bladder, which facilitates the emulsification of fat and fat-soluble vitamins into lipid micelles within the small intestine (353). Serum lycopene levels have been reported to be higher after consumption of lycopene rich tomatoes cooked with olive oil than they were without olive oil (354, 355).

However in a study undertaken by us prior to this thesis project, investigating the effects of two lycopene rich, lipid-lowering diets (high carbohydrates and high MUFA) on serum lycopene concentrations in healthy individuals (342), the results indicated that with increased consumption of lycopene rich tomato paste and tomato soup, the serum lycopene concentrations are increased to similar levels, irrespective of the amount of fat (15 or 36% of energy) in the diet. These results were different from the previous studies (354, 355) with respect to serum lycopene concentrations, probably because the source of fat was different (virgin olive oil vs. MUFA rich sunflower oil) and/or the dietary intervention periods in our study were longer (5 days vs. 14 days). It may also be a case of none versus small amount of fat intake. Three to four week long dietary intervention studies comparing effects of different amounts of fat on serum concentration of β-carotene and vitamin A, in school children, have suggested that the threshold level of fat needed for intestinal carotene uptake lies between three and five grams, as no further increase was seen while comparing the effects of carotenoid rich meals cooked with five or ten gram of fat (337, 338).

In our study (270) of lycopene rich lipid lowering diets (high carbohydrates and high MUFA), the oleic acid and vitamin E content of MUFA diet was higher than the high
carbohydrate diet and so was the lag phase for LDL oxidation. As the serum lycopene concentrations were similar after the two diets, we were unable to state whether or not lycopene had any effect on LDL oxidation. We concluded that the longer lag phase on the MUFA diet was probably the individual or joint result of higher oleic acid and vitamin E content rather than the lycopene.

Although measuring the resistance of LDL to in vitro oxidation by determination of lag time for conjugated diene formation, is the most common method, using isolated LDL has limitations as an indicator of in vivo resistance to oxidation. This is because most of the serum water soluble antioxidants and pro-oxidants are not present in the isolated LDL, and these may play an important role in resisting or augmenting the oxidation process. Similarly HDL which has antioxidative, anti-inflammatory, and anti-aggregatory activities (14) is excluded. The use of serum thus probably provides a better representation of the in vivo situation and there is a need to investigate the effects of lycopene rich olive oil diets and lycopene rich carbohydrate diets on serum lipid oxidation.

1.2.3 Chilli

In the last few years, the natural additives, herbs, spices and condiments, traditionally added to food in different cuisines for taste and flavour have been under the scrutiny of nutritionists and pharmacists. According to the Oxford English dictionary (www.oed.com), “an herb is a plant of which the stem does not become woody and persistent (as in a shrub or a tree), but remains more or less soft and succulent, and dies down to the ground (or entirely) after flowering”. Spice is defined as “one or other of various strongly flavoured or aromatic substances of vegetable origin, obtained from tropical plants, commonly used as condiments or employed for other purposes, on
account of their fragrance and preservative qualities”. Chilli, a fruit and a spice, is used in small quantities to add flavour and taste to food. Commonly, chilli is dried and ground to use as a spice but is also used fresh in salads and curries. Chilli is thought to increase energy expenditure, so that over a long period of time (combined with less energy intake than energy expenditure) it may reduce body weight and obesity, a major risk factor for CVD. Chilli is one of the richest sources of antioxidant vitamin C. The active ingredient of chilli, capsaicin, is also a potent antioxidant. The antioxidative and increasing thermogenesis properties of chilli may make it suitable dietary component to fight against the increased risk of CHD. This literature review briefly outline the types, nutritional composition, consumption of chilli in different populations, and the research available on chilli and glucose and lipid metabolism.

1.2.3.1 History and Types

Although chillies are thought to be an integral part of Asian/Indian cuisine, they were not known in Asia/India until the 16th century (356). Chilli is a Mexican word, representing the red hot fruit belonging to the Solanaceae family and Capsicum species. Capsicum species are probably the oldest cultivated plants of the Americas. Archaeologists have observed the seeds of capsicum on the floors of caves that served as human dwelling about 9000 years ago (357). It was probably brought to cultivation about 2000 years later. It is believed that Columbus introduced chilli peppers from the ‘New World’ (the Americas) to the ‘Old World’ (Europe) in 1494 and from then on, they were rapidly accepted and dispersed throughout the tropical, subtropical and temperate zones in Europe and Asia, to be used as a vegetable and spice. Red pepper is probably the only cultivated species that is known for its use as vegetable, spice,
condiment, ornamental and medicinal plant (357). In south India, dried red whole chillies are hung at the front door to ward off the evil spirits.

The most common species of chilli pepper are

- *Capsicum annum*, which includes bell peppers, paprika, jalapeños, and chiltepin.
- *Capsicum frutescens*, which includes cayenne and tabasco peppers.
- *Capsicum chinense*, which includes habaneros and Scotch bonnets.
- *Capsicum pubescens*, which includes the South American rocoto peppers.
- *Capsicum baccatum*, which includes the South American aji peppers.

Figure 1.8 Some varieties of chilli

![Cayenne](http://en.wikipedia.org/wiki/Chile_pepper) ![Scotch bonnets](http://en.wikipedia.org/wiki/Chile_pepper)

![Chiltepin](http://en.wikipedia.org/wiki/Chile_pepper) ![Bell Pepper](http://en.wikipedia.org/wiki/Chile_pepper)

Photos taken from http://en.wikipedia.org/wiki/Chile_pepper
### 1.2.3.2 Active ingredient/pungent principle

The pungent principle of chilli is called capsaicin (N(4-hydroxy-3-methoxybenzyl)-8-methyl-trans-6-nonanenamide). P.A. Bucholtz in 1816 first discovered that the pungent principle of peppers could be extracted from the macerated pods with organic solvents (358). In 1846, L. T. Thresh reported that the pungent principle could be extracted in a crystalline state and named this substance as capsaicin (358). Capsaicin (Table 1.2) is accompanied by other capsaicinoids including dihydrocapsaicin, mordihydro-, homo-, homodihydro-, nor- and nornor-capsaicin (358). However, 80-90% of capsaicinoids in capsicum fruit are mixture of capsaicin and dihydrocapsaicin (359). The total capsaicin and capsaicinoids content of red hot peppers varies between 0.5 to one percent, while that of mild paprika ranges from 0.01 to 0.3 percent (359).

#### Table 1.2 Capsaicinoids – structure, amount and hotness

<table>
<thead>
<tr>
<th>Capsaicinoid name</th>
<th>Typical relative amount</th>
<th>Scoville heat units</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>69%</td>
<td>16,000,000</td>
<td><img src="capsaicin.png" alt="Capsaicin" /></td>
</tr>
<tr>
<td>Dihydrocapsaicin</td>
<td>22%</td>
<td>16,000,000</td>
<td><img src="dhcap.png" alt="Dihydrocapsaicin" /></td>
</tr>
<tr>
<td>Nordihydrocapsaicin</td>
<td>7%</td>
<td>9,100,000</td>
<td><img src="nhcap.png" alt="Nordihydrocapsaicin" /></td>
</tr>
<tr>
<td>Homodihydrocapsaicin</td>
<td>1%</td>
<td>8,600,000</td>
<td><img src="hdc.png" alt="Homodihydrocapsaicin" /></td>
</tr>
<tr>
<td>Homocapsaicin</td>
<td>1%</td>
<td>8,600,000</td>
<td><img src="hc.png" alt="Homocapsaicin" /></td>
</tr>
</tbody>
</table>

### 1.2.3.3 Pungency

The pungency threshold of capsaicinoids is extremely low, with a detectable level as 0.0625g in $10^6$mL and the intensity of pungency increases sharply with very small increases in the concentration of the capsaicinoids (359). This means that 1g of
capsaicin dissolved in 160,000 L water can be detected on the human tongue.

Dihydrocapsaicin is as pungent as capsaicin. The hotness of chilli is measured in units called ‘scovilles’. The unit was invented in 1912 by an American pharmacologist Wilbur L. Scoville, while working on the use of capsaicin in the muscle pain-relieving ointment ‘Heet’. Scoville used a subjective organoleptic method to quantify the pungency of chilli (360). Essentially this was a taste test, where chilli was ground and mixed with sugary water and was diluted till the taster could no longer notice the burning sensation of chilli. So if the chilli extracts needs to be diluted as 1: 50,000 for it to cease causing the hot sensation on human tongue, its hotness is 50,000 scoville heat units. The more dilute the solution, the hotter the chilli. Bell peppers (also known as capsicum) rate at zero Scoville units, habanaro chilli at 300,000 and the pure capsaicin at 15,000,000 to 16,000,000 scoville units (360). Today the pungency of chillies is measured by reverse phase high pressure liquid chromatography. Figure 1.9 presents a relationship graph for Scoville heat unit and capsaicinoid content of different types of chilli (361). In general, small, thin chillies are believed to be hotter than larger chillies with broader shoulders (362).

Figure 1.9 Relation between Scoville heat unit and capsaicinoid content of chilli.
1.2.3.4 Nutritional composition

The nutritional composition of fresh red chilli, canned red chilli and chilli powder is presented in Table 1.3. Chilli peppers are among the richest sources of vitamin C, with highest amount reaching up to 340mg/100g of pepper fruit (359). While drying chilli reduces the vitamin C content by about 75%, cooking fresh chilli reduces the vitamin C content by only 30% (357). Red peppers are also a good source of vitamin E and carotenoids namely capsanthin, β-carotene, zeaxanthin and lutein (357).

Table 1.3 Nutrient composition of Chilli

<table>
<thead>
<tr>
<th>Nutrient/100g</th>
<th>Red Hot Chilli Raw</th>
<th>Canned Red Chilli (no seeds) solid &amp; liquid</th>
<th>Dry Chilli Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>166</td>
<td>89</td>
<td>1314</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>1.87</td>
<td>0.9</td>
<td>12.26</td>
</tr>
<tr>
<td>carbohydrate (g)</td>
<td>8.81</td>
<td>5.1</td>
<td>54.66</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.44</td>
<td>0.1</td>
<td>16.76</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>1.03</td>
<td>0.5</td>
<td>14.25</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>143.7</td>
<td>68</td>
<td>64.10</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>0.69</td>
<td>0.69</td>
<td>29.05</td>
</tr>
<tr>
<td>β-carotene (µg)</td>
<td>534</td>
<td>6664</td>
<td>15000</td>
</tr>
<tr>
<td>Cryptoxanthin (µg)</td>
<td>40</td>
<td>495</td>
<td>3490</td>
</tr>
<tr>
<td>Lutein + zeaxanthin (µg)</td>
<td>709</td>
<td>444</td>
<td>310</td>
</tr>
</tbody>
</table>


1.2.3.5 Chilli/capsaicin - absorption, distribution, metabolism and excretion

Capsaicinoids consumed with food are rapidly and almost completely absorbed in the gastro-intestinal tract, hydrolysed to a small extent during absorption, then transported through the portal vein and further hydrolysed to vanillylamine and iso-C10 acid mainly in the liver and kidney (359). In rats, intragastrically administered capsaicinoids are readily absorbed and metabolised in the liver before reaching the general circulation and
extra hepatic organs (363). High concentrations of $^{14}$C radioactively of a 100mg of capsaicin/kg body weight of rat (given orally or subcutaneously) have been shown in liver, adrenal, fat and thyroid. In male rats after 48 hours of oral administration of dihydrocapsaicin, an excretion of 8.7% of the dose in urine and less than 10% in faeces has been reported (364). Metabolites in urine include vanillylamine (4.7%), vanillin (4.6%), vanillyl alcohol (37.6%) and vanillic acid (19.2%) in free form, or as glucuronides (364), similar to those observed after the intake of vanillin, a safe food flavouring compound (357, 359).

1.2.3.6 Chilli/capsaicin - consumption

The average consumption of chilli (capsicum spice) has been reported to be 2.5g/person/day in India; 5g/person/day in Thailand, 5g/person/day in Korea and about 15g/person/day in Mexico (359). Assuming that the capsaicinoids content of these spices be 1%, the daily intake of capsaicinoids in these countries would range from 15 to 150mg/person/day. The capsaicinoid content of mild chillies and paprika, mainly used in U.S.A. and Europe ranges from 0.01 to 0.3 percent, making an equivalent of 0.005 to 1.5 mg capsaicinoid/person/day (359).

1.2.3.8 Chilli/Capsaicin – palatability and energy intake

Chilli consumption may affect satiety, palatability and hunger for the food. Chilli added to high carbohydrate or high fat meals reduces palatability and the desire to eat and increases the perceptibility of oiliness of high carbohydrate meals (365, 366). This may affect total energy and fat intake, and thus may be used as a tool for converting high fat diets to low fat diets.
Single day studies have shown that addition of capsaicin as a supplemental capsule or red pepper to the appetizers and/or *ad libitum* lunch and dinner meals, reduces total energy (366-368), and carbohydrate intake in Caucasian men (366). In women, the intake of protein and fat consumption has also been shown to be reduced (366). The authors suggest that this effect may be due to the increased SNS activity caused with capsaicin intake. In rats, capsaicin has been shown to activate neurons that release a wide range of neuropeptides including cholecystokinin (369), which is thought to increase SNS activity and suppress food intake (202, 370, 371).

The reduced intake of energy and macronutrient could also be the result of increased water and coffee intake so as to soothe the “hotness” of food. The *ad libitum* foods available in the study by Yoshioka (367) included meat sauce spaghetti, water and coffee for lunch and enchiladas, water and coffee for dinner. If the *ad libitum* diet also contained full-fat milk/yogurt/ice cream, the energy intake may have been higher. Capsaicin is lipophilic and these fat rich dairy foods are more helpful (personal experience and anecdotal evidence) in soothing the hotness of chillies than water or coffee.

**1.2.3.7 Chilli/capsaicin - gastric motility**

Contradictory data from both animal and humans studies is available, on the effect of chilli/capsaicin consumption on gastric emptying time and whole gut transit time. Some studies have suggested quickening (372-374) while others have reported a delay (375-377) in gastric emptying time. Similarly, the results for whole gut transit time have also been contradictory showing either quickening (376), no change (377), or even delay (375). These results do not seem to be dependent on the amount of capsaicin, but may be attributable to the time course of measurement and the source of capsaicin. In healthy
human adults, Debreceni et al. (373) showed an increase in gastric emptying rate with 400µg capsaicin intake (as capsaicin glucose solution) tested over four hours, whereas Gonzalez et al. (375) showed a delay in gastric emptying time with 420µg of capsaicin intake (taken intraesophageally as tabasco sauce and water), tested over three hours.

1.2.3.9 Chilli/capsaicin - medicinal use

Chillies have been used medicinally for hundreds of years for the treatment and cure of numerous conditions, including: arthritis, bleeding, chills, colds, coughs, digestive disorders, dysentery, toothache, headache, pain, poor circulation and sinus congestion (357, 362). Chillies were used in traditional medicine in India, as an adjunct to bitter tonics to treat loss of appetite (362), however (as discussed earlier) recent research suggests a role of chilli in reducing food intake. Chillies were also used for treating cholera, rheumatism and gout; in South America, as a remedy for cough in combination with honey; in Africa, as an antiseptic for wound healing (362).

In modern medicine, capsaicin has been extensively used in the management of pain. Capsaicin sensitive neurons release glutamate and a number of neuropeptides including substance P, somatostin and calcitonin generated peptide as neurotransmitters of nociceptive signals (378). Capsaicin has been demonstrated to cause activation followed by desensitization of sensory neurons on short and long treatments, respectively. The activation is responsible for the pain, hypersensitivity and inflammation caused by transdermal application of single dose of capsaicin (360, 378). However, repeated use provides analgesic and anti-inflammatory effects. Although the exact mechanism of the action of capsaicin is not known, it is hypothesized to be based on neuropeptide release with single dose and then depletion with repeated use (360, 378).
In animal studies, topical use of capsaicin and its analogues produce localised vasodilatation, possibly through increased release of calcitonin gene-related peptide and/or increased synthesis of NO (379). However, at higher concentrations, capsaicin and its related compounds produce vasoconstriction (379, 380) and increased oxygen consumption (380). In rats, ingestion of chilli increases the blood flow to the gastrointestinal tract (381). Human studies report vasodilatation at the site of topically applied capsaicin, with men responding more readily than women (382). Younger men are reported as better responders than older men (383) and chronic application leads to desensitization (384). Transdermal use of capsaicin has also been shown to improve ischemic threshold in patients with stable coronary disease, possibly through arteriole vasodilation (385).

1.2.3.10 Chilli/capsaicin - lipid metabolism

Although chilli/capsaicin has been used in modern medicine (as an analgesic) for about a century, research on the effects of chilli/capsaicin in other areas is limited. Small number of researchers has studied the effects of chilli/capsaicin on lipid metabolism. The earliest research, reported that rats maintained on high fat diets had repeatedly and consistently shown a pronounced lipotropic activity for red pepper and, natural and synthetic capsaicin (386). Furthermore, the liver fat deposition in the whole red pepper group was almost same as that of the capsaicin group and it was suggested that the lipotropic activity of the former was almost entirely due to its capsaicin component. It was also noted that chilli and capsaicin lowered cholesterol and total lipid content in liver of the rats fed a high fat red pepper diet. Simultaneous to this reduction in liver lipids, there was an increase in serum lipids and the authors hypothesized that the mechanism of action was related to lipid transport or lipid oxidation. However, this finding and theory has been refuted by the subsequent researchers who have reported a
reduced serum lipid content in rats with capsaicin intake (387, 388). Kwada et. al. (387) suggested that capsaicin (0.021% of the diet) stimulates lipid mobilization from adipose tissue and lowers the perirenal adipose tissue weight and serum triglyceride concentration in lard-fed rats. Another study in rats demonstrated a lower serum LDL cholesterol, increased HDL and triglycerides after three consecutive days of 3mg/kg body weight of intraperitoneal capsaicin treatment but not after 1mg/kg body weight treatment (389). One human study (390) investigated the effects of capsaicin supplement intake (135mg/day for nine weeks) on the serum lipids and lipoproteins but demonstrated no differential effects when compared to the placebo treatment.

### 1.2.3.11 Chilli/capsaicin – LDL oxidation

In addition to lipid and lipoprotein profile, some researchers have also studied the incubation effects of chilli extract, capsaicin and its different analogues on metal-induced oxidation of linoleic acid and/or isolated LDL. These studies have shown that capsaicin and its analogues have antioxidative activities and delay the initiation of oxidation and/or rate of oxidation of linoleic acid and/or isolated LDL (391-394). Intraperitoneal treatment of rats with 3mg/kg bodyweight of capsaicin for three days decreases the oxidative stress, measured as malondialdehyde, in the liver, lung, kidney and muscle (389). Chilli fruit (*Capsicum frutescens*) was also shown to up regulate the activity of the LDL receptor in human liver (HepG2) cells (391). The effect of capsaicin on plasmid DNA oxidation has also been examined using a strand scission assay. High levels of capsaicin (0.2 – 1.0mM) reduced oxidative strand breakage caused by a mixture of FeSO₄ and H₂O₂ in a dose dependent manner (395). Chilli has also been shown to have higher anti-oxidative capacity than ginger, garlic, mint and onion (396).
1.2.3.12 Chilli/capsaicin - glucose and energy metabolism

Monsereenusorn in 1978, reported that chilli inhibits the glucose transport in the everted sacs of rats and hamster in vitro (397). In subsequent research Monsereenusorn (398) demonstrated a dose-dependent decrease in fasting glucose concentrations at 30 min after with the oral intake of Capsicum annuum extract and glucose (100mg/ml/200g body weight) compared to glucose alone. The amount of capsaicin administered to rats ranged from 200mg/kg body weight to 1200mg/kg body weight. Monsereenusorn further showed that in addition to the inhibition of intestinal absorption of glucose, chilli extract administered through non-oral route (in this case intracardially) also reduced the plasma glucose concentration. It was then hypothesised that capsaicin could act systemically, especially on liver, to inhibit gluconeogenesis or stimulate glycogenesis. Ensuing research showed lower fasting blood glucose in dogs treated with chilli extract administered via endogastric tube (399). Subsequently, Tolan et. al. (400) showed a lower plasma glucose but a higher plasma insulin concentrations with reduced number and affinity of insulin binding receptors in dogs subjected to oral glucose tolerance test with/without 0.5g of chilli. The explanation given for these results was that the peripheral organs namely liver, pancreas and adrenal medulla that intervene with glucose homeostasis have capsaicin sensitive nerves (401) and increased insulin secretion due to the stimulation of pancreatic nerves may be the cause for hypoglycemia, and the decreased number and affinity of insulin receptors may the protective mechanism against hypoglycaemic coma. Similarly, another study examining the in vivo effect of capsaicin (10mg/kg body weight administered subcutaneously) on insulin secretion in rats, reported higher plasma insulin concentrations an hour after capsaicin than after vehicle treatment (402).
There is only one human study that has reported a significantly lower plasma glucose concentration after ingestion of chilli. Chaiyata et al. (403), in ten women (45-60 year old), reported a 20% lower plasma glucose 30 min after ingestion of 5g of fresh chilli with a glucose drink than after the glucose drink alone.

Studies in rats have also demonstrated that capsaicin (administered intravenously) enhances adrenal medullary catecholamine secretion (especially epinephrine) and increases energy expenditure (404, 405). In another study, intraperitoneal administration of capsaicin, has been shown to increase respiratory quotient (higher carbohydrate oxidation), serum glucose, insulin and free fatty acid concentrations and lower liver glycogen levels (406). From here it was suggested that the increased oxygen consumption, energy expenditure and carbohydrate oxidation with capsaicin was due to the activation of SNS and the subsequent release of catecholamines epinephrine and norepinephrine from adrenal medulla and stimulation of β-adrenergic receptors (406, 407).

Although it is believed that chilli/capsaicin increases energy expenditure, to my knowledge there are only three human studies that have shown this effect. These studies (365, 367, 408) were conducted in small groups (n = 8 to 13) of lean (BMI 22 to 24 kg/m²) healthy individuals. Henry et al. (408), in 12 subjects (mean age 21 years, BMI 22 kg/m²), compared the effects of two meals (3226 kJ, 51% carbohydrate, 32% fat and 16% protein) with/without 3 gram of chilli and mustard sauce and reported a 25% higher thermogenesis after the meal containing chilli and mustard sauce than the control meal. Yoshioka et al. (365), compared the effects of high fat (1883 kJ carbohydrate 40%, fat 45% and protein 15%) and high carbohydrate (1883 kJ carbohydrate 60%, fat 25% and protein 15%) meals with/without chilli in a group of 13 healthy (mean BMI
22kg/m²) Japanese women. The results showed highest thermogenesis after high carbohydrate chilli meal followed by high carbohydrate plain meal, high fat chilli meal and high fat plain meal. In another study, Yoshioka et al. (367) measured the 24 hour energy expenditure in a group of eight Caucasian males (mean age 25 years and BMI of 24.3kg/m²) and observed a lower total energy intake (~3960kJ) but a higher expenditure (~320 kJ) when the intake for these 24hrs included chilli and caffeine with other food. Other studies that have attempted to compare the effects of chilli and control meals, have either shown a tendency towards (409) increased thermogenesis or no difference between the meals (410). One study (411) also compared the meal-induced thermogenesis effects of meals with/without capsaicin in lean (n=8, mean BMI 19.6 kg/m²) and overweight women (n=8, mean BMI 28.8 kg/m²), matched for lean body mass (~47kg). The results exhibited a significantly higher thermogenesis after the capsaicin meal compared to the control meal in lean women, but not in overweight women. In fact, overweight women showed almost no difference in the sympatho-vagal activity or responsiveness between the control and the capsaicin meal (411).

The data for the effect of chilli/capsaicin on substrate oxidation is also sketchy. While higher carbohydrate oxidation has been observed with capsaicin in rats, human data show an increase in carbohydrate oxidation in men (409, 410) but fat oxidation in women (365). These differences between men and women have been attributed to the differences in the macronutrient composition of the meals between different studies (365).

As discussed earlier, increased SNS activity increases energy expenditure and reduces total food intake and thus may help in reducing body weight. However, increased SNS activity is also a risk factor for CVD due to its tendency to cause insulin resistance and
hypertension. Today, humans spend most waking hours in a postprandial state (eating every 3-4 hours), and if chilli were to be added in all the meals, chronically increased SNS activity may increase the risk of CVD.

Although the data for the effects (metabolic as well as vascular) of chilli is limited and equivocal, it is still regularly discussed in scientific as well as the non-scientific community as a possible tool for increasing energy expenditure and reducing body weight. Additionally, the available data supporting this hypothesis comes from small studies in lean healthy individuals and not from people with higher BMI wanting/trying to lose weight. There is thus a need for further research on the effects of chilli on a range of metabolic and vascular functions particularly to determine whether any effects seen in single meal interventions are sustained over medium-long term, especially in people with high BMI. As medium and long-term habitual diets may affect the activity and responsiveness of receptors involved in the regulation and transportation of nutrients, it is also important to ascertain if any metabolic and vascular effects of meals containing chilli differ with different background/habitual diets. Further, it remains to be determined whether consumption of chilli induces vasodilation, as has been seen with transdermal use, or provides significant antioxidant protection to serum lipoproteins as has been reported by in vitro isolated LDL incubation studies.

The following chapters in this thesis comprise part of the investigations of the effects of different dietary components (tomato and chilli) on some metabolic and vascular parameters of CVD risk, being undertaken at the School of Human Life Sciences, University of Tasmania.
Research Aims

The aims of the research presented in this thesis were to investigate the effects of

1. a lycopene rich high monounsaturated fat (light olive oil) diet and a lycopene rich high carbohydrate diet (each of 10 days duration) on serum lycopene concentrations, lipid profile and oxidation of serum lipids in human adults.

2. a chilli-supplemented diet (of four week duration) on a range of metabolic and vascular parameters in human adults.

3. a chilli diet (of three weeks) on endothelial dependent and independent vasodilation, after the administration of glyceryl trinitrate and salbutamol, compared to the effects of a bland diet.

4. a chilli-supplemented diet (of four week duration) on copper induced serum oxidation in humans.

5. incubation of whole serum with active ingredients of spices (in different concentrations) including chilli (capsaicin and its analogue dihydrocapsaicin), turmeric (curcumin), piprine (black pepper) and the colour pigment of tomatoes (lycopene) on the in vitro copper-induced oxidation of serum lipids.

6. meals containing chilli with or without a background of chilli diet on a range of postprandial metabolic and vascular parameters and to compare to a bland meal with the background of a bland diet.
Chapter 2 covers the aims, methodology, results and discussion of the lycopene-olive oil study (Research aim 1). Chapter 3 covers the aims, methodology, results and discussion on particular aspects of the chilli research (Research aims 2, and 3). Chapter 4 covers the aims, methodology, results and discussion for the effects of regular chilli consumption on \textit{ex vivo} serum oxidation (Research aim 4). Chapter 5 covers the aims, methodology, results and discussion of the \textit{in vitro} capsaicin, dihydrocapsaicin and curcumin incubation study (Research aim 5). Chapters 6 and 7 cover the aims, methodology, results and discussion on the postprandial effects of chilli consumption on a range metabolic and vascular function (Research aim 6). Chapter 8 brings together the key results and integrates discussion of the previous chapters, and provides suggestions for future research.

The subject group for the lycopene-olive oil study (Research aim 1) was different from the chilli study (Research aim 2, 3, 5 and 6). Only 15 subjects from four-week chilli trial took part in the chilli-drug study (Research aim 4). All participants from four week chilli study (Research aim 2) took part in the acute meal studies (Research aim 5, 6), however the number of subjects vary between chapters 3, 4, 6 and 7, due to unavailability of the data (from each test point) for some subjects.

Some repetition between the chapters is the result of these chapters being written as scientific papers for publication in various peer reviewed journals (details on page xii).