PHYSICAL CONDITIONING, TOTAL PLASMA HOMOCYSTEINE CONCENTRATION AND CARDIOVASCULAR FUNCTION IN MIDDLE-AGED MEN WITH CORONARY HEART DISEASE RISK FACTORS

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"Obstacles are those frightful things you see when you take your eyes off your goal"

- Henry Ford -

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SUMMARY

Background:

In the past 37 years, increased efforts have been directed toward a better understanding of the importance of Hcy in disease and it has now become clear that hyperhomocysteinemia is a major independent risk factor for CVD. Extensive research on the influence of vitamin supplementation leading to reductions in Hcy levels and improvements in cardiovascular function has been done. The importance of exercise in the lowering of cardiovascular risk factors, as well as its favourable influence on cardiovascular function has also been indicated in several studies, however, the limited number of studies investigating the effect of exercise on Hcy concentrations revealed contradicting results. Furthermore, a relationship between Hcy concentration and cardiovascular function with the intervention of an exercise training and a vitamin supplementation programme respectively has also not been investigated.

Objective:

The objective of this study was to examine the effect of a 12-week exercise training and a 12-week vitamin supplementation intervention respectively on tHcy concentrations and cardiovascular function, and whether the change in tHcy concentration within the different interventions correlated with the change in cardiovascular function.

Methods:

In a randomised controlled cross-over intervention study, 52 men matched for age, cardio-respiratory fitness levels and cardiovascular risk factors were randomly assigned to one of 3 groups (Group A = exercise training programme, 20-30min. at 70-80% of HR_{max}; Group B = 400 µg folic acid and 25 µg vitamin B_{12} supplement; Group C = control). Group A and B were crossed over for phase II, and Group C remained the control. The questionnaires were completed, and the body composition variables (BMI, WHR and body fat percentage), cardiovascular function (Finometer), tHcy concentrations and VO_{2max} were measured before and after each 12-week intervention period. A 6-week washout period separated the crossovers.

Results:

The ANCOVA, adjusted for age and BMI, showed that the percentage change from baseline to end, corrected for baseline of the tHcy concentration increased significantly ($p \leq .05$) by 9.7% with the exercise training intervention and decreased significantly ($p \leq .05$) by 12.9%, with the vitamin supplementation intervention. The ANCOVA of the percentage change from baseline to end in cardiovascular function showed that the vitamin supplementation intervention resulted in improvements in cardiovascular function (decreased resting MAP, TPR and increased resting SV, CO, C_w) in comparison to the impairment in cardiovascular function with the exercise training intervention (increased resting DBP, MAP and TPR). The relationship between the tHcy concentration and cardiovascular function at baseline and within each of the different interventions were assessed by partial correlations adjusted for age, BMI and VO_{2max}. Significant ($p \leq .05$) relationships only occurred within the vitamin supplementation intervention, where decreased percentage change in tHcy concentration significantly correlated with increased percentage change of resting SV and CO and decreased percentage change of resting TPR.

Conclusion:

The general conclusion that can be drawn is that a 12-week vitamin supplementation intervention showed increased health related results, e.g. a significant reduction in tHcy concentration, an improvement in cardiovascular function and a significant positive relationship between these two factors, in comparison to the 12-week exercise training intervention that significantly increased the tHcy concentration and did not show increased health related results. Due to inadequate compliance to the exercise training intervention, no conclusion can be drawn with regard to the effect of exercise training on tHcy concentrations and cardiovascular function.

Key words:

Homocysteine, exercise, cardiovascular function, cardiovascular risk factors, vitamins, VO_{2max}, CVD, arterial compliance, blood pressure, cardiac output and stroke volume

OPSOMMING

Agtergrond:

Die afgelope 37 jaar was daar 'n toename in die aantal pogings tot 'n beter begrip van die rol van Hcy in siekte toestande. Dit is dus nou duidelik dat verhoogde Hcy vlakke 'n onafhanklike risiko faktor vir kardiovaskulêre siektes (KVS) is. Daar is alreeds baie navorsing oor die verlagende effek van vitamien aanvullings op Hcy vlakke, asook vitamienes se verbeterde effek op kardiovaskulêre funksie. Baie studies het ook al die belangrikheid van oefening op die verlaging van kardiovaskulêre risiko faktore, asook 'n verbetering op kardiovaskulêre funksie bewys. Daar is egter 'n beperkte aantal studies oor die effek van oefening op Hcy konsentrasies met teenstrydige resultate. Die verband tussen Hcy konsentrasie en kardiovaskulêre funksie met die intervensie van 'n oefen program en 'n vitamien aanvulling onderskeidelik is ook nog nie nagevors nie.

Doelstelling:

Die doel van hierdie studie was om die effek van 'n 12-week oefening program en 'n 12-week vitamien aanvulling program onderskeidelik op tHcy konsentrasies asook op kardiovaskulêre funksie te bepaal, en of daar 'n verband bestaan tussen die verandering in tHcy konsentrasie en die verandering in kardiovaskulêre funkie met die verskillende intervensie programme.

Metodes:

In die gekontroleerde ewekansige oorkruis intervensie studie, is 52 mans wat bymekaar pas t.o.v. ouderdom, kardiorespiratoriese fiksheid vlakke en kardiovaskulêre risiko faktore, ewekansig in 3 groepe verdeel (Groep A = oefen program, 20-30min. teen 70-80% van HR_{maks}; Groep B = 400 µg foliensuur en 25 µg vitamien B_{12} aanvulling; Groep C = kontrole). Groep A en B is vir fase II oorkruis, en die kontrole groep het dieselfde gebly. Die vraelyste is ingevul, en die liggaamsamestelling (LMI, MHR en persentasie liggaamsvet), kardiovaskulêre funksie (Finometer), tHcy konsentrasies en VO_{2maks} is voor en na elke 12 weke intervensie periode bepaal. 'n Ses weke uitwas periode het na elke oorkruis gevolg.

Resultate:

Die ANCOVA, wat gekorrigeer is vir ouderdom en LMI, het gewys dat die persentasie verandering vanaf basislyn tot einde, gekorrigeer vir basislyn waardes veroorsaak het dat die tHcy konsentrasie met die oefening intervensie statisties betekenisvol ($p \leq .05$) toegneem het met 9.7%, en met die vitamien aanvulling intervensie statisties betekeninsvol ($p \leq .05$) gedaal het met 12.9%. Die ANCOVA vir die persentasie verandering vanaf basislyn tot einde in kardiovaskulêre funksie het getoon dat die vitamien aanvulling intervensie bygedra het tot verbeteringe in kardiovaskulêre funksie (verlaagde rustende MAP, TPR en verhoogde rustende SV, KO, C_w) in vergelyking met die oefening intervensie wat verswakte kardiovaskulêre funksie teweeg gebring het (verhoogde rustende DBD, MAP en TPR). Parsiële korrelasies wat gekorrigeer is vir ouderdom, LMI, en VO_{2maks}, is gebruik om die verband tussen tHcy konsentrasie en die kardiovaskulêre funksie in elkeen van die intervensies te bepaal. Daar is slegs met die vitamien aanvulling intervensie statistiese betekenisvolle ($p \leq .05$) verbande gevind. Die verlaagde persentasie verandering in tHcy konsentrasie het statisties betekenisvol gekorreleer met 'n toename in persentasie verandering in rustende SV en KO en 'n afname in persentasie verandering in rustende TPR.

Gevolgtrekking:

Die algemene gevolgtrekking is dat 'n 12-week vitamien aanvulling intervensie 'n toename in gesondheid vertoon het, m.a.w. 'n statistiese betekenisvolle afname in tHcy konsentrasie, 'n verbetering in kardiovaskulêre funksie en 'n statistics betekenisvolle positiewe verband tussen hierdie twee faktore getoon het, in vergelyking met die 12-week oefening intervensie wat die tHcy konsentrasie statiesties betekenisvol laat styg het en nie 'n toename in gesondheid getoon het nie. Die gebrek aan deelname aan die oefening intervensie maak dit onmoontlik om tot 'n gevolgtrekking te kom t.o.v. die effek van oefening op tHcy konsentrasies en kardiovaskulêre funksie.

Sleutelwoorde:

Homosisteïen, oefening, kardiovaskulêre funksie, kardiovaskulêre risiko faktore, vitamiene, VO_{2maks}, KVS, arteriële meegewendheid, bloeddruk, kardiale omset en slag volume

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LIST OF ABBREVIATIONS

A	**ACSM**	=	American College of Sports Medicine
	ADMA	=	asymmetric dimethylarginine
	ANCOVA	=	analysis of co-variance
	ANOVA	=	one-way analysis of variance
B	**B**	=	baseline
	beats/min	=	beats per minute
	BHMT	=	betaine:homocysteine methyltrasferase
	BMI	=	body mass index
C	**CAD**	=	coronary artery disease
	CBS	=	cystathionine beta-synthase
	CGL	=	cystathionine gamma-lyase
	CHD	=	coronary heart disease
	cm	=	centimetre
	CO	=	cardiac output
	CO_2/O_2	=	carbon dioxide per oxygen *(see R)*
	CRP	=	coronary risk profile
	CVD	=	cardiovascular disease
	C_w	=	Winkessel arterial compliance
D	**d_1**	=	fat-free compartment
	d_2	=	fat compartment
	D_b	=	the density of the body (D = Mass/Volume)
	DBD	=	diastoliese bloeddruk
	DBP	=	diastolic blood pressure

	DMG	=	dimethylglycine
	DNA	=	deoxyribonucleic acid
	DPC	=	diagnostic products corporation
	DTT	=	dithiothreitol
E	**E**	=	end
	ECG	=	electrocardiograph
	EDTA	=	ethylenediaminetetra-actetic acid
	e.g.	=	*exempli gratia* (for example)
	et al	=	*et alii* (and others)
F	**FMD**	=	flow-mediated dilatation
	FMS	=	Finapress Medical Systems
G	**g/cc**	=	gram per cubic centimeter
H	**Hcy**	=	homocysteine
	HDL-C	=	high density lipoprotein cholesterol
	HR	=	heart rate
	HR_{max}	=	maximal age predicted heart rate
	hyper-Hcy	=	hyperhomocysteinemia
I	**i.e.**	=	*id est* (that is)
	IMT	=	intima-media thickness
	Inc.	=	incorporated
	in vitro	=	in a test tube or other laboratory environment
	in vivo	=	natural circumstances
K	**kcal/min**	=	kilocalories per minute
	kg	=	kilogram
	kg/m^2	=	kilogram per metre squared

	km	=	kilometre
	km/day	=	kilometre per day
	KO	=	kardiale omset
	KVS	=	kardiovaskulêre siektes
L	**LDL-C**	=	low density lipoprotein cholesterol
	LMI	=	liggaamsmassa indeks
	l/min	=	litre per minute
	Lp(a)	=	lipoprotein(a)
	Ltd	=	limited
	LVEF	=	Left ventricular ejection fraction
M	**m**	=	metre
	MAP	=	mean arterial pressure
	MAT	=	methionine adenosyltransferase
	m.a.w.	=	met ander woorde
	Max.	=	maximum
	MET	=	energy expenditure (expressed as the metabolic -equivalent score)
	mg	=	milligram
	mg/dl	=	milligram per decilitre
	MHR	=	maag-heup ratio
	MI	=	myocardial infarction
	min.	=	minute
	Min.	=	minimum
	ml	=	millilitre
	ml/mmHg	=	millilitre per millimetre mercury
	mlCO_2/kg/min	=	millilitre carbon dioxide per kilogram per minute
	mlO_2/kg/min	=	millilitre oxygen per kilogram per minute
	mm	=	millimetre
	mmHg	=	millimetres of mercury

	mmHg/ml	=	millimetres of mercury per millilitre
	mmol/l	=	millimole per litre
	MS	=	methionine synthase
	MTHFR	=	methylenetetrahydrofalate reductase
N	**n**	=	number of subjects
	NO	=	nitric oxide
	NOS	=	nitric oxide synthase
O	**O_2^-**	=	superoxide anion
P	**p**	=	significant difference ($p \leq .05$)
	PAI	=	physical activity index
	PP	=	pulse pressure
	Pty	=	proprietary
	PVD	=	peripheral vascular disease
	PWV	=	pulse wave velocity
R	r	=	correlation
	R	=	respiratory exchange rate (CO_2/O_2)
	RDA	=	recommended daily allowance
	RDI	=	recommended daily intake
	ROS	=	reactive oxygen species
	rpm	=	revolutions per minute
S	**s**	=	seconds
	SAC	=	systemic arterial compliance
	SAH	=	S-adenosyl-L-homocysteiene
	SAHH	=	S-adenosylhomocysteine hydrolase
	SAM	=	S-adenosylmethionine
	SBP	=	systolic blood pressure

	SD	=	standard deviation
	SHMT	=	serine hydroxymethyltransferase
	SPSS	=	statistical practice for social science
	SV	=	stroke volume
T	**TC**	=	total cholesterol
	t.o.v.	=	ten opsigte van
	TG	=	triglycerides
	tHcy	=	total plasma or (serum) homocysteine
	THF	=	tetrahydrofolate
	TM	=	thrombomodulin
	TPR	=	total peripheral resistance
U	**USA**	=	United States of America
V	**VCO_2**	=	carbon dioxide production
	V_e	=	minute ventilation
	Vit.	=	vitamin
	vitamin B_{12}	=	hydroxy cobalamin
	vitamin B_6	=	pyridoxine
	VO_{2max}	=	maximal oxygen consumption
	vWF	=	von Willebrand factor
W	**W**	=	watt
	WHR	=	waist-to-hip ratio
Y	**yr**	=	years

LIST OF SYMBOLS

μg	=	micro gram
μmol/l	=	micromole per litre
®	=	registered trademark
°C	=	degrees Celsius
%	=	percentage
%Δ	=	percentage changes from baseline to end
$^{a/a/b}$	=	significance difference
<	=	smaller than
>	=	bigger than
≤	=	smaller or equal to
≥	=	bigger or equal to
–	=	minus
×	=	multiply
/	=	divided
±	=	plus, minus
≈	≈	equal to

Chapter 1

Introduction

1.1 INTRODUCTION

In 1969 McCully (1969:120) made a clinical observation linking elevated homocysteine (Hcy) concentrations with atherosclerotic vascular diseases. Many studies have since confirmed the association of elevated Hcy concentrations in vascular disease (Taylor *et al.*, 1991:128; Welch & Loscalzo, 1998:1048), including cerebrovascular disease, peripheral vascular disease, coronary vascular disease (Clarke *et al.*, 1991:1149), stroke (Perry *et al.*, 1995:1397) and coronary atherosclerosis (Mayer *et al.*, 1996:523).

Hcy, a sulfur-containing amino acid metabolized by either catabolizing enzymes or methionine-conserving enzymes (Ueland *et al.*, 1993:1765), is the most sensitive blood chemistry test for determining the rate of methyl group loss from DNA (Ovokaitys, 2002:16). When 40% of the methyl groups are lost, degenerative death typically occurs (Ovokaitys, 2002:16). Methyl group loss acceleration is caused by aging, smoking, poor nutrition, poor vitamin intake and low exercise levels, to name but a few; the higher the Hcy level, the greater the rate of methyl group loss from DNA (Ovokaitys, 2002:16).

Serum Hcy concentration is directly associated with increased arterial disease and cardiovascular events. In a meta-analysis, Wald *et al.* (2002:1206) calculated that for an increase of 5 μmol/l serum Hcy concentration, the risk increased by 41% for ischaemic heart disease (adjusted for age, sex, smoking, blood pressure and serum cholesterol concentration). Experimental evidence suggests that endothelial dysfunction is the major mechanism by which Hcy exerts its deleterious effect (Woo *et al.*, 1997(a):2542; Welch & Loscalzo, 1998:1047). Although the exact mechanism of the endothelial dysfunction is unknown, there is growing evidence that Hcy exerts its effects by promoting oxidative damage (Welch & Loscalzo, 1998:1047)

The damage to the endothelial cells leads to a reduction of the amount of elastic tissue through the fragmentation and degeneration of elastin, as well as an increase in the amount of collagen that causes the thickening of the arterial wall (Lakatta *et al.*, 1987:42A). This progressive thickening accompanies the stiffening process of the arteries (Sutton-Tyrrell *et al.*, 2001:429)

and may probably be the cause of a decrease in arterial compliance. Arterial compliance has also been described as a predictable marker for vascular disease states (Cohn *et al.*, 1995:508). A decrease in arterial elasticity is an early sign of vascular disease, including atherosclerosis, as well as an independent prognostic marker of morbidity and mortality (Cohn, 1999:S43).

Tanaka *et al.* (2000:1273) found that with aging central arterial compliance decreases whether individuals are physically active or inactive, however, the magnitude of the age-related reduction in central arterial compliance is attenuated in men who regularly participate in vigorous endurance exercise. The treatment of hyperhomocysteinemia varies with the underlying cause but generally involves supplementation with folic acid, vitamin B_{12} (hydroxy cobalamin) and vitamin B_6 (pyridoxine) (Den Heijer *et al.*, 1998:359). A strong inverse association between plasma Hcy concentration and plasma folate has been recognized (Selhub *et al.*, 1993:2693) and short term supplementation with folic acid is associated with a significant enhancement of endothelial function as well as blood pressure reduction (Mangoni *et al.*, 2002:501). A limited number of studies have investigated the effect of physical conditioning through physical activity and/or exercise on total plasma (or serum) homocysteine (tHcy) concentrations. König *et al.* (2003:117) showed a lowering effect on tHcy levels with the high exercise training group, but Duncan *et al.* (2004:898) found that tHcy increases with exercise training. Another study showed that acute exercise had no effect on tHcy levels (Wright *et al.*, 1998:265).

1.2 PROBLEM STATEMENT

The leading Tromso study (Arnesen *et al.*, 1995:706) concluded that in the general population tHcy is an independent risk factor for coronary heart disease (CHD) including an independent predictor of myocardial infarction. Several other studies have also indicated consistently that elevated tHcy levels are an independent risk factor for vascular disease (Ueland *et al.*, 1993:1764; Wald *et al.*, 2002:1202; Brosnan, 2004:779). In a meta-analysis, an estimated 10% of the risk of coronary artery disease (CAD) in the general population is attributable to elevated tHcy levels (Boushey *et al.*, 1995:1049).

Plasma Hcy values between 5 and 15 μmol/l in fasting subjects are considered normal (Ueland *et al.*, 1993:1764). Hcy concentration rises progressively with age in men and women making it an important risk factor for cardiovascular disease (CVD) (Selhub *et al.*, 1993:2695). According to Stampfer *et al.* (1992:880), men with Hcy levels that were above 15.8 μmol/l, had an approximately threefold increase in the risk of myocardial infarction compared to those with lower levels. Causes of elevated tHcy levels include: enzymatic defects in the metabolic pathway (Gallagher *et al.*, 1996:2158), increased age, male sex, cigarette smoking (Nygård *et al.*, 1995:1528), dietary deficiency (folate, vitamin B_{12}, vitamin B_6) (Selhub *et al.*, 1993:2696), liver disorders through impaired methionine metabolism (Kinsell *et* al., 1947:590; Ueland & Refsum, 1989:488), hormonal factors such as hypothyroidism, renal failure (McCully, 1996:389), malignant transformation of cells and certain drugs and toxins, namely methotrexate, nitrous oxide, penicillamine and anti-convulsants (Ueland & Refsum, 1989:488-491).

According to Woo *et al.* (1997(a):2542; 1997(b):S39), hyperhomocysteinaemia was found to be an independent risk factor for arterial endothelial dysfunction. Several different mechanisms have been proposed to explain the association between Hcy levels and atherosclerotic vascular disease, including endothelial cell dysfunction or injury (Welch & Loscalzo, 1998:1047), promotion of the proliferation of smooth muscle cells into the intima, extracellular matrix modification and stimulation of oxidation of low-density lipoprotein (Bellamy & McDowell, 1997:307). Hcy may be the cause of the perturbation of endothelial anti-coagulant protein C activation (Rodgers & Conn, 1990:900), and it is also known to interfere with activity of the endothelial vasodilator and platelet inhibitor nitric oxide (NO) (Romerio *et al.*, 2004:343). In a study by Virdis *et al.* (2001:1106-1115) the profound impact of hyperhomocysteinaemia's impairment on endothelial function (in both healthy subjects and patients with essential hypertension) by producing oxidative stress that reduces NO availability, could be one of the possible mechanisms through which hyperhomocysteinaemia can lead to an increased risk of CVD. This impairment of endothelial vasodilatation through Hcy (Schlaich *et al.*, 2000:388 & Virdis *et al.*, 2001:1106) may be the link to impaired cardiovascular function because of its influence on a loss in arterial compliance.

The relationship of standard cardiovascular risk factors with decreased peripheral arterial compliance corresponds to the relationship of these risk factors to atherosclerotic burden and cardiovascular events (Willens *et al.*, 2003:205). According to Arnett *et al.* (1994:669,675-679), a decrease in arterial distensibility is recognized as a potential marker of subclinical CVD and is associated with a number of well-established cardiovascular risk factors (male gender, age, lipoprotein abnormalities and diabetes) that accompany the initiation and/or progression of hypertension and atherosclerosis. One standard deviation decrease in arterial elasticity was associated with a 15% greater risk of hypertension and suggests that lower arterial elasticity is related to the development of hypertension (Liao *et al.*, 1999:203). Large artery stiffness, a major determinant of myocardial ischaemic threshold (Kingwell *et al.*, 2002:773), appears to be an independent risk factor for future cardiovascular events (Meaume *et al.*, 2001:874) and is associated with CAD (Gatzka *et al.*, 1998:578).

Vitamin requirements of Hcy metabolism are clinically significant in that deficiencies of each of them (folic acid, vitamin B_{12} and vitamin B_6) are associated with elevated tHcy levels (Selhub *et al.*, 1993:2696; Eikelboom *et al.*, 1999:365). A meta-analysis estimated that Hcy levels can be reduced by 25% using folic acid (0.5 to 5 mg daily), and supplementation of vitamin B_{12} (0.5 mg daily) produced an additional 7% reduction (Clarke & Armitage, 2000:342). This has led to the proposal that lowering tHcy concentrations by increasing the intake of folic acid (or combinations with other B-group vitamins), might be an effective means of decreasing cardiovascular risk (Coffey *et al.*, 2003:28). In a study of Wilmink *et al.* (2000:188), supplementation with folic acid enhanced endothelial function and according to Mangoni *et al.* (2005:22,24), enhanced endothelium-dependent vasodilatation in patients with type II diabetes was independent of baseline tHcy concentrations or its reduction. Blood pressure reduction using folic acid supplementation in healthy chronic smokers was also independent from its Hcy lowering effect (Mangoni *et al.*, 2002:501).

Terenzi (2000:30) suggested that enhanced arterial compliance had a beneficial cardio protective effect associated with aerobic training. According to Cameron *et al.* (1999:653), a positive association exists between systemic arterial compliance (SAC) and fitness level in healthy older

individuals and an inverse association between SAC and systolic blood pressure. This increase in SAC, which is greater due to changes in blood pressure, is linearly related to change in VO_{2max} (Cameron & Dart, 1994:H693). In another study, moderate aerobic training, but not high-resistance strength training, reduces large artery stiffness in young individuals (aged 30 to 59 years), whereas older individuals (aged 57 to 80 years) with established isolated systolic hypertension are resistant to such adaptation (Kingwell, 2002:216). In a study group of 73 men with chronic heart failure, the exercise training group showed an 11% decrease in resting heart rate (HR) and a 6% increase in resting cardiac output (CO) from baseline to 6-month follow-up period (Hambrecht *et al.*, 2000:3099). The 6-month exercise training period also led to a significant decrease in resting total peripheral resistance (TPR) of 8% and a significant increase in their mean stroke volume (SV) of 23% as well as VO_{2max} of 26% during peak exercise, compared with the control group (Hambrecht *et al.*, 2000:3099).

The importance of physical activity and/or exercise in the lowering of morbidity and mortality has been indicated in several studies (Blair *et al.*, 1996:205; Kajula *et al.*, 1998:443; Hakim *et al.*, 1999:9), as well as its favourable influence on cardiovascular function (Cameron *et* al., 1999:654; Hambrecht *et al.*, 2000:3098,3099; Tanaka *et al.*, 2000:1273; Terenzi, 2000:29). However research on the effect of an acute exercise session as well as an exercise training programme on tHcy concentrations is limited with contradicting results. This limited research concluded that a 30-minute bout of moderate intensity acute exercise had no effect on tHcy levels in young healthy men aged 24 to 39 years (Wright *et al.*, 1998:265). In another study, an exercise training period of 12 weeks (36 exercise sessions) produced a 12% reduction in Hcy levels in normolipidaemic patients with CAD and hyperhomocysteinaemia. This decrease is expected to produce a 20% to 30% reduction in CAD risk (Ali *et al.*, 1998:1544). However, Duncan *et al.* (2004:899) indicated that higher intensity exercise leads to elevated tHcy concentrations.

The positive associations of raised Hcy levels' effect on components of the cardiovascular function, such as increased pulse pressure, a marker of large vessel stiffness (Davis *et al.*, 2001:327) and an increase in arterial stiffness (Nestel *et al.*, 2003:85) have been indicated.

However, there is currently no extensive research on the influence of a change in tHcy concentration on cardiovascular function with the intervention of an exercise training and a vitamin supplementation programme respectively.

The focus of this study will be on determining the effect of an exercise training and a vitamin supplementation programme respectively on tHcy concentrations and cardiovascular function in men with three or more cardiovascular risk factors. The study will also investigate whether the change in tHcy concentration through an exercise training and a vitamin supplementation programme respectively has an influence on cardiovascular function.

If a exercise training programme and/or vitamin supplementation programme has a lowering effect on tHcy concentrations with related improvements in cardiovascular function, it will be logical to presume that these positive influences of exercise training and/or vitamin supplementation will have an accumulating effect on each other and will then also influence other risk factors in a positive manner. This may indicate the importance of exercise training and/or vitamin supplementation as a less expensive alternative to improve lifestyle while also leading to lower morbidity and mortality rates.

The scientific question to be answered is: How will a 12-week exercise training and a 12-week vitamin supplementation intervention respectively change tHcy concentrations and cardiovascular function in men aged 45 to 60 years with three or more cardiovascular risk factors, and what is the relationship between the change in tHcy concentration and the change in cardiovascular function?

The results obtained in this study may lead to a better understanding of the possible underlying mechanism involved in the influence of tHcy concentrations on cardiovascular function during exercise training and vitamin supplementation respectively.

1.3 OBJECTIVES

The objectives of this study are to determine:

- The tHcy concentrations and cardiovascular function of untrained men aged 45 to 60 years with three or more cardiovascular risk factors
- the effect of a 12-week exercise training and a 12-week vitamin supplementation intervention respectively on tHcy concentrations and cardiovascular function in men aged 45 to 60 years with three or more cardiovascular risk factors
- the relationship between the change in tHcy concentration and the change in cardiovascular function with a 12-week exercise training and a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors.

1.4 HYPOTHESES

The following hypotheses are derived for this study:

- The tHcy concentrations will be elevated and cardiovascular function will be impaired in untrained men aged 45 to 60 years with three or more cardiovascular risk factors
- A significant reduced tHcy concentration and significant improvement in cardiovascular function will result from a 12-week exercise training and a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors
- A reduction in tHcy concentration will be positively related with an improvement in cardiovascular function after a 12-week exercise training and a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors.

1.5 STRUCTURE OF DISSERTATION

The importance of exercise training in the lowering of morbidity and mortality has been indicated in a number of studies. However, in this dissertation the focus is on the influence of tHcy concentrations on cardiovascular function and whether there is a relationship between these two factors with the intervention of an exercise training and a vitamin supplementation programme respectively.

This dissertation consists of five main divisions:

- ✓ An introduction (Chapter 1)
- ✓ Literature review: Mediators for change in homocysteine and cardiovascular function (Chapter 2)
- ✓ Research methods (Chapter 3)
- ✓ Results and discussion (Chapter 4)
- ✓ Summary, conclusions and recommendations (Chapter 5).

After this introductory chapter, a literature review (Chapter 2) about all the present research on the causes, influences and possible mechanisms of elevated Hcy concentrations and impaired cardiovascular function, as well as the interaction between Hcy and cardiovascular function are discussed. The non-pharmacological treatment of vitamin supplementation and exercise training on Hcy concentrations and cardiovascular function are also reviewed. The study design and research methods, namely tHcy concentrations, cardiovascular function measurements and exercise training and vitamin supplementation interventions are discussed in detail in Chapter 3. Chapter 4 (presentation of the results and discussion) investigated the outcome of this study and determined whether exercise training and vitamin supplementation respectively influenced tHcy concentrations and cardiovascular function as well as the relationship between these two factors. A general summary, conclusion, limitations and recommendations are presented in Chapter 5, after which the references and appendices follow.

Chapter 2

Literature review: Mediators for change in homocysteine and cardiovascular function

2.1 INTRODUCTION

Homocysteine (Hcy) was discovered as far back as 1932 by De Vigneaud (as quoted by Ueland & Refsum, 1989:473) as a product of demethylation of methionine. Hcy determination was then introduced into laboratory diagnosis in 1962 when the first patients with the inborn error homocystinuria were described (Carson & Neill, 1962:505-512). In 1969 an autopsy of an 8 year old boy who died of stroke revealed that his arteries had the sclerotic look of blood vessels of an elderly man with coronary heart disease (CHD). His blood also contained excess levels of Hcy (McArdle *et al.*, 2001:900). Thus, in 1969 excessive amounts of the amino acid Hcy were first implicated in the pathogenesis of atherosclerosis (McCully, 1969:111-120). In the past 37 years, increased efforts have been directed toward a better understanding of the importance of this amino acid in disease and it has now become clear that hyperhomocysteinemia (elevated Hcy concentrations) is a major independent risk factor for vascular disease (Clarke *et al.*, 1991:1149; Taylor *et al.*, 1991:128; Mayer *et al.*, 1996:523; Welch & Loscalzo, 1998:1048; Homocysteine Studies Collaboration, 2002:2015-2022). In a meta-analysis by Wald *et al.* (2002:1206) an increase of 5 µmol/l in plasma Hcy concentration raised the odds for ischaemic heart disease by 32% and stroke by 59%. Taylor *et al.* (1999:15-16) also reported that an increase of 1 µmol/l Hcy concentration can be associated with a 5.6% increased possibility of death from cardiovascular disease (CVD), even after adjustment for other risk factors. However, whether the increased risk in vascular disease is mediated directly by Hcy or whether it may simply be a marker for some other disease process remains controversial (Scott, 2000:333-334).

The pathological mechanisms leading to atherothrombosis associated with hyper-homocysteinemia are not completely understood as yet, and endothelial injury/dysfunction may be one mechanism whereby Hcy leads to an increased risk of both arterial and venous disease (Bellamy & McDowell, 1997:307; Faraci, 2003:372). A number of studies indicate that elevated Hcy levels are involved in cellular toxicity (Woo *et al.*, 1997(a):2542; Pruefer *et al.*, 1999:493; Aronow, 2003:22; Faraci, 2003:372), free radical-mediated damage (Upchurch *et al.*, 1997:17015-17016; Signorello *et al.*, 2002:283; Weiss, 2005:27-33) and thrombosis (Lentz & Sadler, 1991:1912; Rees & Rodgers, 1993:347-352; Aronow, 2003:22).

Therefore, Hcy causes detrimental effects on the cardiovascular function through its influence on the vasculature. A positive linear association between plasma Hcy and diastolic blood pressure (DBP), systolic blood pressure (SBP) and heart rate (HR) have been indicated (Nygård *et al.*, 1995:1529). High plasma Hcy also causes arterial stiffness in the central arterial circulation which includes the large elastic arteries such as the aorta, and the rise in Hcy led to an average 21% reduction in systemic arterial compliance (Nestel *et al.*, 2003:85). Furthermore, decreased arterial compliance appears to be an independent risk factor for the development of CVD (Meaume *et al.*, 2001:874; Seals, 2003:68), and as the vessels stiffen, the physical forces that oppose aortic valve opening increase and can contribute to ventricular hypertrophy, aortic root dilation, valvular dysfunction and heart failure (Rowe, 1987:69G-71G; Arnett *et al.*, 1994:678-679; Seals, 2003:69).

From the above mentioned statements it is clear that treatments of elevated Hcy concentrations as well as an impaired cardiovascular function are of utmost importance for a productive and healthy life. Folic acid, as well as vitamin B_{12} and vitamin B_6 effectively reduce elevated Hcy concentrations (Den Heijer *et al.*, 1998:359; Jacques *et al.*, 2001:618; Yao *et al.*, 2003:929; Klerk *et al.*, 2005:98). However, the role of folic acid in lowering cardiovascular risk is not limited to reducing tHcy levels (O'Grady *et al.*, 2002:841). Supplementation with folic acid also enhances endothelial function (Wilmink *et al.*, 2000:188), and reduces blood pressure (Mangoni *et al.*, 2002:501) and arterial stiffness (Williams *et al.*, 2005:29).

Regular endurance exercise training results in VO_{2max} increases of about 15 to 25% (Wilmore, 2003:48), and there is a significant inverse association between blood pressure and exercise (Svetkey *et al.*, 2003:467. Aerobic exercise also increases resting CO and SV and decreases resting HR (Wilmore, 2003:49). Furthermore, moderate aerobic exercise is a potential non-pharmacological therapy to increase systemic arterial compliance (Cameron *et al.*, 1999:655; Mackey *et al.*, 2002:20). Extensive research on the effect of exercise training on high Hcy concentrations is lacking and, therefore, there is no general conclusion on the influence of different modalities, frequencies and intensities of exercise training on Hcy concentrations. After an acute exercise session Hcy concentrations increased according to König *et al.*

(2003:117), and Bailey *et al.* (2000:1062) showed significant reductions in Hcy levels after aerobic exercise training. In contrast a few studies concluded that exercise training did not produce significant differences in tHcy concentrations at all (de Jong *et al.*, 2001:341; Volek *et al.*, 2002:586-588; Chen *et al.*, 2005:36).

In this chapter the causes, influences and possible mechanisms of elevated Hcy concentrations and impaired cardiovascular function are discussed, as well as the relationship between Hcy and cardiovascular function. The non-pharmacological treatment of vitamins and exercise training on Hcy and cardiovascular function are also reviewed.

2.2 HOMOCYSTEINE METABOLISM

In human plasma Hcy exists in several forms. Approximately 70 to 80% is bound to protein, mainly albumin, by a disulphide bond. The remaining Hcy oxides form dimers (homocystine) or combine with cysteine to form a mixed disulphide. Only a small proportion (< 1%) circulates as free Hcy (Doshi *et al.*, 1999:578). A number of techniques are now available for the combined measurement of the multiple forms of plasma Hcy (Still & McDowell, 1998:184-186). The term "total plasma (or serum) homocysteine" (tHcy) refers to the combined pool of free, bound, reduced and oxidized forms of Hcy in the plasma (Hankey & Eikelboom, 1999:407). Hyperhomocysteinemia (hyper-Hcy) may be defined according to arbitrary cut-off points (e.g. 95th percentile) in the distribution of values obtained from the so-called "normal population" (Hankey & Eikelboom, 1999:407). Among fasting individuals, normal tHcy commonly ranges from 5-15 μmol/l and higher fasting values are classified as moderate (16-30 μmol/l), intermediate (31-100 μmol/l) and severe (>100 μmol/l) hyper-Hcy (Hankey & Eikelboom, 1999:407). A more meaningful definition, which remains to be developed, would be one that correlated with risk of serious vascular events (Hankey, 2003:37).

Sections 2.3 to 2.6 provide a brief overview of the genetic and clinical disorders that produce hyper-Hcy; reviews the evidence of elevated tHcy levels as a potential new risk factor for

vascular disease and the possible mechanism by which tHcy exerts its deleterious effect. Subsequently the non-pharmacological treatment of elevated tHcy levels will be discussed.

Hcy is an amino acid that arises from the metabolism of methionine's remethylation and transsulfuration pathways. Unlike sulphur-containing amino acids, i.e. cysteine and methionine, it is not incorporated into proteins and is produced solely as a metabolic intermediate (Brosnan, 2004:775). The three different facets of methionine metabolism are discussed below and demonstrated in Figure 2.1 (Brosnan, 2004:776).

2.2.1 Transmethylation

Methionine metabolism begins with the conversion of methionine to S-adenosylmethionine (SAM), which is the cell's principal methylating agent. No less than forty methyltransferases have been described and there are doubtless many more to be discovered. SAM-dependent methylation reactions probably occur in all cells of the body (Brosnan, 2004:775).

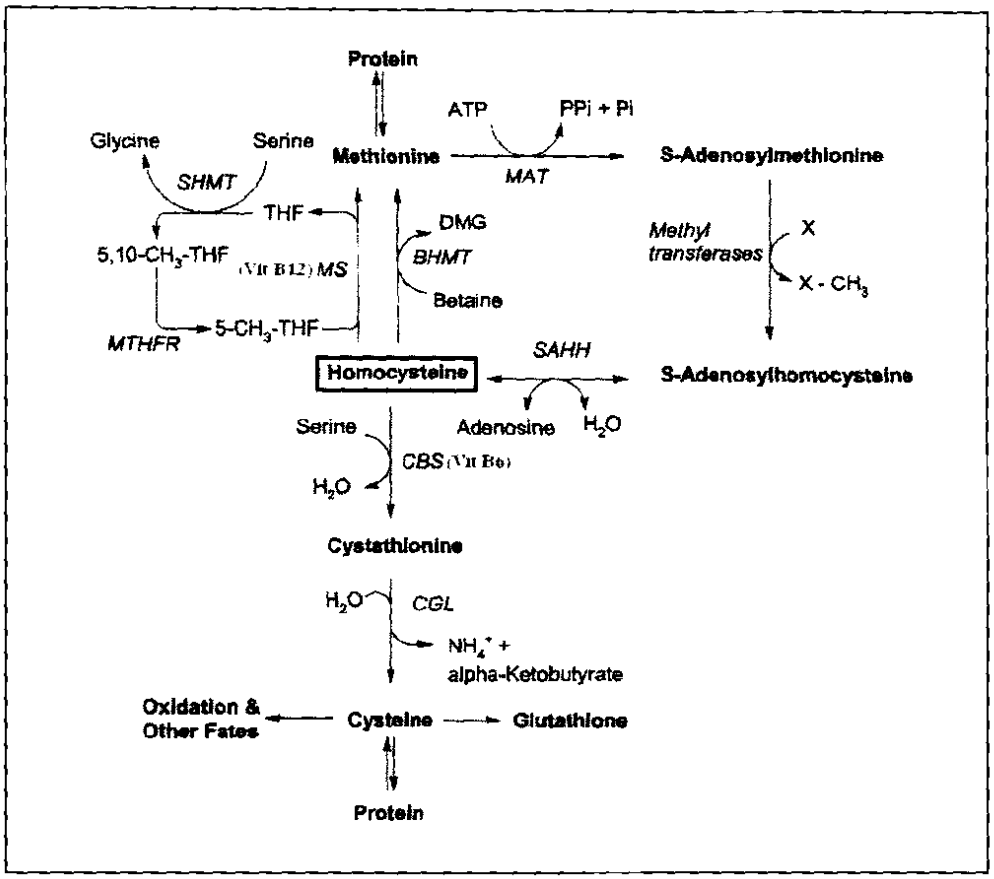
2.2.2 Remethylation

There are two mechanisms by which homocysteine may be remethylated back to methionine. This conserves the carbon skeleton of this essential amino acid and occurs at a higher rate when the methionine supply is limited. One remethylation system derives its methyl group from betaine (a product of choline catabolism) to reform methionine. Betaine:homocysteine methyltransferase (BHMT) has a rather limited distribution, being restricted in humans to the liver and kidney (Brosnan, 2004:775). The other remethylating system, employing 5-methyltetrahydrofolate, is widely distributed and catalyzed by methionine synthase (MS), a vitamin B_{12}-dependent enzyme, to reform methionine (Welch & Loscalzo, 1998:1042; Brosnan, 2004:775).

2.2.3 Transsulfuration

Hcy may be catabolized to cysteine, provided that adequate methionine is available. The irreversibility of this pathway accounts for the fact that methionine cannot be synthesized from cysteine (Brosnan, 2004:775). In this pathway, Hcy condenses with serine to form cystathionine

in a reaction catalyzed by the vitamin B_6-dependent enzyme, cystathionine beta-synthase (CBS). Cystathionine is subsequently hydrolyzed to form cysteine and is dependent on cystathionine gamma-lyase (CGL) (Welch & Loscalzo, 1998:1042; Brosnan, 2004:775). It is abundant in the liver and is also found in the kidney, intestine and pancreas (Brosnan, 2004:775). In addition to the synthesis of cysteine, the transsulfuration pathway effectively catabolizes excess Hcy which is not required for methyl transfer (Bostom & Lathrop, 1997:11).



BHMT = betaine:homocysteine methyltransferase; CBS = cystathionine beta-synthase (Vitamin B6 dependent enzyme); CGL = cystathionine gamma-lyase; DMG = dimethylglycine; MAT = methionine adenosyltransferase; MS = methionine synthase (Vitamin B12 dependent enzyme); MTHFR = methylenetetrahydofolate reductase; SAHH = S-adenosylhomocysteine hydrolase; SHMT = serine hydroxymethyltransferase; THF = tetrahydrofolate.

Figure 2.1: Methionine and homocysteine metabolism (Brosnan, 2004:776)

2.3 FACTORS CONTRIBUTING TO ELEVATED HOMOCYSTEINE CONCENTRATIONS

Hyper-tHcy may be caused by genetic defects of enzymes or their cofactors or co-substrates involved in the metabolism of Hcy, vitamin deficiency, lifestyle factors, diseases, medications, or other factors (Hankey & Eikelboom, 2001:440) (see Table 2.1).

Table 2.1: Factors contributing to hyperhomocysteinemia as compiled from Eikelboom *et al.* (1999:365) and Hankey and Eikelboom (2001:440)

Enzyme deficiencies
Deficiencies in cystathionine synthase, cethionine synthase, MTHFR and MS.

Vitamin deficiencies
Deficiencies of folic acid, vitamin B_{12} and vitamin B_6

Demographic characteristics/Lifestyle
Increasing age, male sex, smoking, physical inactivity, alcohol consumption (wine and spirits) and postmenopausal status.

Various diseases
Renal failure, malignancies, hyperproliferative disorders, psoriasis, diabetes mellitus, hypothyroidism, acute phase of stroke or myocardial infarction (MI), systemic lupus erythematosus and transplantation.

Drugs
Anticonvulsants (phenytoin, carbamazepine), folate antagonists (methotrexate), vitamin B_{12} antagonists (nitrous oxide), vitamin B_6 antagonists, trimethoprim, theophyline, azaribine, estrogen-containing oral contraceptives, lipid lowering drugs (cholestyramine, colestipol, nicotinic acid, fibrates), thiazide diuretics and cyclosporine.

Other factors
Cobalamin mutations, methionine loading (oral, intravenous, peritoneal) and acute-phase response to illness.

2.4 HOMOCYSTEINE, A NEW RISK FACTOR FOR CARDIO-VASCULAR DISEASE?

Age adjusted rates of deaths due to cardiovascular disease have declined by more than 50% in the past 25 years among whites and blacks of both sexes (Coffey *et al.*, 2003:25). This decrease

is due, in part, to improved treatment at the acute care level and to effective primary prevention, such as reducing and controlling known risk factors (Goldman & Cook, 1984:825-836; Rosamond *et al.*, 1998:865-866; Coffey *et al.*, 2003:25). Although many subjects with coronary artery disease (CAD) have a history of cigarette smoking, hyperlipidemia, hypertension or diabetes mellitus, a large proportion of subjects with clinical cardiovascular events do not have these "traditional" risk factors present (Stampfer & Malinow, 1995:328; Meleady *et al.*, 1996:103). Therefore, attention has recently focused on potential new risk factors, of which Hcy is one (Refsum *et al.*, 1998:31-62; Alpert, 1999:858-865; Eikelboom *et al.*, 1999:363-375; Hackman & Anand, 2003:936-937) (see Table 2.2).

Table 2.2: Known and potential new risk factors for cardiovascular disease (Hennekens, 1998:1099 & Hughes, 2003:131-134)

Known risk factors	Potential new risk factors
▪ Cigarette smoking	▪ Homocysteine
▪ Elevated cholesterol	▪ Plasma fibrinogen
▪ Hypertension	▪ Factor V & X
▪ Obesity	▪ Lipoprotein(a)
▪ Physical inactivity	
▪ Diabetes	

2.4.1 Health risks of Hcy

Elevated Hcy concentrations have already been linked with vascular disease in 1969 by McCully (1969:120). Hyper-tHcy is now known as an independent risk factor for CVD (Ueland *et al.*, 1993:1764; Wald *et al.*, 2002:1202; Brosnan, 2004:779), including cerebral arterial and vascular disease (Brattström *et al.*, 1990:59; Taylor *et al.*, 1991:128), peripheral vascular disease (PVD), and coronary artery and vascular disease (Dudman *et al.*, 1993:1259; Welch & Loscalzo, 1998:1048). Clarke *et al.* (1991:1149) reported that the prevalence of hyper-tHcy was 42% among patients with cerebral vascular disease, 28% among patients with PVD and 30% among patients with coronary vascular disease. The relative risk for the occurrence of cardiovascular events or death increases 1% for each increase of 1 μmol/l in tHcy (Moustapha *et al.*, 1998:140).

Boers *et al.* (1985:712) screened 75 patients with premature atherosclerotic vascular disease and found that nearly one third of all patients with occlusive peripheral or cerebral arterial disease had hyper-tHcy. A number of studies accepted the hypothesis of elevated Hcy levels being a risk factor for atherosclerotic vascular disease (Ueland & Refsum, 1989:489; Boushey *et al.*, 1995:1056; Mayer *et al.*, 1996:523; Welch & Loscalzo, 1998:1048; Moustapha & Robinson, 1999:50; Aronow, 2003:27). However, the association between elevated tHcy levels and atherosclerotic vascular disease is dose-related (i.e. the risk is greater with higher tHcy levels) (Hankey & Eikelboom 2001:440).

In the general population elevated tHcy is an independent risk factor for CHD (Arnesen *et al.*, 1995:706; Woo *et al.*, 1997(b):S39). In a nested prospective case-control study by Shai *et al.* (2004:378), a positive association between tHcy levels and CHD risk was found, even after controlling for other established CHD factors. A prospective study by Soinio *et al.* (2004:99) showed that tHcy is an independent risk factor for future CHD event in patients with type-2 diabetes with or without known CHD. Elevated tHcy concentrations are also associated with an increased risk for CAD (Genest *et al.* 1990:1117; Verhoef *et al.*, 1997:994). Elevated tHcy is attributable to an estimated 10% of the risk for CAD (Boushey *et al.*, 1995:1051), and an increase of 5 μmol/l tHcy concentration raised the risk of CAD by as much as an increase of 0.52 mmol/l in the cholesterol concentration (Boushey *et al.*, 1995:1051). According to Anderson *et al.* (2000:1229), Hcy levels >16.5 μmol/l were associated with reduced survival in patients with established CAD.

Aronow (2003:27) concluded that increased tHcy levels is a risk factor for CAD, peripheral arterial disease, extracranial carotid arterial disease, deep vein thrombosis and possibly dementia and Alzheimer's disease in older persons. Den Heijer *et al.* (1996:761) demonstrated that mild hyper-Hcy is an independent risk factor for deep-vein thrombosis and that a tHcy concentration of >21.1 μmol/l was associated with an odds ratio of 4.0 for the development of deep-vein thrombosis. Harpel *et al.* (1996:1285S,1288S) also found a relationship between elevated Hcy levels and arterial as well as venous thrombosis. Selhub *et al.* (2006:1728S) demonstrated that the prevalence of carotid-artery stenosis increases with an increase in tHcy.

In a meta-analysis by Wald *et al.* (2002:1206), an increase of 5 µmol/l in tHcy concentration raised the odds for ischaemic heart disease by 32% and stroke by 59%. Stroke has also been linked with elevated Hcy levels according to a few studies (Perry *et al.*, 1995:1397; Kittner *et al.*, 1999:1556; Aronow, 2003:27). Boysen *et al.* (2003:1260) found a significant difference in tHcy levels between patients with ischaemic and hemorrhagic stroke, suggesting that elevated tHcy is not only a reaction to acute illness but also a risk factor for recurrent stroke. According to Araki *et al.* (1989:139), high levels of tHcy could also be one of the risk factors for cerebral infarction, and in a study by Nygard *et al.* (1997:234) tHcy were strongly related to MI. A nested case-control study by Stampfer *et al.* (1992:880) found that men with Hcy levels that were above 15.8 µmol/l had an approximately threefold increase in the risk of a MI compared to those with lower levels, making it an independent predictor of MI (Arnesen *et al.*, 1995:706). Bots *et al.* (1999:41) calculated that the risk of stroke and MI increased 6 to 7% for every 1 µmol/l increase in tHcy, however, the risk by quintiles of tHcy level was significantly increased only in the group with levels above 18.6 µmol/l. An increased tHcy level independently predicts the risk of development of congestive heart failure in adults, even without prior MI (Vasan *et al.*, 2003:1255).

2.4.2 Hcy: morbidity and mortality

There is increasing evidence that hyper-Hcy is common in the elderly population (Joosten *et al.*, 1993:469; Selhub *et al.*, 1993:2695). According to Moustapha and Robinson (1999:41,50-51), it is advisable to screen for tHcy in older patients determined to be at high risk for CVD. That is: 1) those with a personal or family history of premature CVD; 2) those with arterial occlusive disease; or 3) those who experience a cardiovascular event and have no known risk factors. According to Welch & Loscalzo (1998:1042), patients with mild hyper-Hcy have none of the clinical signs of severe hyper-Hcy or homocysteinuria (increased tHcy due to a genetically determined inborn error of the transsulfuration pathway's CBS), and are typically asymptomatic until the third or fourth decade of life when premature CAD develops, as well as recurrent arterial and venous thrombosis.

Plasma Hcy concentration was identified as an independent risk factor for cardiovascular morbidity and mortality and Taylor *et al.* (1999:15-16) reported that an increase of 1 μmol/l Hcy concentration can be associated with a 5.6% increased possibility of death from CVD, even after adjustment for other risk factors. In another prospective population-based study, each 5 μmol/l increment of tHcy raised the risk of 5-year mortality by 17% in the non-diabetic and by 60% in the diabetic subjects (Hoogeveen *et al.*, 1998(b):69). Nygard *et al.* (1997:230) examined 587 patients with confirmed CAD and found that after a median follow-up of 4.6 years, elevated Hcy was a strong predictor of overall mortality namely 3.8% in those with tHcy < 9 μmol/l and 24.7% in those with tHcy ≥15 μmol/l, even after adjustment for other prognostic determinants. In a community cohort study of 1788 middle-aged and elderly residents, 10% of the 405 deaths recorded were attributable to Hcy levels exceeding 14 mmol/l (Kark *et al.*, 1999:326). In a study of 629 patients, untreated homocysteinuria caused 50% of the occurrence of thromboembolic events by age 29, and mortality was 14% at age 20 and 19% at age 30 (Mudd *et al.*, 1985:2,14,25).

2.4.3 Hcy and known risk factors

Homocysteine is correlated with age, sex, smoking, blood pressure, heart rate and blood cholesterol levels, however, the relationship of homocysteine with vascular disease appears to be independent of these factors (Nygård *et al.*, 1995:1526). Graham *et al.* (1997:1780) concluded that elevated tHcy concentrations conferred an independent risk of vascular disease similar to that of smoking or hypercholesterolemia and also had a multiplicative effect on risk among cigarette smokers and patients with hypertension.

Plasma Hcy levels are positively associated with blood pressure in a healthy population (Araki *et al.*, 1989:145; Malinow *et al.*, 1995:180), and Malinow *et al.* (1989:1185) found that tHcy was significantly elevated in hypertensive patients compared with that in normotensive subjects. Panagiotakos *et al.* (2005:476) demonstrated a positive association between tHcy and total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Wu *et al.* (1994:560) and Glueck *et al.* (1995:133) also showed a correlation between tHcy and LDL-C. Cigarette smoking is associated with increased tHcy

concentrations in a number of studies (Ma *et al.*, 1996:2416; de Bree *et al.*, 2001:152; Chrysohoou *et al.*, 2004:121). Araki *et al.* (1993:155) and Munshi *et al.* (1996:134) demonstrated that diabetic subjects had a higher fasting tHcy level than non-diabetic controls, however, it is not clear whether the higher tHcy levels were due to the diabetic state. Hyper-Hcy also appeared to be a stronger risk factor for CVD in subjects with non-insulin-dependent diabetes mellitus than in subjects with normal or impaired glucose tolerance (Hoogeveen *et al.*, 1998(a):135).

According to the literature there are contradictions regarding Hcy as a risk factor. Verhoef *et al.* (1994:1924) concluded that there was only a small but non-significant association between elevated tHcy levels and risk of ischaemic stroke, and two prospective population-based studies contradict that elevated tHcy levels are an independent risk factor for MI or stroke (Alfthan *et al.*, 1994:17; Lind *et al.* 2003:409). In a study by Brattström *et al.* (1991:55), no significant differences in tHcy concentrations were found between patients with venous thromboembolism and control subjects. Kang *et al.* (1991:543) also noted that there was no correlation between CAD and tHcy. Hence, in a number of studies no evidence for hyper-Hcy as a major risk factor for vascular disease, after adjustment for conventional risk factors, was indicated (Evans *et al.*, 1997:1951; Folsom *et al.*, 1998:208; Kuller & Evans, 1998:196-199). However, according to Ashfield-Watt *et al.* (2001:431), Hcy is associated with an increased risk of CHD, but may not be causally related to CVD except when present in very high concentrations. Ubbink & Delport (2000:673) concluded that the stage has not been reached where an elevated tHcy concentration may be considered a causative factor in atherothrombotic disease because of inconsistent data from prospective studies. The reason for the contradicting results regarding Hcy being a cardiovascular risk factor may be an indication that elevated tHcy levels are only a marker of vascular disease states.

2.5 THE POSSIBLE MECHANISMS OF HOMOCYSTEINE IN VASCULAR DISEASE

The vascular endothelium is a cellular monolayer that plays an important role in cardiovascular physiology in both health and disease. The endothelium is involved in the modulation of

vascular tone, initiation of coagulation and fibrinolysis and the generation of inflammatory mediators. Markers of endothelial function have been widely used in cardiovascular research since the proposal that endothelial damage was an important event in the development of CVD (Moat *et al.*, 2004:69).

Endothelial injury/dysfunction appears to be an early event in the promotion of atherogenesis (Ross, 1993:804-806), correlates positively with increased risk of CAD (Schroeder *et al.*, 1999:736) and has recently been shown to be of prognostic significance for spontaneously occurring acute cardiovascular events, including sudden cardiac death, myocardial infarction, and cerebral infarction, after adjustment for presence of CAD and other cardiac risk factors (Halcox *et al.*, 2002:657). Endothelial injury/dysfunction may be one mechanism whereby Hcy leads to an increased risk of both arterial and venous disease (Bellamy & McDowell, 1997:307 & Faraci, 2003:372). Hcy could potentially be involved in several of the processes in the development of atherosclerosis (Bellamy & McDowell, 1997:309), as well as thrombosis (Soinio *et al.*, 2004:98). However the pathological mechanisms leading to atherothrombosis associated with hyper-Hcy are not completely understood as yet. Experimental data support a range of possibilities (see Figure 2.2), including:

Cellular toxicity

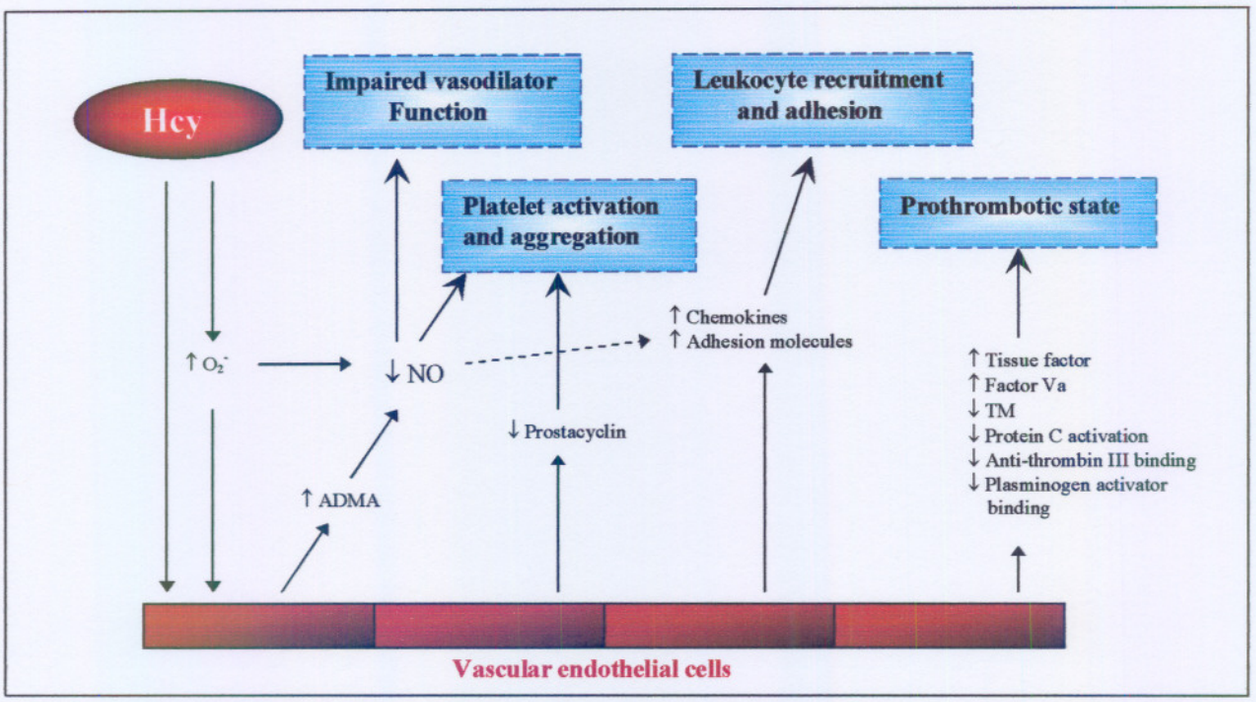
- ✓ Endothelial cell injury/dysfunction (Wall *et al.*, 1980:113-121; Woo *et al.*, 1997(a):2542; Chambers *et al.*, 2001:191; Fenster *et al.*, 2003:224).
- ✓ promotes the proliferation of smooth muscle cells and stimulation of collagen synthesis (Tsai *et al.*, 1996:150-151; Majors *et al.*, 1997:2079; Aronow, 2003:22).
- ✓ decreases bioavailability of the endothelial vasodilator and platelet inhibitor nitric oxide (NO) (Faraci, 2003:372 & Romerio *et al.*, 2004:343; Weiss, 2005:30-32).
- ✓ increases expression of chemokines, adhesion molecules and leukocyte recruitment and binding (Pruefer *et al.*, 1999:493; Weiss *et al.*, 2002:232).

Free radical-mediated damage

- ✓ Enhances arachidonic acid release and reactive oxygen species (ROS) formation in platelets (Signorello *et al.*, 2002:283; Moselhy & Demerdash, 2003,2004:29).
- ✓ oxidative damage through super oxide anion and hydrogen peroxide generation (Heinecke *et al.*, 1987:10099; Upchurch *et al.*, 1997:17015-17016; Weiss, 2005:27-30).
- ✓ suppresses the expression of cellular glutathione peroxidase (intracellular antioxidant) (Upchurch *et al.*, 1997:17015-17016; Das, 2003:687; Weiss, 2005:29).
- ✓ enhances LDL-C oxidation (Hirano *et al.*, 1994:271; Upchurch *et al.*, 1997:17016; Weiss, 2005:33).

Hcy and thrombosis

- ✓ Increases fibrinogen levels and thrombus formation (Rees & Rodgers, 1993:347-352; Aronow, 2003:22).
- ✓ increases activation of coagulant factor V and factor X (Rodgers & Kane, 1986:1913; Aronow, 2003:22).
- ✓ inhibition of cell surface expression of thrombomodulin (TM) and perturbation of protein C mechanism (Rodgers & Conn, 1990:899,900; Lentz & Sadler, 1991:1912).
- ✓ positive association with von Willebrand factor (vWF) (Lentz & Sadler, 1993:687; Manrique *et al.*, 2005:3784).
- ✓ induces tissue factor expression, and reduces plasminogen activator binding (Das, 2003:687; Loscalzo, 1996:6).
- ✓ enhancement of thromboxane formation and platelet aggregation (Signorello *et al.*, 2002:283; Aronow, 2003:22).
- ✓ interferes with fibrinolysis by increasing lipoprotein(a)-fibrin binding (Rees & Rodgers, 1993:351; Aronow, 2003:22).



ADMA = asymmetric dimethylarginine; Hcy = homocysteine; NO = nitric oxide; O_2^- = superoxide anion; TM = thrombomodulin.

Figure 2.2: Some of the putative mechanisms and effects of Hcy-induced endothelial dysfunction (Weiss *et al.*, 2002:229)

2.5.1 Endothelial injury/dysfunction

Endothelial injury/dysfunction is characterized by an imbalance between vasorelaxing and vasoconstriction substances, between pro-coagulant and anti-coagulant factors, between pro-inflammatory and anti-inflammatory mediators and between inhibitors and promoters of vascular cell growth, and is an early and persistent vascular abnormality in the evolution of atherothrombotic disease (Weiss *et al.*, 2002:228). Some of the biochemical markers in endothelial injury/dysfunction as well as the influence of Hcy on each marker are discussed below. These markers are NO, protein C, lipoprotein(a) and fibrinogen.

NO

Endothelium-derived relaxing factor, shown to be NO, is synthesized from L-arginine within the endothelial cell by the constitutive enzyme nitric oxide synthase (NOS) (Leoncini *et al.*, 2003:717). NO partially mediates inhibition of platelet activation, adhesion, and aggregation,

prevents vascular smooth muscle proliferation, and adheres leukocytes to the endothelium. Endothelial products such as NO regulate various aspects of blood vessel homeostasis, including vascular tone, leukocyte-vessel wall interaction, smooth muscle growth, proliferation and survival, local haemostasis and fibrinolysis, and redox state (Fenster *et al.*, 2003:218,219). Dysfunction of the endothelium with reduced NO bioavailability occurs before structural damage is apparent and is widely considered to be a sensitive indicator of vascular pathology (Ross, 1993:804-805). In human platelets Hcy decreases NO formation in whole cells (Leoncini *et al.*, 2003:718) and may then lead to vasoconstriction, platelet aggregation and monocyte adhesion, all of which promote atherosclerosis (Chambers *et al.*, 1999:1160).

One of the mechanisms suggested that Hcy impairs production of NO by increasing oxidative stress, namely elevating hydrogen peroxide and decreasing glutathione peroxidase activity in endothelial cells (Upchurch *et al.*, 1997:17015-17016; Weiss, 2005:32). In a study by Sydow *et al.* (2004:94,97-100), findings support another concept, namely that Hcy causes dysfunctional NOS through the increase in plasma asymmetric dimethylarginine (ADMA), which is a potent endogenous inhibitor of NOS and this accounts for endothelial dysfunction and impaired endothelium-dependent vasodilatation. Hcy also decreases NO formation through the inhibition of L-arginine transport (Leoncini *et al.*, 2003:718; Weiss, 2005:32). When L-arginine is limited, NOS also acts upon O_2 to form super oxide anion (Ogonowski *et al.*, 2000:C140-C142) and generates ROS (Leoncini *et al.*, 2003:718). Thus, decreased vasodilator activity of NO leaves the endothelium vulnerable to Hcy-mediated oxidative injury (Stamler *et al.*, 1993:312).

Protein C

Protein C is a serine protease that circulates in the blood as a zymogen (Harpel *et al.*, 1996:1286S). It is activated by interaction with thrombin that is bound to a platelet and the endothelial cell membrane receptor thrombomodulin (TM), consisting of anti-thrombotic properties. After activation, protein C catalyzes the inactivation of factors V and VIII to inhibit their coagulant activity (Esmon, 1989:4743). The importance of the protein C pathway is further emphasized by the finding that inherited defects in the protein C pathway are associated with thromboembolic disease (Gladson *et al.*, 1988:20).

There are several mechanisms by which Hcy might reduce TM activity. The biggest possibility is that Hcy may directly interact with TM to impair TM-thrombin or TM-protein C interactions (Rodgers & Conn, 1990:899). Rodgers and Conn (1990:900) observed that Hcy competitively inhibits the thrombin-TM interaction, thus endothelial cell protein C mechanism can be perturbed by Hcy.

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is synthesized in the liver and closely related to LDL-C, but with the addition of a specific protein [apoprotein(a)] linked to apoprotein B by a disulphide bond (Bellamy & McDowell, 1997:313). High levels of Lp(a) are thought to be atherosclerotic and prothrombotic (Reguero *et al.,* 1995:434). Lp(a) interferes with the process of fibrinolytic degradation of blood clots, thus promoting progression of atherosclerotic lesions (Jenner *et al.,* 1993:1139). Lp(a) has been linked to the atherothrombosis pathologically by its participation in foam cell formation and LDL-C oxidation (Scanu, 1998:29Q-30Q), and promotes thrombosis by competing with plasminogen for binding sites on fibrin-coated surfaces (Bellamy & McDowell, 1997:313; Stein & Rosenson, 1997:1171). Therefore, Lp(a) is established as a significant and independent risk factor for the development of atherosclerosis, CAD (Jenner *et al.,* 1993:1140; Kwiterovich, 1994:S55-S70), CHD (Stein & Rosenson, 1997:1171-1174) and ischemic heart disease (Reguero *et al.,* 1995:432). Hcy promotes Lp(a) binding to fibrin at concentrations as low as 8 μmol/l, much lower than that required to alter other vascular hemostatic activities (Rees & Rodgers, 1993:351).

Fibrinogen

Fibrinogen is a circulating glycoprotein present in blood plasma that, under the proper physiological circumstances, is converted in fibrin threads that form the basis of a blood clot. In addition, fibrinogen stimulates blood platelet aggregation, encourages smooth muscle cell migration, proliferation and increased blood viscosity (Thomas *et al.,* 2003:642-643). Thus, high levels of fibrinogen increase the likelihood of internal clot formation (Plowman & Smith, 1997:197). One of the inflammatory markers that predicts future CVD events is fibrinogen levels (Huges, 2003:134). Cardiovascular risk, the extent of atherosclerosis and the severity of

CAD are also directly related to the level of plasma fibrinogen (de Maat *et al.*, 1996:189; Wang *et al.*, 1997:249). Hcy may play a role in increased fibrinogen levels and subsequent thrombus formation (Harker *et al.*, 1974:539-543).

Thus Hcy-related endothelial injury/dysfunction may be involved in the initiation and progression of atherosclerosis and/or thrombosis.

2.5.2 Atherosclerosis

Atherosclerosis is an inflammatory disease and does not result simply from the accumulation of lipids (Ross, 1999:123). Hcy is only one of the possible causes of endothelial dysfunction leading to atherosclerosis (Ross, 1999:116).

According to Ross (1999:115), the endothelial dysfunction that results from the injury leads to compensatory responses that alter the normal homeostatic properties of the endothelium. The different forms of injury increase the adhesiveness of the endothelium with respect to leukocytes or platelets, as well as its permeability. The injury also induces the endothelium to have pro-coagulant instead of anti-coagulant properties and to form vasoactive molecules, cytokines and growth factors. If the inflammatory response does not neutralize or remove the offending agents effectively, it can continue indefinitely. In doing so, the inflammatory response stimulates migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation to form an intermediate lesion (Ross, 1999:115). If these responses continue unabated, proliferation of the arterial wall ensues, which compensates by gradual dilation, so that up to a point, the lumen remains unaltered (Glagov *et al.*, 1987:1374-1375), a phenomenon termed "remodelling" (Ross, 1999:115).

Continued inflammation results in increased numbers of macrophages and lymphocytes, which both emigrate from the blood and multiply within the lesion (Ross, 1999:115). Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines, and growth factors, which can induce further damage and eventually lead to focal necrosis. Thus, cycles of accumulation of mononuclear cells, migration and proliferation of smooth-muscle cells and

formation of fibrous tissue lead to further enlargement and restructuring of the lesion, so that it becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue, a so-called advanced, complicated lesion. At some point, the artery can no longer compensate by dilation and the lesion may then intrude into the lumen and alter the flow of blood (Ross, 1999:116).

Therefore, the importance of reducing elevated Hcy levels is emphasized because of its detrimental effect on vascular function and will be discussed in the next section.

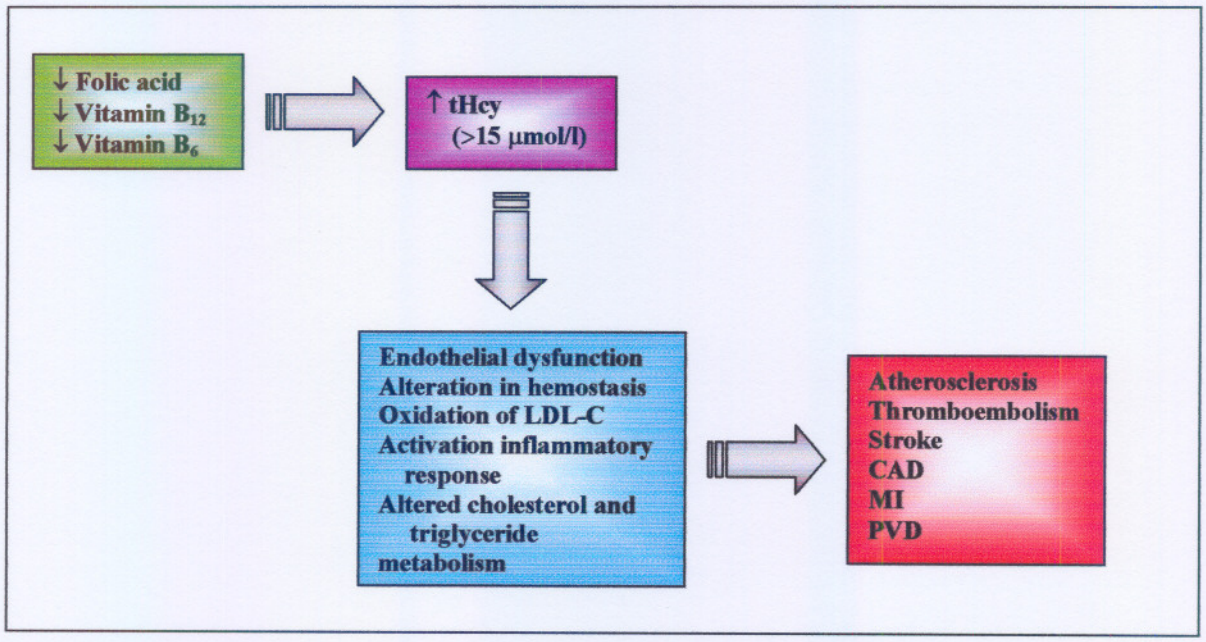
2.6 NON-PHARMACOLOGICAL TREATMENT OF HYPERHOMOSISTEINEMIA

2.6.1 Vitamin supplementation

The metabolism of methionine and Hcy (see Figure 2.1) views an example of the involvement of vitamins as cofactors and the effects of vitamin deficiency on metabolism. Folic acid and vitamin B_{12} protect against the build-up of Hcy in the blood by conversion to methionine. Vitamin B_6 also prevents the accumulation of Hcy by converting it to cysteine and other compounds excreted in urine (O'Grady *et al.*, 2002:839). Vitamin requirements of Hcy metabolism are clinically significant in that deficiencies of each of them (folic acid, vitamin B_{12} and vitamin B_6) are associated with elevated tHcy levels (Selhub *et al.*, 1993:2696; Eikelboom *et al.*, 1999:365). Indeed, since the advent of folic acid fortification, the mean tHcy of the USA population has decreased by about 7% (with a marked decrease in the number of people displaying high Hcy levels) (Brosnan, 2004:777).

A number of studies found that folic acid, probably the most important dietary determinant of tHcy levels (Saw *et al.*, 2001:237), as well as vitamin B_{12} and vitamin B_6 effectively reduced elevated Hcy concentrations (Den Heijer *et al.*, 1998:359; Jacques *et al.*, 2001:618; Yao *et al.*, 2003:929; Klerk *et al.*, 2005:98). Hence, it is an efficient, safe, and inexpensive means to reduce elevated tHcy (Refsum *et al.*, 1998:51). A meta-analysis estimated that Hcy levels can be reduced by 25% using folic acid (0.5 to 5 mg daily) and supplementation of vitamin B_{12} (0.5 mg daily) produced an additional 7% reduction (Clarke & Armitage, 2000:342). Moustapha and Robinson (1999:41) suggested that co-administration of vitamin B_{12} may be needed to prevent

irreversible neurologic damage. A diet rich in fruits, vegetables and low fat dairy products and reduced in saturated and total fat can also lower tHcy concentrations (Appel *et al.*, 2000:856).



CAD = coronary artery disease; LDL-C = low density lipoprotein cholesterol; MI = myocardial infarction; PVD = peripheral vascular disease; tHcy = total plasma homocysteine.

Figure 2.3: Effects of reduced levels of folic acid, vitamin B_{12} and vitamin B_6 on the vasculature (Coffey *et al.*, 2003:28)

According to a meta-analysis, treatment with 0.5 to 5.0 mg folic acid daily can lower tHcy by 16 to 39% within approximately 6 weeks (Homocysteine lowering trialists' collaboration, 1998:896-897), and there is an inverse relationship between dietary intake of folic acid and subsequent risk of CVD (Moat *et al.*, 2004:67). This has led to the proposal that lowering tHcy levels by increasing the intake of folic acid (or combinations with other B-group vitamins), might be an effective means of decreasing cardiovascular risk (see Figure 2.3). Vermeulen *et al.* (2000:521) found that folic acid and vitamin B_{12} treatment aimed at decreasing tHcy concentrations is associated with decreased risk of atherothrombotic disease. Schnyder *et al.* (2001:1598) demonstrated both a decreased Hcy level in the group treated with folic acid, as well as a significantly decreased coronary restenosis rate. A meta-analysis of 30 studies found that a 25% lower Hcy level was associated with an 11% lower risk of ischaemic heart disease and a

19% lower risk of stroke (The homocysteine studies collaboration, 2002:2021). In a meta-analysis by Wald *et al.* (2002:1206), a decrease in tHcy of 3 μmol/l from current levels (achievable by daily intake of about 0.8 mg folic acid) should reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis by 25% and stroke by 24%. Boushey *et al.* (1995:1053) already estimated in 1995 that an increase of 350 μg folic acid fortification per day would potentially prevent more than 49 000 deaths per year from CHD, however, a sample of 10 500 to 21 500 patients would be required to power a study adequately to detect such modest reductions in mortality rate (Anderson *et al.*, 2004:163).

A number of intervention trials are ongoing throughout the world to test the effectiveness of vitamin supplementation on the morbidity and mortality associated with CVD (Brosnan, 2004:778). Results of some of these studies are already available and concluded that vitamin supplementation reduced tHcy levels, but failed to reduce the risk of major cardiovascular events in 5522 patients with vascular disease (The Heart Outcomes Prevention Evaluation 2 investigators, 2006:1576) and did not lower the risk of recurrent CVD after acute MI in 3749 patients (Bønaa *et al.*, 2006:1586). A harmful effect on CVD from combined B vitamin treatment was even suggested (Bønaa *et al.*, 2006:1578).

2.6.2 Physical activity and/or exercise training

Researchers know little about the effects of physical activity and/or exercise training on Hcy levels. The limited research that could be found revealed contradicting results and did not permit general conclusions. The Hordaland study was the first study to demonstrate that tHcy levels were inversely related to habitual physical activity (Nygård *et al.*, 1995:1528) and the investigators concluded that the effect of physical activity on Hcy might help explain the beneficial effect of exercise on cardiovascular risk (Nygård *et al.*, 1995:1532). Ali *et al.* (1998:1544) found that an exercise training period of 12 weeks produced a 12% reduction in Hcy levels in normolipidemic patients with CAD and hyper-tHcy. A few studies showed similar results of a reduction in tHcy levels with exercise training (Bailey *et al.*, 2000:1062; König *et al.*, 2003:117). However, Duncan *et al.* (2004:899) indicated that higher intensity exercise leads to elevated tHcy concentrations. Coombes *et al.* (2004:598) showed that cardio-respiratory fitness

and tHcy were significantly negatively related in women but not in men. In contrast, Mennen *et al.* (2002:1286) observed a negative relation between physical activity and tHcy concentrations in men but not in women. A few studies, on the other hand, found no effect of exercise training on tHcy concentrations (De Crée *et al.*, 1999:277; de Jong *et al.*, 2001:343; Saw *et al.*, 2001:237)

The effect of acute, sub maximal exercise of relatively short duration on tHcy levels had contradicting results. Some studies found no effect of an acute exercise session on tHcy levels (Wright *et al.*, 1998:265; De Crée *et al.*, 2000:260; Chen *et al.*, 2005:36), where Gaume *et al.* (2005:129) found a decrease in Hcy concentrations after acute exercise. In contrast, some studies showed that acute, intense exercise produced increases in tHcy levels (Weiss *et al.*, 1998:52; De Crée *et al.*, 1999:276; Herrmann *et al.*, 2003:1521; König *et al.*, 2003:117).

It is interesting that resistance training led to significant decreases in tHcy levels in healthy men (Steenge *et al.*, 2001:1456). Chrysohoou *et al.* (2004:121), however, observed an inverse association between Hcy and endurance, but not with resistance exercise. Weight loss interventions have been shown to result in significant increases in Hcy, which may be due to inadequate intake of folic acid (Henning *et al.*, 1998:41). However, in a 6-week weight loss programme by Volek *et al.* (2002:589), tHcy concentrations were not significantly altered. This might have been due to the multi-vitamin supplements subjects received (Volek *et al.*, 2002:591). Therefore, vitamin supplementation may be an important component of a weight loss programme to prevent increases in Hcy (Volek *et al.*, 2002:591). This proposition is in agreement with other studies which demonstrated that the combined effects of exercise training and a high folic acid and vitamin B_{12} intake could be responsible for the reduction of tHcy concentrations that might be a key for the prevention of many diseases including CVD (König *et al.*, 2003:118; Gaume *et al.*, 2005:130).

Discrepancies in data about physical activity and/or exercise training and tHcy within laboratories may be caused by different methodologies. The addition of other influencing factors, such as age, gender, intensity or duration of exercise and amount of training may explain different values of tHcy (Gaume *et al.*, 2005:129). Further intervention studies are needed to

confirm findings on the beneficial effect of exercise training (if any) on Hcy levels and cardiovascular risk.

2.7 CARDIOVASCULAR FUNCTION

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in developed countries (Oren *et al.*, 2003:949). CVD includes, but is not limited to, CHD (which can lead to MI or heart attacks), cerebrovascular disease (which can lead to strokes), hypertension, congestive heart failure, atherosclerosis and aneurysms, PVD (which can lead to a claudication severe calf pain during walking), and rheumatic heart diseases (Plowman & Smith, 1997:190). Although large artery pathology is a major contributor to CVD, the initiation and progression of pathological changes in arteries are only partially understood (Oren *et al.*, 2003:949). The importance of the optimal functioning of the cardiovascular system is obvious. Table 2.3 and Table 2.4 display the average values of cardiovascular function variables for different age groups.

Table 2.3: Typical resting blood pressure values for males and females of various ages (Ogawa *et al.*, 1992:496; Spina *et al.*, 1993(a):851; Spina *et al.*, 1993(b):102; Fleg *et al.*, 1995:891-893; Plowman & Smith, 1997:92)

Blood pressure value	Age of males (yr)			Age of females (yr)		
	10-15	20-30	50-60	10-15	20-30	50-60
SBP (mmHg)	100	120	134	84	120	130
DBP (mmHg)	60	80	84	40	74	84
MAP (mmHg)	73	93	97	55	88	92

DBP = diastolic blood pressure; MAP = mean arterial pressure; SBP = systolic blood pressure; yr = years.

Table 2.4: Typical resting cardiovascular values for males and females of various ages (Ogawa *et al.*, 1992:496; Spina *et al.*, 1992:2459-2462; Spina *et al.*, 1993(a):851,853; Spina *et al.*, 1993(b):102; Fleg *et al.*, 1995:891-893; Plowman & Smith, 1997:84)

Cardiovascular value	Age of males (yr)			Age of females (yr)		
	10-15	20-30	50-60	10-15	20-30	50-60
HR (beats/min)	82	75	80	85	75	80
SV (ml)	50	90	70	40	75	62
CO (l/min)	4.0	6.5	5.5	3.4	5.5	5.0

CO = cardiac output; HR = heart rate; SV = stroke volume; yr = years.

Section 2.8 to 2.10 provides a brief description of factors that influence cardiovascular function. Health risks associated with an impairment of cardiovascular function and the possible mechanism thereof will be investigated, and the non-pharmacological treatment of this induced impairment will be discussed.

2.8 FACTORS THAT INFLUENCE CARDIOVASCULAR FUNCTION

2.8.1 Age

Aging is a well-documented cardiovascular risk factor. One of the possible physiopathological mechanisms through which increasing age may lead to cardiovascular damage is the promotion of endothelial dysfunction (Taddei *et al.*, 2000:2896). As one ages, arterial compliance declines with the large conducting vessels losing the ability to distend in response to an increase in pressure (Joyner, 2000:1214). With physiological ageing, the left ventricle also undergoes tonic structural and functional changes that include an increase in wall thickness and chamber diameter, concentric remodelling, increased mass, and reduced diastolic function (Lakatta, 2002:29-42; Gates *et al.*, 2003:2213). A change in arterial function, particularly an increase in larger artery stiffness that refers to the inverse of arterial compliance and is primarily determined by the elasticity of large arteries (Seals, 2003:68), is thought to serve as a key physiological stimulus for age-associated changes in the left ventricle (Lakatta, 2002:40).

Tanaka *et al.* (2000:1273) found that central arterial compliance decreases with age, even in healthy physically active men. Aging has a profound effect on arterial compliance because of a) increased fragmentation and decreased density of elastin in the arterial wall; b) increased collagen content mediated by increased synthesis and decreased turnover; and c) increased cross-linking of collagen molecules (Seals, 2003:68-69). Other changes in arterial wall composition that may be involved include hypertrophy of vascular smooth muscle cells and increased interstitial cell adhesion molecules and selective growth factors (Seals, 2003:69). Thus, the decrease in arterial compliance associated with ageing may result from both structural and functional abnormalities of the endothelium (Cohn, 1999:S43). One of these abnormalities of the endothelium is endothelium-dependent vasodilatation, of which age has an influence independent from the presence of classic cardiovascular risk factors (Hirsch *et al.*, 2002:1019). Taddei *et al.* (2000:2898-2899) showed impaired endothelium-dependent vasodilatation caused by oxidative stress-induced reduction in NO availability in elderly controls.

SBP increases substantially with age (Miller, 2003:112), whereas the increase of DPB is less pronounced (Nichols & O'Rourke, 1998:347-395 & Meaume *et al.*, 2001:871). According to Lakatta (2002:38), cardiac output (CO) at rest remains unchanged with aging. Tanaka *et al.* (1998:1153) found that increases in arterial stiffness and systemic vascular resistance with age were inversely related to blood volume, stroke volume (SV) and CO.

2.8.2 Known cardiovascular risk factors

Cigarette smoking

In habitual smokers, the short-term effects of smoking one cigarette caused a sharp increase in blood pressure (6%) and heart rate (14%) (Kool *et al.*, 1993:1884). According to Smilde *et al.* (1998:1961), an increase in carotid intima-media thickness (IMT) is related to smoking. Cigarette smoking is also associated with increased arterial stiffness (Kool *et al.*, 1993:1884; Stefanadis *et al.*, 1997:35) and acute cigarette smoking in healthy volunteers resulted in an impaired flow-mediated dilatation (FMD) of the brachial artery (Lekakis *et al.*, 1997:530). The

increased stiffness is probably based on an increased vascular distension due to an increase in blood pressure in combination with an increased vascular tone (Stefanadis *et al.*, 1997:35-37).

Hypertension

Blood pressure is both a risk marker and a risk contributor (Cohn, 1999:S41). Hypertension is a major determinant of vessel wall injury and its deleterious effect on arterial compliance is well documented (Kupari *et al.*, 1994:389-391; Stepniakowski & Egan, 1995:R567; Leibovitz *et al.*, 2003:717). Vascular stiffness is increased in patients with hypertension, even when clinical arterial disease is absent (Benetos *et al.*, 1993:95-96) and other studies confirmed that arterial stiffness increased with hypertension (Cameron *et al.*, 1995:1721-1722; Safar & Frohlich, 1995:12; Lehman *et al.*, 1998:566-568). SBP is also related to carotid IMT (Smilde *et al.*, 1998:1961). According to Oren *et al.* (1996:666), hypertensive patients not only had higher TPR, reflecting the reduced calibre of the small arteries, but also had lower arterial compliance, reflecting impaired distensibility of the large arteries.

Hypercholesterolaemia

A decrease in arterial compliance was found in subjects with high levels of TC (Lehman *et al.*, 1998:566-568), as well as subjects with mild and familial hypercholesterolaemia (Giannattasio *et al.*, 1996:256). Arterial stiffness has been shown to be positively associated with plasma concentrations of fasting triglycerides (TG) (Mackey *et al.*, 2002:19) and LDL-C and negatively associated with plasma concentrations of HDL-C (Kupari *et al.*, 1994:392). Impaired endothelium-dependent vasodilatation has also been found in subjects with hypercholesterolaemia (Lambert, 1997:29) and LDL-C is related to carotid IMT (Smilde *et al.*, 1998:1961).

Impaired fasting glucose/diabetes

Increased fasting plasma glucose levels were significantly correlated with arterial stiffness in both black and white patients and in men and women, regardless of the presence of diabetes (Salomaa *et al.*, 1995:1432). According to cross-sectional studies an independent association exists between insulin resistance and arterial stiffness (Kupari *et al.*, 1994:393), which was more

pronounced in women than in men (Giltay *et al.*, 1999:218). Diabetic patients have increased arterial stiffness (Salomaa *et al.*, 1995:144) and Oxlund *et al.* (1989:751) found that type I diabetic patients, without clinical atherosclerosis or hypertension, have shown an increased arterial stiffness. Airaksinen *et al.* (1993:944) also found that middle aged diabetic patients with CAD have diminished arterial elasticity. The Strong Heart Study showed that left ventricular mass increased by 6 to 14%, left ventricular function lowered by 5% and arterial stiffness increased by 12% in persons with type II diabetes compared to non-diabetic persons (Devereux *et al.*, 2000:2274-2275).

Obesity

Excess body fat is the dominant factor predisposing to blood pressure elevation in cross-sectional and longitudinal population studies (Beilin *et al.*, 1999:934). Moreover, obese individuals have a 3-fold increased risk for developing hypertension (Miller, 2003:109). According to Danias *et al.* (2003:195), obese subjects had decreased abdominal aortic elasticity, characterized by 24% lower compliance and a 22% higher stiffness index. SBP at rest was also higher in the obese group compared to the lean controls (Danias *et al.*, 2003:198). Among elderly men and women, arterial stiffness was positively associated with measures of abdominal obesity (Sutton-Tyrrel *et al.*, 2001:432; Mackey *et al.*, 2002:19), and in a study by Stepniakowski and Egan (1995:R567), obesity was also associated with reduced venous distensibility. It was found that total body fatness, in particular BMI, during adolescence is positively and independently associated with carotid IMT and both abdominal and truncal subcutaneous fat are independently associated with arterial stiffness (Ferreira *et al.*, 2004:152). In contrast, Oren *et al.* (1996:667) showed that, compared with their non-obese counterparts, obese patients have higher SV, CO, central blood volume and total blood volume, arterial compliance and lower TPR.

Sedentary lifestyle

Physical inactivity, the so-called sedentary lifestyle, induces endothelium-dependant vasodilatation, even in the absence of other cardiovascular risk factors (Suvorava *et al.*, 2004:1324). In a study by Ferreira *et al.* (2002:729), they found that cardiopulmonary fitness (VO_{2max}) was inversely and independently associated with carotid IMT, but only in men and that

current levels of VO_{2max} were positively associated with carotid arterial compliance and arterial distensibility in men and women. According to Mackey *et al.* (2002:19), arterial stiffness was positively associated with decreased physical activity, as well as increased resting HR in elderly participants. A faster HR might accelerate the fatigue of elastic fibres by increasing the lifetime number of repetitive stretching cycles, or by not allowing enough time for the large arteries to relax between each ventricular contraction. Increased HR might also indirectly increase arterial stiffness by increasing the strain on arterial walls from increased CO and blood pressure (Mackey *et al.*, 2002:20).

The relationship of standard cardiovascular risk factors with decreased peripheral arterial compliance parallels the relationship of these risk factors to atherosclerotic burden and cardiovascular events (Willens *et al.*, 2003:205). According to Celermajer *et al.* (1994:1472), classical cardiovascular risk factors known to predispose to atherosclerosis and its complications, are also associated with a decrease in endothelium-dependent vasodilatation.

2.8.3 Biochemical markers for CVD

Endothelial dysfunction is a well-known pathologic condition that is associated with a variety of cardiovascular diseases such as CAD, hypertension and heart failure, to name but a few. The role of biochemical markers and their interaction with tHcy on endothelial injury/dysfunction was discussed in section 2.5. However, in this subsection a brief view is given on biochemical markers' influence on cardiovascular function variables. The mechanism of endothelial dysfunction is multi-factorial and most likely depends on the underlying pathologic process (Suvorava *et al.*, 2004:1324). The markers being discussed are NO, Lp(a), C-reactive protein and fibrinogen and microalbuminuria.

NO

NO exerts important functions contributing to the regulation of vascular tone and blood pressure (Leoncini *et al.*, 2003:717). Flow-mediated dilatation (FMD) is a non-invasive technique to measure vasodilatation, is indicative of endothelial dysfunction and is an index of NO-related endothelium-dependent vasodilatation (Ashfield-Watt *et al.*, 2001:429; Bilsborough *et al.*,

2003:136). Indeed, most risk factors for atherosclerosis have been shown to be associated with impaired endothelium-dependent vasodilatation because of reduced NO availability (Verhaar *et al.*, 1998:237). The precise mechanism responsible for this reduced NO availability is unknown; both impaired formation and increase degradation of NO may be involved (Verhaar *et al.*, 1998:237). Impaired vascular NO activity has emerged as an early marker for CVD (Verhaar *et al.*, 1998:237) and is an independent predictor of vascular morbidity and mortality (Sydow *et al.*, 2004:93).

Lp(a)

Lp(a) is commonly detected in men and women with premature coronary atherosclerosis and CHD (Stein & Rosenson, 1997:1170-1173) and elevated Lp(a) is a strong, independent risk factor for CAD (Foody *et al.*, 2000:449-450). Furthermore, it has been estimated that 15 to 20% of those with premature CVD have elevated Lp(a) levels (Jenner *et al.*, 1993:1137-1140; Huges, 2003:132). Even young men with elevated Lp(a) levels are at high risk of early CAD-related mortality (Rosengren *et al.*, 1990:1250). Lp(a) has also been linked to a reduction of endothelium-dependent vasodilatation (Scanu, 1998:28Q-30Q; Huges, 2003:132).

C-reactive protein and fibrinogen

The inflammatory markers such as C-reactive protein and fibrinogen also predict future CVD events (Huges, 2003:134). Retterstol *et al.* (2002:439) concluded that C-reactive protein was a strong predictor of mortality in patients with premature MI (CHD) and that C-reactive protein was a better indicator of inflammation and a better prognostic marker than fibrinogen. Elevated fibrinogen levels may contribute to cardiovascular risk by increasing blood platelet aggregation and blood viscosity (Thomas *et al.*, 2003:642-643) and was the only biochemical factor according to Guérin *et al.* (2000:1019), significantly associated with arterial calcifications. The presence and extent of arterial calcifications *per se* is associated with arterial stiffening, independent of age and blood pressure (Guérin *et al.*, 2000:1019).

Microalbuminuria

A slightly elevated urinary albumin excretion rate, so-called microalbuminuria, is strongly associated with CVD (Vermeulen *et al.*, 2003:213). Also, an elevated blood pressure in the presence of abnormal albuminuria is a strong cardiovascular risk factor (Mogensen *et al.*, 1992:1193). The most widely held view is that microalbuminuria indicates underlying generalized vascular, possibly, endothelial dysfunction (Stehouwer *et al.*, 1992:322). Vascular stiffness may be one of the factors responsible for the increased cardiovascular risk, especially in insulin-dependent diabetes mellitus patients with microalbuminuria (Lambert *et al.*, 1997:143).

Changes in the biochemical markers associated with increased cardiovascular risk do not occur in isolation. Rather, epidemiologic studies have demonstrated strong positive correlations among the levels of these biochemical markers (Frishman, 1998:24S).

2.8.4 The influence of Hcy on cardiovascular variables

The detrimental influence of Hcy on cardiovascular disease states was discussed (see section 2.4), however, in this subsection more attention is given to Hcy's relationship with cardiovascular variables.

Even mildly elevated Hcy levels caused impairment on endothelial vasodilatation (Virdis *et al.*, 2001:1106; Sydow *et al.*, 2004:97), especially if other cardiovascular risk factors such as hyper-cholesterolaemia are present (Schlaich *et al.*, 2000:388). Hirsch *et al.* (2002:1020) found that a decrease in endothelium-dependent vasodilatation because of hyper-Hcy, occurs in older but not younger healthy people. These observations suggest that hyper-Hcy may not promote the development of atherosclerosis *per se*, but it instead may alter vascular function in a way that increases the risk for complications of atherosclerosis (Lentz, 2001:1385). FMD reflects endothelium-dependent vasodilatation. In a randomised crossover study it was found that an increase in tHcy acutely impairs FMD in healthy subjects (Bellamy *et al.*, 1998:1850). A number of studies showed similar results (Lambert, 1997:191; Tawakol *et al.*, 1997:1120; Woo *et al.*, 1997(a):2543; Nappo *et al.*, 1999:2117; Chambers *et al.*, 2001:191). Even carotid IMT, which reflects atherosclerosis (Ubbink *et al.*, 1994:1927), has been associated with high Hcy

levels through the stimulation of smooth-muscle cell proliferation and collagen synthesis (Malinow *et al.*, 1993:1110; Majors *et al.*, 1997:2079; Marcucci *et al.*, 2005:2492).

Studies confirmed the hypothesis that high tHcy causes arterial stiffness (Smilde *et al.*, 1998:1960; Bortolotto *et al.*, 1999:840). Nestel *et al.* (2003:85) showed that high tHcy caused arterial stiffness in the central arterial circulation that includes the large elastic arteries such as the aorta, and the rise in tHcy led to an average 21% reduction in systemic arterial compliance (Nestel *et al.*, 2003:85). However, Davis *et al.* (2001:331) showed that acute hyper-Hcy increased pulse pressure (PP), a marker of large artery stiffness (Laurent *et al.*, 2001:1236), but had no effect on endothelial-dependent micro vascular vasodilatation.

High Hcy levels are also independently related to isolated systolic hypertension in older individuals (Sutton-Tyrrell *et al.*, 1997:1748). In the Hordaland Homocysteine Study, tHcy showed a positive linear association with DBP and SBP in the younger age group and tHcy was positively related to HR in the younger and older age groups (Nygård *et al.*, 1995:1529).

The possible mechanisms of tHcy's role in vascular disease through endothelial injury/dysfunction were discussed in section 2.5. Probably the most important mechanism is that continued exposure of the endothelium to Hcy compromises the production of adequate amounts of NO (Leoncini *et al.*, 2003:718; Lee *et al.*, 2004:144; Romerio *et al.*, 2004:343) and thus diminishes FMD (Sydow *et al.*, 2004:97-100) . This leads to attenuated blood pressure decline and a positive response of both platelet aggregation and blood viscosity (Nappo *et al.*, 1999:2117). It ultimately leads to unopposed Hcy-mediated injury to the endothelium and initiation of atherosclerosis and/or thrombosis formation or acceleration of existing atherosclerosis (Das, 2003:687). Also, Hcy attenuates the anti-thrombotic action of endothelium by enhancing the production of super oxide anion, which in turn inactivates NO (Das, 2003:687). Hence, via reduced endothelial NO release, Hcy effects large vessel compliance (Davis *et al.*, 2001:331).

2.9 IMPAIRMENT OF CARDIOVASCULAR FUNCTION: HEALTH RISKS AND POSSIBLE MECHANISMS

A reduction in arterial compliance contributes to isolated systolic hypertension (Joyner, 2000:1214; Beltran *et al.,* 2001:1009). Liao *et al.* (1999:205) suggested that a possible mechanism may be hemodynamic factors associated with the development of hypertension. It can be hypothesized that greater arterial stiffness (loss of elasticity) in large and medium-sized arteries represents a cumulative adverse impact of conventional risk factors on the arterial wall and that arterial stiffness, together with its adverse impact on other target organs such as the kidneys, contributes to the development of hypertension (Liao *et al.,* 1999:205).

Moreover, patients with essential hypertension are characterized by endothelial dysfunction caused by reduced NO availability due to oxidative stress (Taddei *et al.,* 1999:1404). The alteration of large artery elasticity is responsible for an inadequate increase in SBP and a decrease in DBP at any given value of mean arterial pressure (MAP) (Benetos *et al.,* 1998:560), thereby causing increased left ventricular after-load and altering coronary perfusion (Nichols & O'Rourke, 1998:347-395; Blacher *et al.,* 1999:2434). The principal outcomes of these changes are left ventricle hypertrophy, aggravation of coronary ischaemia and increased fatigue of arterial wall tissues (Blacher *et al.,* 1999:2434).

Stiffer large arteries cause pulse pressure (PP) elevation through higher systolic and lower diastolic pressure (Kingwell, 2002:214). In a large French population, male subjects aged 40 to 69 yr had increased PP which was a strong predictor of general and cardiovascular mortality, especially coronary mortality (Benetos *et al.,* 1997:1414). Benetos *et al.* (1998:562-563) found that PP is an independent predictor of cardiovascular mortality, including CHD mortality in both normotensive and hypertensive men as well as hypertensive women. The same study found that normotensive men that were in the higher PP group had an increased relative cardiovascular risk of 40% compared with normotensives that belonged in the lower PP group (Benetos *et al.,* 1998:563). Domanski *et al.* (1999(a):377) demonstrated an 11% increase in the risk of stroke and a 16% increase in total mortality for each 10 mmHg increase in PP. MAP was also independently associated with risk of adverse cardiovascular events with a 20% increase in the

risk of stroke and a 14% increase in total mortality associated with each 10 mm Hg rise in MAP (Domanski *et al.,* 1999(a):377). The link between PP and cardiovascular events has also been shown in subjects of post-MI with left ventricular dysfunction (Mitchell *et al.,* 1997:4258).

At a given SV and velocity of ventricular ejection, the mechanisms influencing PP are related to the status of conduit arteries, i.e. the viscoelastic properties of the arterial wall and the timing of the reflected waves (Benetos *et al.,*1998:563). Hence, increased PP is associated with increased carotid IMT (Matthews *et al.,* 1998:1528) and has been associated with reduced endothelium-dependent relaxation, e.g. NO production (Ryan *et al.,* 1995:H361). Thus, increased stiffness and earlier wave reflections within the thoracic aorta, increase the PP because of an increase in SBP and a decrease in DBP (Nichols & O'Rourke, 1990:377-395; Kelly *et al.,* 1992:499; Benetos *et al.,*1998:563).

Chronic elevations in SBP and PP may cause endothelial damage and accelerate atherosclerosis, which contribute to occlusive cerebrovascular, coronary and peripheral vascular diseases (Seals, 2003:69). Consequently, the risk of thrombosis, MI, aneurysms, stroke and heart failure are increased (Chae *et al.,* 1999:636-638; Miller, 2003:108; Seals, 2003:69). Ultimately, higher SBP and PP and lower DBP are independent factors of CVD and CHD morbidity and mortality in the general population (Darné *et al.,* 1989:397; Mitchell *et al.,* 1997:4258; Domanski *et al.,* 2001:795-796).

In healthy young adults arterial stiffness [assessed by aortic pulse wave velocity (PWV)] and sub-clinical atherosclerosis (assessed by carotid IMT) are independent entities of vascular damage (Oren *et al.,* 2003:953-954). It has been suggested that arterial stiffness may play a role in the development of atherosclerosis or *vice versa* (Oren *et al.,* 2003:949). Changes in arterial stiffness over time may serve as a marker for atherosclerotic changes in the arterial wall (Arnett *et al.,* 1999:175), also, arterial stiffness increases with atherosclerosis due to tissue degeneration (Wada *et al.,* 1994:480; Kingwell *et al.,* 2002:778). It is evident that atherosclerosis promotes MI not only via coronary artery obstruction but also via large artery stiffening (Kingwell *et al.,* 2002:778). Giannattasio *et al.* (2001:1179) expanded the evidence in favour of the hypothesis

that changes in arterial stiffness precede atherosclerotic development by showing decreased distensibility of the common carotid artery without any increase in wall thickness. In contrast, Barenbrock *et al.* (1995:1716) showed altered arterial compliance only in case of extensive atherosclerosis in patients who clinically manifest CVD, hypertension or hyper-cholesterolaemia.

A prospective study emphasized the possibility of small vessel compliance as a diagnostic indicator of diffuse CAD (Syeda *et al.*, 2003:359). Gatzka *et al.* (1998:577) found that proximal aortic stiffness as well as left ventricular wall thickness is increased in patients with CAD in a general population sample. Lower arterial compliance is even associated with the presence of significant coronary stenoses, independent of conventional cardiovascular risk factors (Herrington *et al.*, 2003:665). While several mechanisms could contribute, one may be that large artery stiffness is an important independent determinant of MI threshold in patients with CAD (Kingwell *et al.*, 2002:776). Endothelial dysfunction, with a reduced release of NO (endothelial vasodilator) might be the root of the large and small arterial compliance reduction. The reduced NO produced vasoconstriction which reduced the compliance. As atherosclerosis progresses, the tunica media thickens and the tunica intima becomes rigid, thus reducing the arterial elasticity (Syeda *et al.*, 2003:359).

Therefore, decreased arterial compliance appears to be an independent risk factor for the development of CVD (Meaume *et al.*, 2001:874; Seals, 2003:68). Previous studies have shown that carotid IMT (Heiss *et al.*, 1991:254-255; Chambless *et al.*, 2000:483) as well as arterial stiffness (Blacher *et al.*, 1999:2437; Laurent *et al.*, 2001:1239) are strong predictors of subsequent CVD morbidity and mortality (Oren *et al.*, 2003:949). The increased mortality risk because of a 5 m/s increase in PWV (a measure of arterial stiffness) is equivalent to that of aging 10 years (Laurent *et al.*, 2001:1240).

Additionally, as the vessels stiffen, the physical forces that oppose aortic valve opening increase and can contribute to ventricular hypertrophy, aortic root dilation, valvular dysfunction and heart failure (Rowe, 1987:69G-71G; Arnett *et al.*, 1994:678-679; Seals, 2003:69). Also, components of congestive heart failure, characterized by reduced ventricular performance, are elevated

peripheral vascular resistance (Parnell *et al.*, 2002:1) and impaired endothelium-dependent vasodilatation (Hambrecht *et al.*, 1998:2713). Impaired endothelium-dependent vasodilatation of coronary or peripheral vasculature correlates positively with an increased risk for cardiovascular events and is an independent predictor of vascular morbidity and mortality (Schächinger *et al.*, 2000:1903; Heitzer *et al.*, 2001:2676).

According to Mensink and Hoffmeister (1997:1408), increased HR was a significant predictor of cardiovascular mortality, especially coronary mortality in men but not in women. Kerzner *et al.* (2003:288-289) found that predictors of mortality in patients with heart failure differ by age and by whether left ventricular ejection fraction (LVEF) was reduced or preserved. Their long-term prognosis is more favourable than patients with reduced LVEF. In the SOLVD study, patients who had LVEF dysfunction were indicated by a multivariate analysis to have a strong association of all-cause mortality and cardiovascular death with an increase in PP (Domanski *et al.*, 1999(b):954-955).

Arterial mechanical properties have, therefore, become a target for intervention in the management of hypertension and CVD, with increase in arterial compliance and improved mechanical performance considered of benefit in overall cardiovascular integrity (Cameron *et al.*, 1999:653). The clustering of risk factors suggests that effective treatment of one may have positive effects on the other risk factors (Frishman, 1998:24S).

Vitamin supplementation plays a role in the improvement of cardiovascular function (Mangoni *et al.*, 2002:501; Weiss *et al.*, 2002:234; Marcucci *et al.*, 2005:2492; Williams *et al.*, 2005:29), and there is a strong, graded and independent association of higher levels of cardio-respiratory fitness with lower incidence of CVD and all-cause mortality (Blair *et al.*, 1996:210). Prescription of physical activity and/or exercise training has, therefore, emerged as a tool not only for the primary, but also for the secondary prevention of CVD (Thompson *et al.*, 2003:1319-1320). These factors will be discussed in the next section.

2.10 NON-PHARMACOLOGICAL TREATMENT OF IMPAIRED CARDIOVASCULAR FUNCTION

2.10.1 Vitamin supplementation

The role of folic acid in lowering cardiovascular risk is not limited to reducing tHcy levels (O'Grady *et al.*, 2002:841). In a 2-year trial conducted among healthy individuals, treatment with folic acid and vitamin B_6 were associated with a 3.7 mmHg decrease of SBP and a 1.9 mmHg decrease of DBP (Van Dijk *et al.*, 2001:2076). Lowering tHcy levels by folic acid supplementation also improves endothelial dysfunction indicated by the improvement of endothelium-dependent vasodilator function (Weiss *et al.*, 2002:234), as well as significant reduction of carotid IMT (Marcucci *et al.*, 2005:2492). Supplementation with folic acid enhanced endothelial function (Wilmink *et al.*, 2000:188) in CAD subjects (Doshi *et al.*, 2001:1199), CHD subjects (Ashfield-Watt *et al.*, 2001:431) or hyper-tHcy subjects (Woo *et al.*, 1999:2005; Weiss *et al.*, 2002:234; Lee *et al.*, 2004:146). Short-term supplementation with folic acid was associated with a significant enhancement of endothelial function and blood pressure reduction in healthy chronic smokers (Mangoni *et al.*, 2002:501), independent from its Hcy lowering effect. Verhaar *et al.* (1998:240) also concluded that folic acid can restore endothelial function in hypercholesterolaemic patients, probably by a reduction in ROS, independent from its Hcy lowering effect. Till *et al.* (2005:134) found that a 1-year vitamin supplementation significantly decreased carotid IMT in 60 yr old subjects, independent of Hcy-lowering capacity. According to Doshi *et al.* (2001:1201), folic acid resulted in improvement in FMD and enhanced endothelium-dependent vasodilatation in patients with type II diabetes (Mangoni *et al.*, 2005:22,24) and was independent of baseline tHcy or its reduction. However, in the last study folic acid did not affect arterial stiffness (Mangoni *et al.*, 2005:24). In a study by Williams *et al.* (2005:29), short-term dietary supplementation with folic acid reduced PP and consequently arterial stiffness in young men with normal or mildly elevated SBP. There was also no significant relation between changes in tHcy concentrations and changes in blood pressure or arterial stiffness (Williams *et al.*, 2005:29).

This underscores folic acid's importance in the prevention and treatment of atherosclerosis and clinical conditions that accelerate atherosclerotic processes (Das, 2003:689), as well as

potentially reducing cardiovascular risk in high-risk patients (Van Dijk *et al.*, 2001:2078; Mangoni *et al.*, 2002:502). A possible mechanism is that folic acid enhances NO production, reduces super oxide anion generation and, therefore, improves endothelial dysfunction (Das, 2003:686-689), independent of tHcy concentrations. Vitamin B_6 also has anti-inflammatory actions (Das, 2003:687).

The antioxidant effect of vitamin C not only scavenges super oxide anions or inhibits LDL-C oxidation, but also spares intracellular glutathione, another intracellular antioxidant (Virdis *et al.*, 2001:1114). Nappo *et al.* (1999:2117) suggested that pre-treatment with antioxidant vitamins C an E prevents endothelial dysfunction caused by hyper-tHcy. A review article by Fenster *et al.* (2003:222,224) also demonstrated the treatment of endothelial dysfunction with vitamin C and E and an inverse association between vitamin E and fibrinogen levels has been shown (James *et al.*, 2000:392).

Vitamin supplementation depends on the baseline Hcy concentrations and is greater for higher baseline levels. Recommended doses are 0.2 to 0.8 mg folic acid, 3 to 30 μg vitamin B_{12} and 2 to 6 mg vitamin B_6. However, ideal supplementation for moderate hyperhomocysteinaemia is 1 to 5 mg folic acid, 100 to 600 μg vitamin B_{12} and 6 to 25 mg vitamin B_6 (Stanger *et al.*, 2003:1399).

2.10.2 Physical activity and/or exercise training

Drug therapy does not address the major underlying (non-genetic) causes of elevated blood pressure, each of which can be addressed through lifestyle modification. Importantly, in contrast to most drug therapies, lifestyle modifications, e.g. physical activity and/or exercise training that reduces blood pressure, can also prevent or control other chronic conditions (Svetkey *et al.*, 2003:462).

Regular physical activity and/or exercise training results in improvements in cardiovascular function, which is most readily demonstrated by an increase in the functional capacity of the cardiovascular system (that is, an increase in VO_{2max}) (Plowman & Smith, 1997:156). Even in

elderly males and females VO_{2max} and LVEF increase as a result of endurance training. Resting SV increases are generally small, they show lower resting HR and resting CO is unchanged (Green & Crouse, 1993:336; Plowman & Smith, 1997:163-164).

Physical activity contributes significantly to weight loss and blood pressure lowering. The relation between habitual physical activity, including leisure-time activities, and blood pressure has been extensively studied. The majority of these studies indicate a significant inverse association between blood pressure and physical activity. Moreover, this inverse association has been observed in all sexes, ages and is independent of weight loss (Miller, 2003:110). The PREMIER Interventions often showed reductions in blood pressure of 5-10 mmHg with physical activity in efficacy studies (Svetkey *et al.*, 2003:467).

According to Tanaka *et al.* (2000:1274), central arterial compliance was 40% higher in aerobic endurance-trained older men than in their sedentary peers, emphasising the importance of therapeutic methods for increases in arterial compliance. Moderate aerobic exercise is a potential non-pharmacological therapy to increase systemic arterial compliance, highlighting the possibility that part of its benefit may be an associated improvement in functional capacity in older individuals (Cameron *et al.*, 1999:655; Mackey *et al.*, 2002:20). Terenzi (2000:29) and Ferreira *et al.* (2003:1675) found that increased VO_{2max} were inversely and significantly associated with large artery stiffness. Moreover, habitual aerobic endurance exercise attenuated age-associated increases in arterial stiffness in healthy men (Gates *et al.*, 2003:2219), as well as in elderly men and woman aged 70 to 79 yr (Havlik *et al.*, 2003:163). Hunt *et al.* (2001:2426) showed similar results and suggested that regular aerobic conditioning (training duration from 21 to 56 yr) is indeed related to lesser carotid stiffness. Aerobic exercise training, which reduces arterial stiffness, may confer particular benefits in CAD patients by improving ischemic threshold (Dart & Kingwell, 2001:979-980). Hence, improving VO_{2max} by increasing physical activity levels may, therefore, contribute to a reduction in mortality from CVD through decreasing arterial stiffness (Ferreira *et al.*, 2003:1677-1678).

Exercise training might modify these changes in several ways. First, when humans exercise there is an increase in arterial pressure and HR. These changes and the physical forces acting on the large conducting vessels might cause the vessels to deform and act in a manner that is similar to "stretching exercise" in skeletal muscle. In other words, occasional periods of increased deformation of the large blood vessels may combat some of the connective tissue cross-linking that occurs as a result of aging (Joyner, 2000:1214). Secondly, skeletal muscle vasodilates dramatically during exercise and at least some of this vasodilatation in resistance vessels propagates upstream to large conducting vessels (Joyner, 2000:1214).

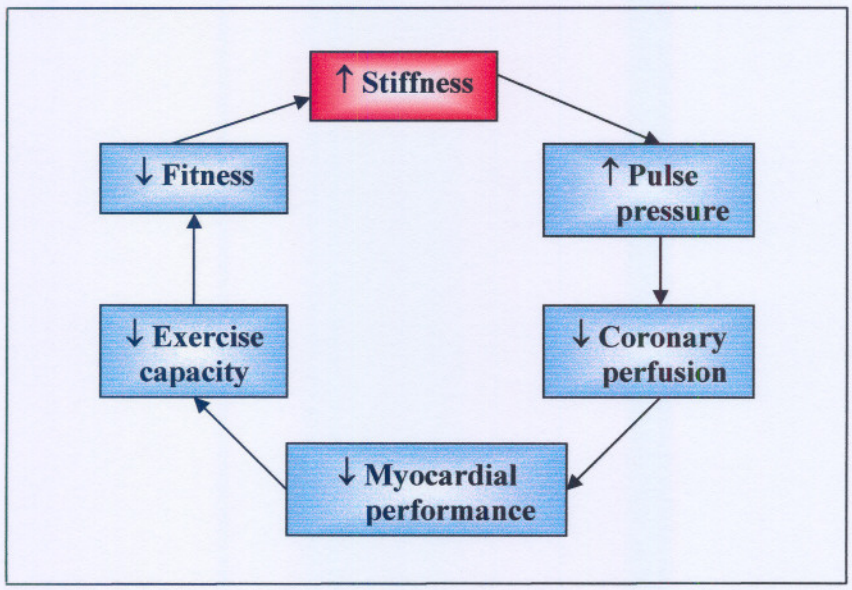


Figure 2.4: Diagram illustrating the circular nature of the relationship between large artery stiffness, physical work capacity and cardiac risk (Kingwell, 2002:215)

Hambrecht *et al.* (2003:3156) showed that 4-weeks of exercise training improved endothelium dependent vasodilatation in patients with CAD. Suvorava *et al.* (2004:1326) also proposed that in patients with CVD, regular exercise reduces the degree of endothelial dysfunction, whereas in young healthy individuals normal physical activity and/or moderate exercise might delay the development of cardiovascular disorders by maintaining normal endothelial function. A possible mechanism involved may be that during exercise, heart rate increases, which in turn increases blood flow, leading to higher intra-luminal shear forces which stimulate the endothelium to

release relaxing factors (Ferreira *et al.,* 2003:1677; Suvorava *et al.,* 2004:1326), thus, induces an increased expression of vascular endothelial NOS (Kojda *et al.,* 2001:2842; Hambrecht *et al.,* 2003:3157-3158). This adaptation most likely improves the bioavailability of NO, resulting in arterial vasodilatation/compliance (Joyner, 2000:1214; Taddei *et al.,* 2000:2899; Hambrecht *et al.,* 2003:3157-3158). These results suggest that impaired endothelium-dependent vasodilatation, even in patients with chronic heart failure, can be restored by long-term aerobic exercise training (Hambrecht *et al.,* 1998:2713), and suggest that increased NO production induced by exercise may also slow the progression of vascular disease (Suvorava *et al.,* 2004:1320). An independent association between exercise and lower levels of fibrinogen (Elwood *et al.,* 1993:186), C-reactive protein (Pitsavos *et al.,* 2003:370), as well as lower levels of Lp(a) (Von Duvillard, 1997:1414) have been reported.

According to a review by Stone *et al.* (1991:211-227), high volume resistance training has positive effects on various parameters associated with cardiovascular fitness and disease risk. It is a common misconception that resistive training is directly responsible for the hypertension seen among some strength power athletes. However, more likely causes of resting hypertension include essential hypertension, chronic overtraining and the use of androgens or perhaps gaining large amounts of body mass. Hence, lower resting SBP and DBP have been shown because of resistance training (Stone *et al.,* 1991:215-216). Besides increased strength with resistance training, peak VO_2 and SV also increased and resting HR was lower (Stone *et al.,* 1991:212-213,217,219).

In contrast, Ferreira *et al.* (2003:1675) concluded that longitudinal changes in VO_{2max} and physical activity levels were not associated with carotid IMT and despite improvement in VO_{2max}, an 8-week exercise programme failed to improve large artery stiffness in patients with isolated systolic hypertension (Ferrier *et al.,* 2001:224). This data suggest that the large artery stiffening associated with isolated systolic hypertension is resistant to modification through short-term aerobic training (Ferrier *et al.,* 2001:224). According to Seals *et al.* (2001:510), salt restriction had a greater effect on blood pressure and arterial stiffness reduction than exercise.

Regular physical activity and/or exercise improves cardiovascular function variables (Cameron & Dart, 1994:H698; Tanaka *et al.*, 2000:1274), as well as endothelial function (Hambrecht *et al.*, 2003:3156). It may be speculated that the decrease in Hcy with regular physical activity and/or exercise training (Bailey *et al.*, 2000:1062; König *et al.*, 2003:117; Duncan *et al.*, 2004:899) may also be beneficial for cardiovascular function variables and endothelial function because of the influence that Hcy has on cardiovascular function. These influences of physical activity and/or exercise training may have an accumulating effect on each other and may then also influence other cardiovascular risk factors in a positive manner.

2.11 EXERCISE TRAINING

Exercise training is defined as planned, structured, repetitive and purposeful physical activity (McArdle *et al.*, 2001:871). Exercise training is beneficial to a number of cardiovascular function variables (Hambrecht *et al.*, 2000:3100; Tanaka *et al.*, 2000:1274; Havlik *et al.*, 2003:163) and plays a major role in reducing the risk of CVD, including CAD, CHD, stroke and hypertension (Lee *et al.*, 2000:985, Lee & Paffenbarger, 2001:37-52; Wannamethee & Shaper, 2001:101-114; Wilmore, 2003:51). A number of studies have reported a relationship between exercise training and Hcy, but with equivocal results (Ali *et al.*, 1998:1544; Bailey *et al.*, 2000:1062; König *et al.*, 2003:117).

Epidemiological studies showed that the risk of CAD associated with physical inactivity is nearly the same as the risk associated with smoking, abnormal lipid levels and hypertension. However, inactivity is about 2 to 3 times more prevalent than smoking, abnormal lipid levels or hypertension (Wilmore, 2003:51). High levels of cardio-respiratory fitness, even only occasional exercise sessions, seem to provide protection against the force of combinations of CVD and other mortality predictors on deaths (Blair *et al.*, 1996:209-210; Sundquist *et al.*, 2004:25-26). Increasing evidence has shown that regular participation in moderate-intensity exercise training is associated with health benefits, even when aerobic fitness (e.g. VO_{2max}) remains unchanged (Balady *et al.*, 2000:137).

Section 2.12 to 2.15 will briefly mention exercise prescription according to the ACSM's recommendations, discuss the general health benefits of exercise, as well as the influence of exercise training on a potential new cardiovascular risk factor, Hcy. Exercise training's influence on the cardiovascular function and its variables will also be discussed.

2.12 EXERCISE PRESCRIPTION

The ACSM recommendations for devising an average aerobic training programme are summarized in Table 2.5. The exercise prescription, however, should be developed with careful consideration of the individual's health status (including medications), risk factor profile, behavioural characteristics, personal goals, and exercise preferences (Armstrong *et al.*, 2006:135).

Table 2.5: ACSM recommendations for compiling an aerobic training programme (Armstrong *et al.*, 2006:139-148)

COMPONENTS	RECOMMENDATIONS
Mode	Any activity that uses large muscle groups over prolonged periods in activities that are rhythmic and aerobic in nature (e.g. walking, running, swimming, cycling or rowing).
Intensity	Low to moderate intensity exercises of ± 55-65% of maximal age predicted heart rate (HR_{max}), which slowly progresses within a few weeks/months to higher intensity exercises of ± 70-85% of HR_{max}.
Duration	20-60 min., gradually progressing.
Frequency	Although de-conditioned persons may improve cardio respiratory fitness with only twice-weekly exercise sessions, optimal training frequency appears to be achieved with 3-5 workouts/week.

It should be noted that health benefits may be obtained from activities at lower intensities if the accumulated (not necessarily continuous) duration is at least 30 min. and the frequency is daily (Plowman & Smith, 1997:143). Lee *et al.* (2000:984) stated that the accumulation of shorter

sessions of activity is associated with equivalent benefits (at least with regard to CHD risk) compared with longer sessions, as long as the total amount of energy expended is similar.

Feigenbaum and Pollock (1999:38-44) also prescribed resistance training for health and disease. Resistance training should be performed 2 to 3 days/week with a typical workout consisting of a minimum of 1 set of 8 to 10 exercises to cover the large muscle groups of the upper and lower body. When 15 repetitions of an exercise can be completed with little difficulty, the weight should be increased by 2.3 to 4.5 kg to ensure a progressive muscle overload (Feigenbaum & Pollock, 1999:39; Stewart, 2002:1628).

2.13 HEALTH BENEFITS OF EXERCISE

2.13.1 General benefits

When a sedentary person becomes more active substantial physiological adaptations occur additionally to the increase in respiratory fitness (VO_{2max}) (Ogawa *et al.*, 1992:497; Wilmore, 2003:48). The magnitude of these adaptations is determined by the mode, volume and intensity of training (Wilmore, 2003:48).

Aerobic training produces substantial adaptations within skeletal muscle. The cross-sectional area of type I muscle fibers increases by up to 25% and a transition of type IIb fibers to type IIa fibers can occur. The number of capillaries also increases within the trained muscle, as does the ratio of capillaries to muscle fibers (Green & Crouse, 1993:334; Wilmore, 2003:48). Exercise training, especially resistance training increases strength. Stronger muscles protect the joints they cross and may also increase the maximum strength of tissue including tendons and ligaments. This strengthening effect may reduce the possibility of strains, sprains and other injuries (Stone *et al.*, 1991:219-220). Flexibility increases with stretching exercises and it has been indicated that weight-training generally enhances flexibility (Stone *et al.*, 1991:223). However, care should be taken to use full range of motion movements and that partial movements are not overemphasized (Stone *et al.*, 1991:223).

Moderate exercise training can play a role in improved control of the joint swelling and pain associated with arthritis (Macera *et al.*, 2003:122). According to Kolden *et al.* (2002:448), exercise participation led to increased functional capacity, improved mood, increased self-esteem, decreased distress, improved body image, decreased fatigue, decreased emotional distress and reduced depression and anxiety. Moderate exercise training also enhances immune function and may play an important role in determining an individual's susceptibility to infection (Plowman & Smith, 1997:202-206). Exercise is also associated with lower prevalence and mortality rates for cancers involving the colon, breast, prostate and lung (Woods & Davis, 1994:147-154; Plowman & Smith, 1997:205).

2.13.2 Benefits of exercise on cardiovascular risk factors

In a review article of Durstine *et al.* (2001:1042), careful evaluation of the literature indicated that exercise training, more often than not, resulted in unaltered total cholesterol and LDL-C levels. In some instances regular exercise can produce small changes in TC and LDL-C of 4 to 7% in both men and women. Reductions in these lipid fractions occur with greater frequency in previously sedentary individuals and when exercise caloric expenditure exceeds 1200 kcal/week (Durstine *et al.*, 2001:1042). In the same review article, regular aerobic exercise can increase HDL-C and decrease TG levels in men and women. The effect of exercise on HDL-C is similar in both males and females, however, TG changes are more commonly reported in males (Durstine *et al.*, 2001:1042). Regular exercise can raise HDL-C levels by 2 to 8 mg/dl and lower TG from 5 to 38 mg/dl in men and women. The training volumes that elicit energy expenditures ≥1200 kcal/week are most frequently associated with elevations in HDL-C levels in both genders and reduced TG levels in men (Durstine *et al.*, 2001:1045). Kim *et al.* (2001:945) concluded that exercise frequency may be more important than intensity in improving HDL-C, LDL-C:HDL-C and TC:HDL-C ratios in men with CHD.

In a study by Andersen *et al.* (1999:337), 40 obese women attended 3 step aerobics classes/week. Classes lasted 15 min. at first, which build up to 45 min. After 16 weeks, weight loss was 8.3 kg, body fat loss 7.4 kg, body fat percentage reduced to 41.9% and fat free mass increased by 0.5 kg. Significant reductions in TC, TG, LDL-C and HDL-C were also observed after the 16-week

treatment. Resting SBP also decreased significantly and maximum oxygen uptake increased significantly (Andersen *et al.*, 1999:339). After a 6-month aerobic exercise training programme of 3 sessions/week, Boileau *et al.* (1999:379-381) found that there was a significant decrease in body weight of 2.1%, and a significant increase in VO_{2max} of 12.1% compared to the control group.

Overweight patients with CHD completed a 4-month exercise training programme. The programme primarily consisted of walking 60 to 90 min., 5 to 7 days/week, at an intensity of 50 to 60% of peak VO_2. In addition to the aerobic training, patients also incorporated weight training consisting of 1 set of 10 repetitions of 6 different exercises targeting major muscle groups (Savage *et al.*, 2003:319). After the 4-month exercise intervention, subjects had decreased total body mass by 4.9%, BMI by 3.5%, waist circumference by 5%, fat mass by 13.3% and percentage body fat by 9.6%. Peak VO_2 also increased by 16.6% (Savage *et al.*, 2003:319). Their lipid profile also showed favourable results with TG significantly improved by 23.7%, as well as decreased insulin levels by 22.3%. TC decreased by 6.2%, HDL-C increased by 6.7% and LDL-C decreased by 8.5% (Savage *et al.*, 2003:320,322). According to Manson *et al.* (1999:652), women between the ages of 40 and 65 yr who participated in exercise sessions of more than 21.7 MET hours/week, were less likely to be current smokers and, as expected, had a lower body fat percentage and a lower prevalence of reported hypertension, diabetes and hypercholesterolaemia than less active women.

According to Hu *et al.* (1999:1437-1438), both walking and vigorous activities, e.g. callisthenics, aerobics, jogging, running and playing tennis, are associated with substantial reductions in the risk of type II diabetes. Decreased plasma insulin was associated with moderate (45 to 55% of heart rate reserve, 3 to 4, 30 min. sessions/week) and high intensity exercise training (65 to 75% of heart rate reserve, and 5 to 7, 30 min. sessions/week). However, higher intensity exercise is more effective than moderate intensity exercise in reducing fasting plasma insulin levels (Duncan *et al.*, 2004:896,900). The American College of Sports Medicine states that exercise training, including appropriate endurance and resistance training, is a major therapeutic modality for type II diabetes (Albright *et al.*, 2000:1349). It is recommended that type II diabetics should

engage in exercise training of three 10-min. sessions, which can later be increased to 30 min. of continued exercise, at least 3 to 5 days/week at a low-to-moderate intensity (of 40 to 70% of VO_{2max}) (Albright *et al.*, 2000:1349-1350). An evidence-based review also found that the effect of aerobic or resistance training on glycemic control in type II diabetes is generally positive (Kelley & Goodpaster, 2001:S500).

In a 2-phase crossover-treatment study, each phase involved a 9-day energy deficit period and a 5-day follow-up energy repletion period. A 25% energy deficit was achieved by either food restriction or exercise (consisting of activities such as walking, jogging, swimming and bicycling) (Tsai *et al.*, 2003:541). The exercise group resulted in more favourable results than the food restriction group (Tsai *et al.*, 2003:543-548). Food restriction resulted in a greater reduction in body weight, however, exercise resulted in a greater reduction in body fat percentage as well as maintaining lean body mass. Exercise also led to a more desirable blood lipid profile than food restriction (decreased TC, LDL-C and increased HDL-C). It is, therefore, desirable to include exercise in a weight reduction programme (Tsai *et al.*, 2003:541).

Volek *et al.* (2002:586-587) included dietary, exercise and vitamin supplementation in an 8-week weight-loss programme. The overweight subjects participated in a supervised exercise programme of 4 to 5 days/week where they could choose from a variety of exercises, e.g. walking, jogging, cycling, rowing or stairmaster. Exercise duration and intensity were prescribed and progressed individually based on their level of conditioning and in accordance with the ACSM's guidelines for exercise prescription (Volek *et al.*, 2002:587). The morphological outcomes, BMI, WHR and waist girth decreased significantly in men and woman. Decreases in body weight and fat mass were significant in men and woman after the 8 weeks, but only a significant decrease in body fat % was present in men. TC and LDL-C significantly decreased in men but not in woman (Volek *et al.*, 2002:588).

Dunn *et al.* (1999:331) concluded that a structured exercise prescription (50 to 85% of maximal aerobic power for 20-60 min., 5 days/week for 24 months) resulted in a 10% increase in cardio respiratory fitness from baseline. Mean DBP decreased by 2.66 mmHg, TC, LDL-C and HDL-C

decreased significantly and the TC to HDL-C ratio increased significantly (Dunn *et al.*, 1999:332). However, the major finding of this study was that both the lifestyle and the structured interventions produced significant and comparable beneficial changes and that a lifestyle approach to increasing physical activity in previously sedentary healthy adults is as effective over 24 months as more traditional structured exercise approaches (Dunn *et al.*, 1999:332-333).

Prabhakaran *et al.* (1999:191) studied the effects of a supervised, progressive, 14-week resistance training programme with 45-50 min. sessions 3 times/week on lipid profile and body fat percentage in healthy, sedentary, pre-menopausal women. The 14-week resistance training programme resulted in a significant 9% decrease in TC, 14% decrease in LDL-C and a significant 14.3% decrease in the TC/HDL-C ratio (Prabhakaran *et al.*, 1999:192-193). In addition to the significant increase in strength, there was not a significant decrease in body mass, however, a small but significant decrease in the body fat percentage was seen (Prabhakaran *et al.*, 1999:193).

A review article of the health and performance-related potential of resistance training (Stone *et al.*, 1991:210-227) found that resistance exercise training had a beneficial effect on serum lipid levels, but it appeared that the benefits reside in the volume of training. Over a 12-week period during the highest volume of training an increase in HDL-C and a significant decrease in TC, TC/HDL-C and LDL-C/HDL-C ratios were observed (Stone *et al.*, 1991:219). Weight training may also beneficially alter glucose tolerance and insulin sensitivity. The mechanism of these effects may be related to body composition alterations as a result of exercise training (Stone *et al.*, 1991:219). Cross-sectional longitudinal studies of highly weight-trained male and female athletes showed that they possessed higher than average lean body mass and lower than average percentage body fat (Stone *et al.*, 1991:223-224). Again a key factor appeared to be the total volume of resistance training thus using higher repetitions (8-12) and multiple sets (Stone *et al.*, 1991:227).

Adjusted all-cause death rates were from 17 to 39% lower in moderately fit men compared with low-fit men who smoked cigarettes, had elevated blood pressure, elevated cholesterol levels or were unhealthy. The lower mortality rates for similar analyses for women ranged from 48 to 67% (Blair *et al.*, 1996:209). Thus, maintaining an active lifestyle throughout life appears to be critical to maintain independence and health (Wilmore, 2003:51).

2.13.3 Epidemiological evidence of exercise and CVD

The potential value of rehabilitation with exercise training in individuals with CHD was recognized nearly as early as the clinical description of the disease itself (O'Conner *et al.*, 1989:234). In a review article of Lee and Paffenbarger (2001:37-52), the favourable influence of exercise on CHD was discussed. Moderate exercise training is likely to improve CHD risk factors and also reduce CHD risk. A half hour of vigorous exercise expends as much energy as moderate exercise carried out for twice or three times as long and provides greater CHD benefits (Lee & Paffenbarger, 2001:52). Wannamethee and Shaper (2001:101-114) reported similar results and concluded that light or moderate exercise that does not need to be strenuous or prolonged in middle or older age confers significant benefits for CVD and all-cause mortality.

Elderly men who walked >2.4 km/day were at half the risk of developing CHD as men who walked < 0.4 km/day (Hakim *et al.*, 1999:11-12). Among women who either walked briskly at least 3 hours/week or exercised vigorously for 1.5 hours/week, the risk of coronary events was reduced by 30 to 40%. Increasing walking time or combining walking with vigorous exercise appears to be associated with even greater risk reductions (Manson *et al.*, 1999:657).

O'Connor *et al.* (1989:234-243) overviewed randomized trials of rehabilitation with exercise after myocardial infarction. This overview concluded that cardiac rehabilitation with exercise indicated a moderate reduction of about 20% in total and cardiovascular-related mortality (O'Connor *et al.*, 1989:239). It is reasonable to expect exercise rehabilitation to have a beneficial effect on cardiovascular morbidity and mortality because exercise training improves functional work capacity thereby decreasing the metabolic and circulatory demands of daily activities (O'Connor *et al.*, 1989:243).

In a review of 48 studies using exercise intervention for CHD patients, the outcome results confirmed the benefits of exercise-based cardiac rehabilitation in terms of cardiac and all-cause mortality, as well as demonstrated significant improvements in a number of primary risk factors (e.g. TC, TG and SBP) that appear to be sustained in the present era of cardiovascular therapy provision (Taylor *et al.*, 2004:684-688). These findings support the hypothesis that reductions in mortality may also be mediated via the indirect effects of exercise through improvements in the risk factors for atherosclerotic disease (Taylor *et al.*, 2004:689). These benefits are not limited to particular CHD patient subgroups or particular models of exercise intervention (Taylor *et al.*, 2004:690).

2.14 EXERCISE TRAINING AND HOMOCYSTEINE

The favourable alterations of exercise training on known risk factors are well documented (Albright *et al.*, 2000:1349-1350; Durstine *et al.*, 2001:1042-1045; Savage *et al.*, 2003:319-322), but the effects of exercise training on a potential new risk factor, tHcy, and its possible influence on modulating the risk of CHD is not clear (Wright *et al.*, 1998:262). Recent data have pointed out that duration and intensity of exercise are relevant factors to modulate tHcy levels (Gaume *et al.*, 2005:126). Therefore, in order to understand the influence of exercise on homocysteine, it is important to distinguish between acute, chronic and resistance training.

2.14.1 Acute exercise session

An acute exercise session is an intense bout of exercise for a relatively short period of time. In a study of 12 trained and untrained middle-aged men (aged 50 to 56) that performed an incremental acute exercise session until exhaustion on a cycle ergometer (Gaume *et al.*, 2005:127), the resting tHcy concentrations were significantly lower in trained than untrained men (Gaume *et al.*, 2005:127).

De Crèe *et al.* (1999:272), on the other hand, found that the acute effect of 2 exercise-to-exhaustion sessions (cycle ergometer) during 2 different phases of the menstrual cycle in 15 untrained woman with a mean age of 18 yr, resulted in a 17 and 16% increases in tHcy levels respectively. Weiss *et al.* (1998:52) showed increased Hcy levels in 13 trained males after

completing a 2.5 hour run. Another study also found that acute exercise increases Hcy levels (Herrmann *et al.*, 2003:1521). In this study 100 recreational athletes (87 males and 73 females, aged 37 to 40) participated in a marathon race, a 100 km run or a 120 km mountain bike race (Herrmann *et al.*, 2003:1519). The most impressive results were found in marathon runners, where post-race Hcy was 64% higher than before the race (Herrmann *et al.*, 2003:1521). Also, 39 healthy, well-trained triathletes, aged between 19 and 49, who participated in a competitive exercise sprint triathlon showed increased Hcy concentrations after the exercise session (König *et al.*, 2003:117).

In contrast, Wright *et al.* (1998:265) concluded that a 30 min. bout of acute, moderate intensity exercise (at 70% of HR_{max}) had no effect on tHcy concentrations in 20 normal healthy men aged 24 to 39. A 1-hour 60% VO_{2max} exercise session (cycle ergometer) had no influence on tHcy levels in 7 young healthy men with a mean age of 20 yr (De Crèe *et al.*, 2000:258). In a study by Chen *et al.* (2005:36) using similar study characteristics but performing a 30-min. 80% VO_{2peak} exercise session, similar results were found.

It appears that untrained, middle-aged subjects performing an incremental exercise session to exhaustion showed decreased tHcy concentrations and trained, young to middle-aged athletes performing an intense acute exercise session showed increases in tHcy concentrations. However, healthy, young to middle-aged subjects performing moderate acute exercises showed no change in tHcy concentrations. It is evident that further research, combining different age groups, levels of fitness and intensity of acute exercise sessions are necessary to determine the exact contribution of an acute exercise session on tHcy concentrations.

2.14.2 Aerobic exercise training

According to Ali *et al.* (1998:1543), 65 men (mean age of 63 yr) who had a recent CAD event participated in a cardiac rehabilitation and aerobic exercise training programme of 12 weeks consisting of 36 exercise sessions. A 12% reduction in Hcy levels in these normolipidemic patients with CAD and hyper-tHcy was seen. This 12% reduction in Hcy levels would be expected to produce 20 to 30% reductions in CAD risk (Ali *et al.*, 1998:1544). Bailey *et al.*

(2000:1062) showed that a 4-week exercise training programme in young healthy men with a mean age of 22 yr was associated with an 11% reduction in tHcy concentrations. Training involved 4 weeks of cycling exercises performed 3 days/week for 20 to 30 min. at 70 to 80% of HR_{max} (Bailey *et al.*, 2000:1060). In another study 39 healthy, well-trained triathletes aged between 19 and 49 were divided in a low-training group (9.1 training hours/week) and a high-training group (14.9 training hours/week) (König *et al.*, 2003:115). The results concluded that athletes in the high-training group had significantly lower Hcy levels at the end of the 4-week training period than those in the low-training group (König *et al.*, 2003:117).

However, in Duncan *et al.* (2004:894,896) 324 sedentary adults with a mean age of 49 yr were randomly assigned to 4 groups of training programmes consisting of walking either 45 to 55% (moderate intensity) or 65 to 75% (high intensity) of individual heart rate reserve and a frequency of either 3 to 4 (low frequency) or 5 to 7 (high frequency) days/week. After the 6 months of exercise training tHcy increased in all 4 exercise groups combined, moreover, tHcy increased significantly in both higher intensity groups (Duncan *et al.*, 2004:898).

In contrast, 15 untrained women with a mean age of 18 yr participated in two 5-day periods of identical exercise-to-exhaustion tests on a cycle ergometer (De Crée *et al.*, 1999:273). Results showed that the short-term effect of intensive training did not significantly alter tHcy concentrations, although the results were dependent on the menstrual cycle (De Crée *et al.*, 1999:275). A randomized controlled intervention of 217 frail elderly subjects (≥ 70 yr) found no significant differences in Hcy concentrations (De Jong *et al.*, 2001:341). The intervention consisted of a 17-week supervised intervention of moderate, gradually increased intensity group sessions (skills training through walking, stooping and chair stands using different materials), 2 days/week for 45 min. sessions (De Jong *et al.*, 2001:3339-340). Also, a weight-loss programme consisting of a supervised aerobic exercise programme as well as nutritional supplements and nutritional education in 12 overweight women (mean age of 42 yr) and 10 overweight men (mean age of 40 yr) resulted in no change in tHcy concentrations (Volek *et al.*, 2002:586-588). The exercise programme was performed 4 to 5 days/week and involved a variety of exercises, e.g. walking, jogging, cycling, rowing and stairmaster. The exercise

duration and intensity were prescribed and progressed individually based on the level of conditioning and in accordance with the ACSM guidelines for exercise prescription (Volek *et al.*, 2002:587).

Elderly subjects participating in moderate aerobic exercise of about the same period of time in 2 different studies showed contrasting results. One study showed no significant differences in Hcy concentrations and the other study showed a reduction in Hcy levels. The contrasting results may be because the baseline tHcy concentrations were higher in the study consisting of subjects with recent CAD events and, therefore, resulted in decreased Hcy levels. However, healthy young men participating in moderate exercises also resulted in reductions of tHcy concentrations. Another study, also including sedentary, middle aged subjects assigned to different training intensities, showed significantly increased tHcy levels in different intensity groups. Therefore, no conclusion can be drawn from these studies due to controversy that exists, and further research on the effect of chronic exercise training on tHcy concentrations is necessary.

2.14.3 Resistance training

In Steenge *et al.* (2001:1455), 20 moderately active healthy women (aged 19 to 38) participated in 8 weeks of prescribed resistance training, 3 days/week, consisting of a number of exercises using multigym equipment. This study resulted in a very small, nonsignificant decrease in tHcy concentrations (Steenge *et al.*, 2001:1456). Furthermore, the ATTICA study demonstrated that endurance exercise was associated with significant lower Hcy levels compared with resistance exercise or a sedentary lifestyle in men as well as in woman (Chrysohoou *et al.*, 2004:120). The ATTICA study is a population-based cohort of 1128 adult men and 1154 women (Chrysohoou *et al.*, 2004:117). Endurance exercise involving walking or bicycling of light (< 4 kcal/min) to moderate (4 to 7 kcal/min) intensity showed the lowest Hcy levels compared to the resistance exercise (weight lifting) (Chrysohoou *et al.*, 2004:118-1119).

The limited number of studies investigating the influence of resistance training on tHcy concentrations makes it impossible to draw a conclusion with regard to the effect of resistance training on tHcy concentrations.

In this section the beneficial effect of acute, chronic and resistance training respectively on tHcy concentrations are indicated. However, no conclusions can be drawn because studies with similar characteristics also showed that acute, chronic and resistance training respectively resulted in no change as well as increased tHcy concentrations.

2.15 EXERCISE TRAINING AND CARDIOVASCULAR FUNCTION

2.15.1 Aerobic exercise training

A meta-analysis of 44 randomized controlled trials including 2674 participants demonstrated average reductions in SBP and DBP of 2.6 and 1.8 mmHg in normotensive subjects and 7.4 and 5.8 mmHg in hypertensive subjects respectively after training from 3 to 5 days/week, 30 to 60 min./session at an intensity of 40 to 50% of net maximal exercise performance (Fagard, 2001:S484-S485). An association exists between increased systemic arterial compliance, decreased beta-stiffness index, decreased TPR, decreased resting SBP and increased VO_{2max} in 18 to 32 yr old sedentary males after a 4-week supervised exercise training programme (30 min at 75% VO_{2max}, 3 days/week) (Cameron & Dart, 1994:H693-H696).

Hambrecht *et al.* (2000:3098-3099) demonstrated statistically significant improvements in 73 patients (70 yr or younger) with chronic heart failure after 6 months of exercise training compared with controls. Patients began with 2 weeks of in-hospital ergometer exercise for 10 min., 4 to 6 times/day, followed by 6 months of home-based ergometer exercise training for 20 min./day at 70% of peak oxygen uptake (Hambrecht *et al.*, 2000:3096). After the 6 months of exercise training significant increases in maximum ventilation, exercise time and exercise capacity were observed in the exercise training group. Resting HR decreased by 9 beats/min., resting SV significantly increased, increased resting CO and significantly increased maximum CO were observed. In addition, LVEF improved in the exercise training group as well as a

significant decreased resting TPR (Hambrecht *et al.*, 2000:3098-3099). In another study of 21 chronic heart failure patients (mean age of 55 yr), 8-weeks of aerobic exercise (three 30 min. sessions/week at 50 to 60% of HR_{max}, that increased to 60 min./day for 5 to 7 days/week), led to decreased resting HR and significantly increased systemic arterial compliance (Parnell *et al.*, 2002:2-4). Hence, the maintenance and improvement of arterial compliance in response to an exercise training programme may be of great benefit to chronic heart failure patients by reducing left ventricular after load and improving coronary perfusion (Parnell *et al.*, 2002:6). Moreover, an overview of randomized trials of cardiac rehabilitation with exercise indicated a moderate reduction of about 20% in total and cardiovascular-related mortality (O'Connor *et al.*, 1989:239).

According to Tanaka *et al.* (2000:1272), 20 middle-aged and older (mean age of 53 yr) sedentary healthy men participated in a 12-week exercise intervention that started with a 25 to 30 min./day, 3 to 4 days/week walk at 60% of HR_{max}, and increased to 40 to 45 min./day, 4 to 6 days/week at 79% of HR_{max} (Tanaka *et al.*, 2000:1272). Results showed that regular aerobic exercises produced a 25% increase in central arterial compliance and a 20% reduction in the beta-stiffness index (Tanaka *et al.*, 2000:1273). Moreau *et al.* (2003:864) showed that exercise training in 12 healthy women (mean age of 58 yr), increased carotid arterial compliance and distensibility coefficient by 40% and decreased beta-stiffness index by 25%. The exercise intervention of 12 weeks consisted of walking 40 to 45 min./day, 4 to 5 days/week at a moderate intensity (65 to 80% of HR_{max}).

In contrast, Balkenstein *et al.* (1999:1835) concluded that a 3-month supervised aerobic exercise programme had no effect on blood pressure and carotid arterial compliance on 37 overweight men (aged between 18 and 50). The exercise programme consisted of four 1 hour sessions/week at 40% VO_{2max} (Balkenstein *et al.*, 1999:1832). In another study, 35 healthy women (≥50 yr of age) participated in 3 months of aerobic exercise training (Seals *et al.*, 2001:508). During the initial few weeks subjects were asked to walk for 30 min./day at an intensity of 40 to 50% of HR_{max}, 3 to 4 days/week, walking then increased to 40 to 45 min./day at 65 to 80% of HR_{max} (Seals *et al.*, 2001:508). Their SBP, DBP and PP were significantly lowered, however, no differences in any measure of arterial stiffness were found (Seals *et al.*, 2001:508,510). This

suggests that it may take more than 3 months before the effects of physical exercise on large artery wall properties become clear. The level of exercise may also play a role (Balkenstein *et al.*, 1999:1835).

Moderate aerobic exercise training shows great beneficial effects on cardiovascular function, e.g. decreases blood pressure, resting HR and TPR and improves resting CO and SV. However, in general, significant improvements in large artery wall properties, e.g. arterial compliance and arterial stiffness, moderate intensity aerobic exercise training consisting of a training period of at least 12 weeks and longer are necessary. The exception is chronic heart failure patients where an aerobic exercise training period of 8 weeks at a moderate intensity was sufficient to improve arterial compliance significantly.

2.15.2 Resistance training

A review article about the health and performance-related potential of resistance training demonstrated that resistance training can indeed have positive effects on the cardiovascular function (Stone *et al.*, 1991:210-227). Resistance training resulted in lower resting HR and is likely to be accompanied by an increased SV because there is an increased venous return per ventricular contraction (Stone *et al.*, 1991:212-213). Lower resting SBP and DBP have also been shown in a number of studies according to Stone *et al.* (1991:215-216).

The degree to which these parameters can be affected by resistance training is related to the volume, intensity and type of exercise used as well as the length of the programme, where high volume resistance training (e.g. 8 to 12 repetitions and multiple sets) generates the most favourable effects (Stone *et al.*, 1991:224-227). However, individuals should not rely solely on resistance training to improve cardiovascular function and cardio respiratory fitness, instead a resistance training programme should be used in conjunction with an aerobic exercise training programme (Plowman & Smith, 1997:166).

Resistance training shows improvements in cardiovascular function. However, a combined programme consisting of aerobic exercise training at a moderate intensity and a training period

of at least 12 weeks, as well as high volume resistance training, is necessary to show significant improvements in cardiovascular function.

2.16 CONCLUSION

This literature review has demonstrated the detrimental influences of elevated tHcy concentrations on CVD and endothelial injury/dysfunction through which Hcy exerts these damaging effects. Therefore, a positive relationship exists between increased Hcy concentrations and an impaired cardiovascular function, and the negative consequences of an impaired cardiovascular function on CVD and mortality rates are highlighted.

Extensive research on the influence of vitamin supplementation leading to the lowering of tHcy concentrations has been done, but more randomized, controlled studies focusing on efficacy, mechanism and effectiveness of various modes, intensities, durations and frequencies of exercise training on tHcy are recommended for healthy individuals as well as CVD patients. Furthermore, the health benefits of vitamin supplementation and exercise training on cardiovascular function have been indicated in a number of studies. This confirms the importance of vitamin supplementation and exercise training as less expensive alternatives for a better lifestyle that can also lead to lower morbidity and mortality rates.

Chapter 3

Research methods

3.1 INTRODUCTION

In this study the effect of an exercise training and a vitamin supplementation programme respectively on total plasma homocysteine concentrations and cardiovascular function in men aged 45 to 60 years with cardiovascular risk factors was investigated. Subjects with three or more cardiovascular risk factors were recruited because they are more likely to have elevated total plasma homocysteine concentrations. In order to obtain the relevant information to answer the scientific questions asked, fasting total plasma homocysteine concentrations were obtained, as well as selected cardiovascular variables determined by means of the Finometer apparatus. Subjects were then exposed to a 12-week exercise training and a 12-week vitamin supplementation intervention respectively to determine whether these two different interventions will influence total plasma homocysteine concentrations as well as cardiovascular function. In this chapter the methods for obtaining the above measurements will be described.

3.2 STUDY DESIGN

A randomised controlled cross-over intervention study design was used. The Ethical committee of the North-West University approved this study (code number 04M08).

The study was performed under free-living conditions. The subjects that complied with the selection criteria were each allocated with a number at recruitment. The numbers were then randomly allocated to a group until there were an equal number of subjects in each group (Group A, B, and C). During phase I, Group A was submitted to an exercise training programme and Group B was submitted to the folic acid and vitamin B_{12} supplement treatment. Group C was the control group that received no treatment during either phase (see Table 3.1). All the subjects were asked to continue with their daily routine (eating pattern, smoking habits and alcohol consumption) and requested to keep a diary of illnesses and medication used (if absolutely necessary) for the duration of the study. After the subjects followed the respective treatments for 12 weeks, a washout period of 6 weeks was introduced. The cross-over for phase II was performed on the intervention groups (Group A and B) and Group C remained the control

(Table 3.1). Questionnaires, blood samples and all other variables measured were at rest and determined at baseline and the end of each phase. Written feedback was given to the subjects at the end of the study.

Table 3.1: A schematic presentation of the study design

Group	A	B	C
Baseline	Pre-test	Pre-test	Pre-test
Phase I (12 weeks)	*Exercise training*	*Vitamins and normal daily routine*	*Control*
End	Post-test	Post-test	Post-test
Washout	(6 weeks)	(6 weeks)	(6 weeks)
Group	**A**	**B**	**C**
Baseline	Pre-test	Pre-test	Pre-test
Phase II (12 weeks)	*Vitamins and normal daily routine*	*Exercise training*	*Control*
End	Post-test	Post-test	Post-test
Washout	(6 weeks)	(6 weeks)	(6 weeks)

The respective intervention periods were for a duration of 12 weeks, as this is the period necessary for exercise training to result in physiological changes. A 6-week washout period followed each intervention to ensure a complete washout of the vitamin supplement. Six weeks is also sufficient to obtain de-conditioning from the exercise training programme.

3.3 SUBJECTS

Fifty-two men aged 45 to 60 years were recruited to participate voluntarily in this study. Informed consent for participation in the study (Appendix A) was obtained after the test procedure was explained to the subjects. Subjects using vitamin supplements were asked to

abstain from the supplements for 2 weeks prior to enrolling in the study. The following criteria were used for inclusion and exclusion of the subjects.

Inclusion criteria:

- Caucasian men
- 45 to 60 years of age
- three or more cardiovascular risk factors (e.g. family history, cigarette smoking, hypertension, hypercholesterolemia, diabetes, obesity)
- inactive for previous 3 months.

Exclusion criteria:

- Kidney and liver disease
- hypothyroidism
- chronic medication that has an influence on homocysteine metabolism
- serious medical conditions as determined by the principal investigator
- allergy to the supplement.

3.4 MEASURING INSTRUMENTS/APPARATUS

3.4.1 Demographics

Demographic information (Appendix B) was obtained and subjects were asked to complete the following questionnaires prior to testing: Coronary artery disease risk factor thresholds with ACSM risk stratification (adapted from Armstrong *et al.*, 2006:22,40,41) was completed first to determine each participant's medical history and the number of cardiovascular risk factors present (Appendix C). Thereafter, a physical activity index (PAI) (adapted from Sharkey, 1997:7,8) was completed (Appendix D), where the intensity, frequency and time of activity in summer and winter were multiplied and added to determine the PAI. An index of ≤ 25 indicated physical inactivity. A coronary risk profile (CRP) (Bjurstrom & Alexiou, 1978:524) was also completed (Appendix E) where a specific value was allocated to each risk factor's grade of intensity. The sum of all allocated values for all the risk factors indicated the risk. A value ≥ 31

indicated a high coronary risk profile and the participant is at a high risk for developing coronary heart disease (CHD). All the subjects completed a quantitative food frequency questionnaire (Appendix F) to determine their individual baseline daily vitamin intake (MacIntyre *et al.*, 2001:63-71). The vitamin intake was analysed by the Food-Finder 3 (Medical Research Council, Tygerberg, South Africa) computer programme.

3.4.2 Body composition

The heights (m) of the subjects were measured to the nearest 0.5 cm using a vertical stadiometer. Subjects stood without shoes, erect with the feet together and the head in the Frankfort plane. Body mass (kg) was measured with the calibrated electronic scale of the BOD POD® to the nearest 0.1 kg. Seven skin folds (mm), the triceps, subscapular, suprailiac, abdomen, front thigh, chest and midaxillary skinfolds were measured to the nearest 1.0 mm with a Slimguide® skinfold caliper and waist and hip girth (cm) were taken to the nearest 0.1 cm with a Mabis plastic measuring tape. The body mass index (BMI) was calculated as body mass divided by the height squared (kg/m^2). The formula of seven skinfolds for the fat percentage according to Armstrong *et al.* (2006:62,63) (adapted from Jackson & Pollock, 1985:76-90) was determined and the waist-to-hip ratio (WHR) was calculated as minimum waist girth divided by maximum hip girth.

BOD POD®:

The body mass (kg), percentage body fat (%), fat mass (kg) and lean mass (kg) of the subjects were determined with the BOD POD® Body Composition System using the air displacement plethysmographic method (Dempster & Aitkens, 1995:1692; Alemán-Mateo *et al.*, 2004:367). The BOD POD® consists of a dual-chamber plethysmograph, an electronic scale and a computer. The device has 2 chambers (a test chamber and a reference chamber) separated by a diaphragm which is precisely controlled by an electronic servo system. The system determined the body volume through an air displacement method. A volume-perturbing element oscillated under computer control and produced complemented volume perturbations in the 2 chambers. These volume perturbations produced small pressure fluctuations that allowed the calculation of the chamber air volume with and without the subject (body volume = chamber volume empty - chamber volume subject inside), and allowed the calculation of the volume of the subject

(McCrory *et al.*, 1995:1687,1688). Considering adjusted body volume and body mass, the body density was calculated using the equation developed by Siri (Siri, 1961:230; Lohman, 1992:7; Roche *et al.*, 1996:5) to determine percentage body fat of normal subjects (as indicated below). The Brozek equation (Brozek *et al.*, 1963:113-140; Lohman, 1992:7; Roche *et al.*, 1996:5) was used for the very lean or obese subjects (as indicated below). These formulas are based on a sample of Caucasian Adults in Life Measurement, Inc. (2003:45).

** Siri 1961	% fat = $[(4.95/D_b) - 4.50] \times 100$
** Brozek 1963	% fat = $[(4.570/D_b) - 4.142] \times 100$

$$\% \text{ fat} = [(1/D_b)(d_1 \times d_2) / (d_1 - d_2)] - d_2/d_1 - d_2$$

D_b = the density of the body (D = Mass/Volume)

d_1 = 1.100 g/cc fat-free compartment (known)

d_2 = 0.9 g/cc fat compartment (known)

In order to perform a reliable and repeatable measure with the BOD POD®, a few preparations had to be adhered to, namely:

Preparation of the subjects:

- Minimal, tight fitting clothing and a swim cap had to be worn
- no food, drink or exercise 2 hours prior to testing
- all jewellery, watches, eyeglasses had to be removed
- subject was asked to void the bladder
- subject had to be relaxed, dry and at a normal body temperature prior to testing (Life Measurement, Inc., 2003:10).

During measurements:

- Ensure that the correct height and age were entered
- the subjects were asked to enter the BOD POD®, put their hands in lap and relax

- was important to ensure that there was minimal movement from the subject as well as no talking or laughing
- the measurement was taken and predicted lung volume was used for the lung volume correction (Life Measurement, Inc., 2003:18, 21,22).

3.4.3 Finometer

A continuous blood pressure measurement was recorded for a period of at least 7 min. with the Finometer apparatus (FMS, The Netherlands). The Finometer computed all cardiovascular variables online and stored the data in result files on a hard disk. The Beatscope 1.1 software programme integrated the subject's gender, age, height and weight and this information was further integrated to obtain the systolic blood pressure (SBP) (mmHg), diastolic blood pressure (DBP) (mmHg), mean arterial pressure (MAP) (mmHg), heart rate (HR) (beats/min), stroke volume (SV) (ml), cardiac output (CO) (l/min), total peripheral resistance (TPR) (mmHg/ml) and Windkessel arterial compliance (C_w) (ml/mmHg). The mean values of all the cardiovascular function variables were estimated in the last 2 min. of the 7 min. measuring time and the data were exported to an excel spreadsheet for the statistical analyses. The vascular unloading technique of Penáz together with the Physiocal criteria of Wesseling provided reliable, noninvasive and continuous estimations of the cardiovascular function variables (Imholz *et al.*, 1998:606; Silke & McAuley, 1998:403; Schutte *et al.*, 2004:79).

3.4.4 Blood sampling

The blood samples were taken after a fasting period of 8 hours. A qualified nursing sister used a 21-gauge scalp infusion set (Butterfly and syringe) and collected venous blood samples (5 ml). The EDTA blood samples for the total plasma homocysteine analyses were immediately placed on ice and centrifuged for 15 min. within one hour of sampling to yield plasma. The plasma was placed in aliquots and stored at -82 °C in a bio-freezer until the analyses were performed.

3.4.5 Total plasma homocysteine analyses

Total plasma Hcy concentrations (µmol/l) were measured by means of competitive immunoassay (IMMULITE® 2000, Homocysteine: DPC, USA, Catalogue number L2KH02). The

IMMULITE® 2000 performed an on-line one-cycle sample pre-treatment of patient's plasma with S-adenosyl-L-homocysteiene (SAH) hydrolase and dithiothreitol (DTT) solution in a reaction tube containing no bead. After a 30-min. incubation, the treated sample was transferred to a second reaction tube containing a SAH-coated polystyrene bead and an alkaline phosphatase-labelled antibody specific for SAH. During the 30-min. incubation, the converted SAH from the sample pre-treatment competed with immobilized SAH for binding alkaline phosphatase-labelled anti-SAH antibody. Unbound enzyme conjugates were removed by centrifugal wash. Substrate was added and the procedure continued as described for typical immunoassays.

3.4.6 Cardio-respiratory fitness

Each subject's cardio-respiratory fitness was determined by means of direct maximal oxygen consumption (VO_{2max}) ($mlO_2/kg/min$). It was measured with the Mijnhardt Para-Magnetic Analyzer (Model UG-61D; Gerb. Mijnhardt B.V., Odijk, Holland) that continuously sampled expired air and rate of oxygen consumption (VO_2) ($mlO_2/kg/min$), carbon dioxide production (VCO_2) ($mlCO_2/kg/min$), minute ventilation (V_e) (l/min) and the respiratory exchange rate (R) (CO_2/O_2), calculated every 30 s by an on-line computer system. The VO_{2max} test determined the subjects' functional capacity at each baseline and after each intervention period. The test was performed on a Monark cycle ergometer (Model 834E; Monark Exercise AB, Vansbru, Sweden). The subjects were connected to a 5-lead electrocardiograph (ECG) as a safety precaution for the duration of the test.

The resting blood pressure (mmHg) and heart rate (beats/min) were determined with a mercury blood pressure monitor (Baumanometer®) and stethoscope. The subjects completed a warm up of 1 min. at 50 revolutions per min. (rpm) at a resistance of 50 Watt (W). For the duration of the test a constant speed of 60 rpm was cycled. Every 30 s heart rate was registered and every 60 s the resistance was increased by 25 W. When VO_{2max} was reached according to the ACSM's criteria (Balady *et al.*, 2000:117), e.g. 1) failure of heart rate to increase with further increases in exercise intensity; 2) a plateau in oxygen uptake with increased workload; 3) a respiratory exchange ratio greater than 1.15; 4) a post-exercise venous lactic acid concentration of more than

8 mmol; 5) a rating of perceived exertion of more than 17 (6-20 scale); or additionally 6) the subject requested the test to be terminated; the heart rate and systolic and diastolic blood pressure were measured. If the test was terminated before the VO_{2max} was reached due to ECG and/or blood pressure abnormalities, a VO_{2peak} was obtained. The weights were removed and the recovery period began at a speed of 30-40 rpm with a resistance of 50 W for 5 min. Heart rate and blood pressure were taken after a 1-min. recovery period and then after 3 and 5 min. respectively.

3.5 EXPERIMENTAL PROCEDURE

Subjects were requested not to participate in exercise training 24 hours prior to the pre and post-tests. Subjects arrived, completed an informed consent form and all the questionnaires. The body composition variables were determined next, followed by the Finometer measurements. Total plasma Hcy concentrations were taken and then the VO_{2max} test.

Exercise training intervention

The exercise training intervention consisted of a basic 12-week exercise training programme. All the subjects had to exercise at least 3 times per week. The exercise training programme included aerobic, resistance and stretch exercises. The target heart rate (beats/min) for training was individually calculated at 70% of maximal age predicted heart rate (HR_{max}) according to Karvonen's formula (modified by Armstrong *et. al.*, 2006:145) in order to prescribe the 12-week exercise training programme.

In the first 4 weeks the aerobic exercises were performed at 70-80% of HR_{max} for 20 min. on a bicycle ergometer. The duration was increased by 5 min. every 4 weeks. After each aerobic exercise session, the subjects performed 4 stretch exercises. The hamstring, quadriceps, calves and pectoral muscles were stretched respectively for 2 sets of 30 s each. Ten resistance exercises were then performed for the strengthening of the quadriceps, hamstring, biceps, triceps, chest, back, shoulders, calves and abdominal muscles. The repetitions and intensity of these 10 exercises were increased every 4 weeks. In the first 4 weeks, 2 sets of 15 repetitions were

performed, with 2 sets of 20 repetitions in the next 4 weeks, and in the last 4 weeks 3 sets of 20 repetitions.

Vitamin supplementation intervention

A multi-vitamin, containing folic acid and vitamin B_{12} [Pharma Natura (Pty) Ltd.] were used. Each tablet contained 200 μg folic acid and 12.5 μg vitamin B_{12}. The subjects received a 4 week supply of the tablets at a time for the 12 weeks. Two tablets had to be taken per day in the morning before eating.

Compliance to interventions

Compliance to the vitamin supplementation intervention was determined by tablet counting. Compliance to the exercise training intervention was done by reporting the maximal heart rate attained during every aerobic exercise session and by keeping record of the attendance of exercise training sessions.

3.6 STATISTICAL ANALYSES

Statistical analysis was performed with SPSS for Windows (version 14.0; SPSS Inc., Chicago, Ill.). Mean baseline characteristics presented as minimum, maximum, mean and standard deviations of the subjects as a total group, as well as randomised to each of the different interventions were performed with descriptive statistics. Differences between the average characteristics (mean and standard deviation) at baseline, as randomised to the different interventions, were assessed by the one-way analysis of variance (ANOVA), and followed by the Tukey HSD *post hoc* test for significance ($p \leq .05$). Frequency analyses were performed to determine the distribution of cardiovascular risk factors present in the subjects at baseline. The baseline to end changes (mean and standard deviation) during each of the different interventions were also assessed by ANOVA, followed by the Tukey HSD *post hoc* test for significance ($p \leq .05$). Analysis of co-variance (ANCOVA), adjusted for age and BMI, was performed to determine the statistical significant percentage change from baseline to end (%Δ), corrected for baseline of all the variables within the different interventions. Correlations between %Δ of tHcy

concentration in relationship to %Δ in cardiovascular function at baseline and within each of the different interventions were assessed by two-tailed partial correlations adjusted for age, BMI and VO_{2max}, because of their influence on blood pressure, arterial stiffness and arterial compliance. The statistical analyses were done on the bases of intention-to-treat in which the outcomes of all patients were analysed with the group to which they were originally assigned whether or not they completed the protocol (Lang & Secic, 1997:19).

Chapter 4

Results and discussion

4.1 INTRODUCTION

There are inconclusive results regarding the effect of exercise on total plasma homocysteine (tHcy) concentrations. No research could be found on the relationship between the percentage change of tHcy concentration and percentage change of cardiovascular function with the intervention of an exercise training and a vitamin supplementation programme respectively. The objective of this study is, therefore, to determine the effect of a 12-week exercise training and a 12-week vitamin supplementation intervention respectively on tHcy concentrations as well as on cardiovascular function, and whether the change in tHcy concentration within the different interventions related to the change in cardiovascular function.

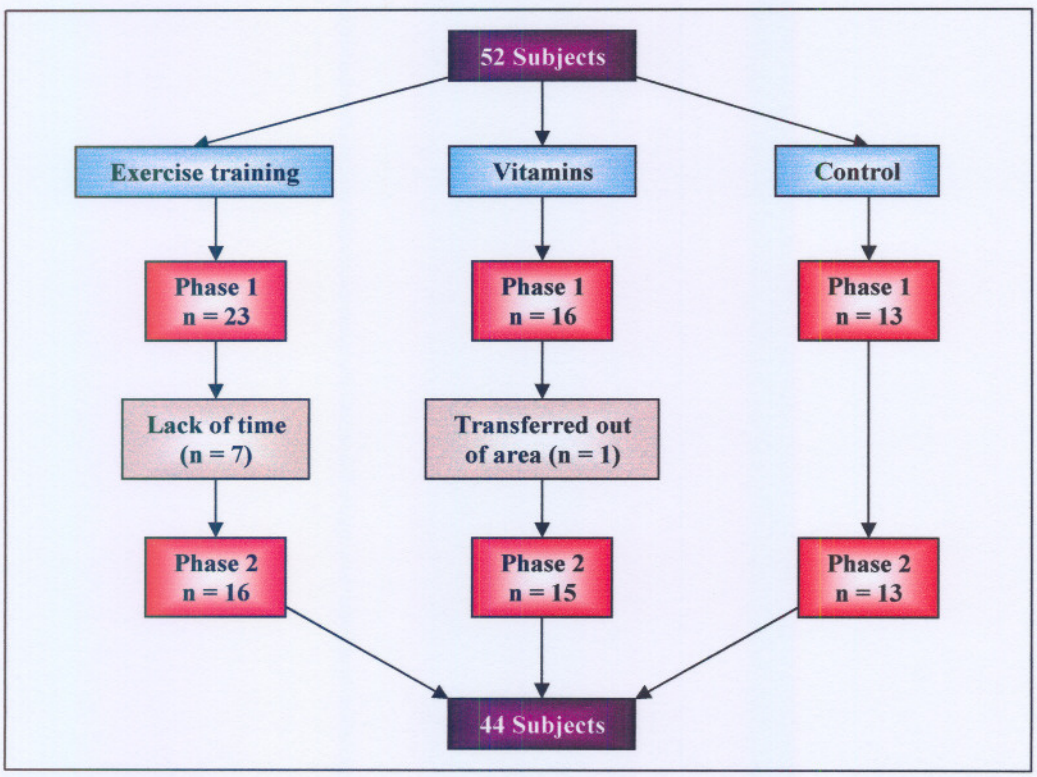
A randomised controlled cross-over intervention study design was used. The 52 men adhering to the inclusion criteria were randomly assigned to one of 3 groups. Group A was submitted to an exercise training programme, Group B was submitted to the folic acid and vitamin B_{12} supplement and Group C was the control group that received no treatment. Group A and B were crossed over for phase II, with Group C remaining the same. Each intervention period lasted for 12-weeks, with a 6-week washout period separating the crossover.

The results will be presented and discussed in this chapter. In order to report and discuss the data in a logical order, the results are presented in the following manner: Firstly, a description of the baseline characteristics of the subjects is given as a total group (Table 4.1 and Figure 4.2) and as randomized to the different interventions (Table 4.2). Secondly, the baseline to end changes of the anthropometric characteristics, cardio respiratory fitness (VO_{2max}), tHcy concentrations and cardiovascular function variables of the total group within the different interventions (Table 4.3) are presented. Thirdly, the percentage change from baseline to end (%Δ) of the anthropometric characteristics, VO_{2max}, tHcy concentrations (Figure 4.3) and cardiovascular function variables (Figure 4.4) of the total group within the different interventions are presented. Lastly, the partial correlations between percentage change of Hcy (%Δ tHcy) concentration and %Δ in cardiovascular function is given at baseline (Table 4.4), with the exercise training intervention (Table 4.5.), vitamin supplementation intervention (Table 4.6) and control group

(Table 4.7). A summarized version is also given of the partial correlations between %Δ tHcy concentration and %Δ of cardiovascular function within the different interventions (Table 4.8).

4.2 RESULTS

Fifty-two subjects were initially recruited for the study. Due to lack of time to adhere to the exercise training intervention, 7 subjects refrained from participation in the study. One subject moved out of the area (Figure 4.1). The data of the subjects that participated in the exercise training intervention, vitamin supplementation intervention and control group respectively were combined in order to increase statistical power. Only 58% of the subjects complied with the requested 36 exercise sessions (e.g. 3 days/week for 12 weeks). An average of 79% of the subjects complied with the vitamin supplementation intervention (2 tablets/day for 12 weeks).



n = number of subjects

Figure 4.1: The trial profile of the subjects in the study

4.2.1 *Baseline characteristics*

The baseline characteristics of all the subjects are summarized in Table 4.1. The mean age of the subjects was 51.5 ± 5.3 years. All the men were inactive according to the physical activity index (PAI) scale (4 ± 9), as required by the inclusion criteria. The mean coronary risk profile (CRP) (38 ± 9) indicated a high risk of coronary heart disease (CHD) among the subjects. The mean number of cardiovascular risk factors was 3.9 (Figure 4.2). Fifty-one of the 52 subjects were inactive and the most prevalent risk factors were hypertension and obesity and the least prevalent risk factor diabetes (Figure 4.2).

The results of the quantitative food frequency questionnaire (Table 4.1) indicated that the subjects had low intakes of dietary folic acid (266.8 ± 146.9 µg/day) compared to the recommended daily intake (RDI) of 400 µg/day (Institute of Medicine, 1998). Subjects ingested 6.3 ± 2.9 µg/day of dietary vitamin B_{12}, which is more than the RDI (2.4 µg/day) (Institute of Medicine, 1998). The reason for the high intake of vitamin B_{12} may be due to the fact that all the subjects reported a high red meat intake.

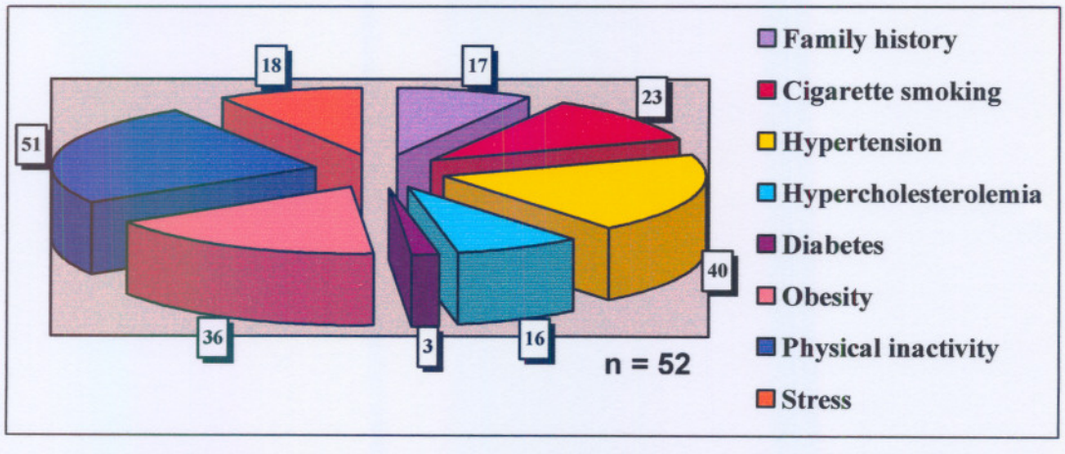


Figure 4.2: Distribution of the number of cardiovascular risk factors present in the subjects at baseline

Table 4.1: Baseline characteristics (minimum, maximum, means, and standard deviations) of all the male subjects (n = 52) with 3 or more cardiovascular risk factors

Variable	Min.	Max.	Mean ± SD
Age (yr)	42.0	61.0	51.5 ± 5.3
BMI (kg/m^2)	22.5	42.5	31.2 ± 5.4
WHR	0.80	1.10	0.97 ± 0.06
BOD POD (body fat %)	9.5	56.5	30.9 ± 9.8
VO_{2max} ($mlO_2/kg/min$)	2.4	29.0	17.9 ± 5.3
tHcy (µmol/l)	5.0	22.0	8.9 ± 3.1
SBP (mmHg)	105	174	138 ± 14
DBP (mmHg)	49	111	83 ± 11
MAP (mmHg)	70	129	104 ± 11
HR (beats/min)	47	99	69 ± 13
SV (ml)	37.0	182.3	104.5 ± 32.3
CO (l/min)	2.2	12.7	7.1 ± 2.3
TPR (mmHg/ml)	0.40	3.00	1.02 ± 0.48
C_w (ml/mmHg)	1.1	3.5	2.1 ± 0.5
Physical activity index (PAI)†	0	32	4 ± 9
Coronary risk profile (CRP)‡	24	63	38 ± 9
Dietary Vit. B_{12} (µg/day)	1.5	14.0	6.3 ± 2.9
Dietary folic acid (µg/day)	86.0	650.0	266.8 ± 146.9

BMI = body mass index; CO = cardiac output; C_w = Windkessel arterial compliance; DBP = diastolic blood pressure; HR = heart rate; MAP = mean arterial pressure; Max. = maximum; Min. = minimum; SBP = systolic blood pressure; SD = standard deviation; SV = stroke volume; tHcy = total plasma homocysteine concentration; TPR = total peripheral resistance; Vit. = vitamin; VO_{2max} = maximal oxygen consumption; WHR = waist-to-hip ratio; † = inactivity ≤ 25; ‡ = high risk ≥ 31.

In Table 4.2 differences between the average characteristics (mean ± standard deviation), of the different interventions at baseline were assessed by the one-way analysis of variance (ANOVA), followed by the Tukey HSD *post hoc* test for significance ($p \leq .05$). After the subjects were

randomly assigned to Group A, B or C no statistically significant differences between the groups for all the baseline characteristics were observed (Table 4.2).

Table 4.2: Means and standard deviations of the ANOVA for the baseline characteristics of the subjects when randomised to the different groups

Variable	Group A (n = 23) Mean ± SD	Group B (n = 16) Mean ± SD	Group C (n = 13) Mean ± SD
Age (yr)	53.0 ± 5.8	51.7 ± 4.5	48.6 ± 4.4
BMI (kg/m^2)	32.0 ± 5.0	30.6 ± 6.3	30.8 ± 5.0
WHR	0.99 ± 0.05	0.96 ± 0.07	0.97 ± 0.06
BOD POD (body fat %)	32.8 ± 10.7	29.7 ± 11.5	29.2 ± 5.0
VO_{2max} ($mlO_2/kg/min$)	17.5 ± 5.0	18.8 ± 6.5	17.3 ± 4.4
tHcy (µmol/l)	9.1 ± 3.8	8.5 ± 1.8	9.2 ± 3.2
SBP (mmHg)	139 ± 14	137 ± 12	137 ± 18
DBP (mmHg)	83 ± 11	84 ± 9	84 ± 13
MAP (mmHg)	104 ± 12	104 ± 8	104 ± 14
HR (beats/min)	69 ± 15	65 ± 10	73 ± 11
SV (ml)	108.5 ± 27.0	101.2 ± 41.4	101.3 ± 31.4
CO (l/min)	7.4 ± 2.3	6.6 ± 2.9	7.2 ± 1.8
TPR (mmHg/ml)	0.94 ± 0.34	1.20 ± 0.69	0.95 ± 0.38
C_w (ml/mmHg)	2.1 ± 0.5	2.0 ± 0.6	2.1 ± 0.5

BMI = body mass index; CO = cardiac output; C_w = Windkessel arterial compliance; DBP = diastolic blood pressure; HR = heart rate; MAP = mean arterial pressure; n = number of subjects; SBP = systolic blood pressure; SD = standard deviation; SV = stroke volume; tHcy = total plasma homocysteine concentration; TPR = total peripheral resistance; VO_{2max} = maximal oxygen consumption; WHR = waist-to-hip ratio.

The mean body mass index (BMI) of subjects in all the different interventions was ≥ 30 kg/m^2 and the mean body fat percentage ≥ 29% as measured with the BOD POD®. This indicated that all the subjects were obese (Armstrong *et al.*, 2006:22,66). The mean maximal oxygen consumption (VO_{2max}) (between 17.3 and 18.8 $mlO_2/kg/min$) indicated that all the subjects in the different interventions had low cardio-respiratory fitness levels (Armstrong *et al.*, 2006:79), as

was required. The mean tHcy concentrations were between 8.5 and 9.2 μmol/l, which is within the normal range of tHcy (Hankey, 2003:37). This indicated that all the subjects had normal ranges of tHcy although they had more than 3 cardiovascular risk factors.

The resting cardiovascular variables at baseline e.g. the resting diastolic blood pressure (DBP), heart rate (HR), stroke volume (SV) and cardiac output (CO) appeared to be within the normal range for their age (Table 4.2) (Ogawa *et al.*, 1992:496; Spina *et al.*, 1993(a):851,853; Plowman & Smith, 1997:84,92). There are no standard resting values for total peripheral resistance (TPR) and Windkessel arterial compliance (C_w). The resting mean systolic blood pressure (SBP) (between 137 and 139 mmHg) and mean arterial pressure (MAP) (104 mmHg) were slightly above the normal resting range of 134 mmHg and 97 mmHg respectively (Table 4.2) (Spina *et al.*, 1993(b):102; Plowman & Smith, 1997:92).

4.2.2 Changes in descriptive variables

In Table 4.3 the means and standard deviations of the ANOVA of baseline to end changes during each of the different interventions are displayed. Baseline as well as end variables were all taken at rest.

After the 12-week exercise training intervention the anthropometric variables showed more positive results in comparison to the vitamin supplementation and control interventions (Table 4.3). Both the BMI changes (31.6 ± 5.5 kg/m^2 to 31.2 ± 5.7 kg/m^2) and body fat percentage changes (32.1 ± 10.9% to 30.4 ± 10.2%) showed small but non-significant decreases with the exercise training intervention, whereas the vitamin supplementation and control interventions caused small increases or had no influence on the anthropometric variables. The VO_{2max} showed small non-significant increases in the 2 different interventions, as well as in the control group (Table 4.3). Plasma Hcy concentrations, on the other hand, increased non-significantly with the 12-week exercise training intervention from 9.3 ± 3.3 μmol/l to 10.1 ± 4.6 μmol/l, but decreased non-significantly after the 12-week vitamin supplementation intervention from 9.6 ± 3.8 μmol/l to 8.4 ± 3.1 μmol/l. The control group also showed non-significant decreased tHcy concentrations (9.7 ± 3.2 μmol/l to 8.4 ± 2.7 μmol/l).

The resting cardiovascular variables, e.g. the baseline and end values of the resting blood pressure variables (SBP, DBP and MAP) with the different interventions did not show significant changes (Table 4.3). However, resting HR decreased from 69 ± 14 beats/min to 66 ± 13 beats/min with exercise training, whereas the vitamin supplementation as well as the control interventions increased resting HR from 67 ± 12 beats/min to 69 ± 11 beats/min and 73 ± 10 beats/min to 75 ± 9 beats/min respectively. Furthermore, the Tukey HSD *post hoc* test observed a significant difference ($p \leq .05$) between the end resting HR values of the exercise training intervention and the control group.

The 12-week vitamin supplementation intervention showed greater improvements in resting SV and CO than the exercise training intervention within the same period (Table 4.3). During the vitamin supplementation intervention the resting SV improved non-significantly from 104.2 ± 34.3 ml to 114.5 ± 28.6 ml and resting CO improved non-significantly from 7.0 ± 2.5 l/min to 7.9 ± 2.5 l/min. The exercise training intervention resulted in no change in resting SV (114.0 ± 28.5 ml to 114.9 ± 30.4 ml), and a non-significant decrease in resting CO from 7.8 ± 2.5 l/min to 7.5 ± 2.0 l/min. The control group showed a non-significant decrease in resting SV from 100.9 ± 27.3 ml to 98.7 ± 18.8 ml. This implied that the exercise training as well as the vitamin supplementation interventions did result in improvements in resting SV. The fact that the amount of improvements within the vitamin supplementation intervention was greater than within the exercise training intervention may be due to the fact that the compliance of the exercise training intervention was only 58% in comparison to the 79% compliance of the vitamin supplementation intervention.

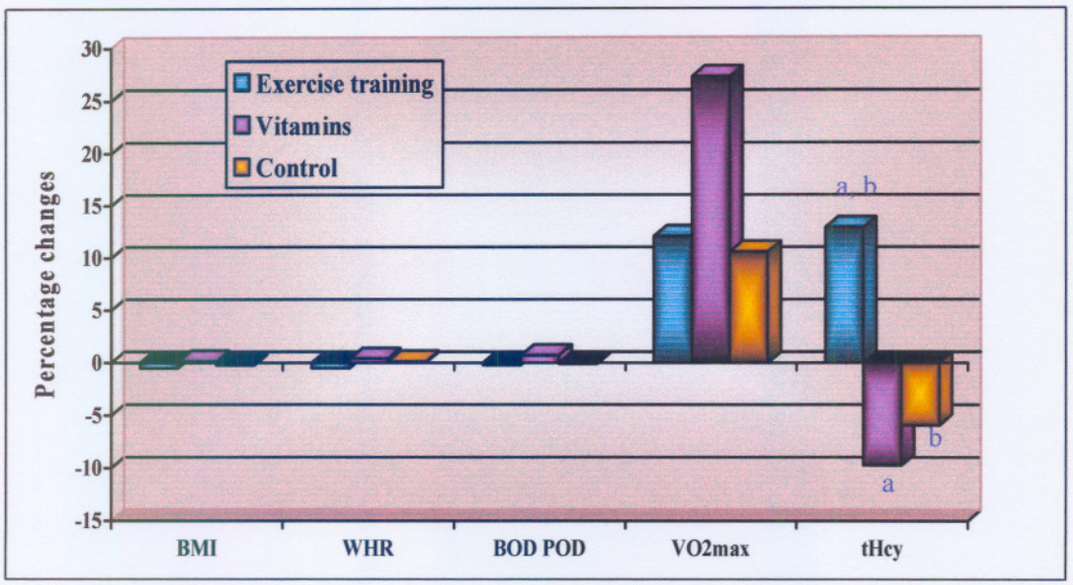
The same trend was observed with the resting TPR and C_w, where the vitamin supplementation intervention showed non-significant decreases in resting TPR (1.04 ± 0.52 mmHg/ml to 0.84 ± 0.26 mmHg/ml) in comparison to the exercise training intervention where no change was observed (Table 4.3). The vitamin supplementation intervention caused a non-significant improvement in resting C_w from 2.1 ± 0.5 ml/mmHg to 2.2 ± 0.5 ml/mmHg, and again, the exercise training intervention indicated no change.

Table 4.3: Means and standard deviations of the ANOVA of baseline to end changes during each of the different interventions

Variable	Exercise training (n = 39) B: Mean ± SD E: Mean ± SD	Vitamins (n = 31) B: Mean ± SD E: Mean ± SD	Control (n = 26) B: Mean ± SD E: Mean ± SD
BMI (kg/m^2)	**B:** 31.6 ± 5.5 **E:** 31.2 ± 5.7	**B:** 30.7 ± 6.8 **E:** 30.7 ± 6.8	**B:** 30.6 ± 4.8 **E:** 31.3 ± 4.6
WHR	**B:** 0.98 ± 0.06 **E:** 0.98 ± 0.06	**B:** 0.96 ± 0.06 **E:** 0.96 ± 0.06	**B:** 0.98 ± 0.05 **E:** 0.98 ± 0.05
BOD POD (body fat%)	**B:** 32.1 ± 10.9 **E:** 30.4 ± 10.2	**B:** 30.4 ± 11.2 **E:** 30.6 ± 11.2	**B:** 29.1 ± 5.0 **E:** 29.9 ± 5.2
VO_{2max} ($mlO_2/kg/min$)	**B:** 19.8 ± 6.0 **E:** 21.9 ± 5.7	**B:** 20.7 ± 8.4 **E:** 21.6 ± 7.7	**B:** 19.2 ± 4.1 **E:** 21.3 ± 4.1
tHcy (µmol/l)	**B:** 9.3 ± 3.3 **E:** 10.1 ± 4.6	**B:** 9.6 ± 3.8 **E:** 8.4 ± 3.1	**B:** 9.7 ± 3.2 **E:** 8.4 ± 2.7
SBP (mmHg)	**B:** 139 ± 14 **E:** 141 ± 14	**B:** 137 ± 15 **E:** 136 ± 15	**B:** 136 ± 14 **E:** 136 ± 13
DBP (mmHg)	**B:** 81 ± 9 **E:** 80 ± 7	**B:** 81 ± 9 **E:** 79 ± 7	**B:** 83 ± 11 **E:** 84 ± 9
MAP (mmHg)	**B:** 103 ± 11 **E:** 103 ± 8	**B:** 103 ± 10 **E:** 101 ± 10	**B:** 104 ± 11 **E:** 104 ± 11
HR (beats/min)	**B:** 69 ± 14 **E:** 66 ± 13*	**B:** 67 ± 12 **E:** 69 ± 11	**B:** 73 ± 10 **E:** 75 ± 9*
SV (ml)	**B:** 114.0 ± 28.5 **E:** 114.9 ± 30.4	**B:** 104.2 ± 34.3 **E:** 114.5 ± 28.6	**B:** 100.9 ± 27.3 **E:** 98.7 ± 18.8
CO (l/min)	**B:** 7.8 ± 2.5 **E:** 7.5 ± 2.0	**B:** 7.0 ± 2.5 **E:** 7.9 ± 2.5	**B:** 7.2 ± 1.7 **E:** 7.3 ± 1.2
TPR (mmHg/ml)	**B:** 0.89 ± 0.33 **E:** 0.89 ± 0.29	**B:** 1.04 ± 0.52 **E:** 0.84 ± 0.26	**B:** 0.94 ± 0.33 **E:** 0.89 ± 0.25
C_w (ml/mmHg)	**B:** 2.2 ± 0.5 **E:** 2.2 ± 0.4	**B:** 2.1 ± 0.5 **E:** 2.2 ± 0.5	**B:** 2.1 ± 0.4 **E:** 2.1 ± 0.4

*B = baseline; BMI = body mass index; CO = cardiac output; C_w = Windkessel arterial compliance; DBP = diastolic blood pressure; E = end; HR = heart rate; MAP = mean arterial pressure; n = number of subjects; SBP = systolic blood pressure; SD = standard deviation; SV = stroke volume; tHcy = total plasma homocysteine concentration; TPR = total peripheral resistance; VO_{2max} = maximal oxygen consumption; WHR = waist-to-hip ratio; * = significant difference between end values of exercise training and control groups (significant difference = $p \leq 0.05$).*

The percentage change from baseline to end (%Δ) at the different interventions was calculated as the %Δ corrected for baseline values. The results of the analysis of co-variance (ANCOVA), that adjusted for age and BMI was performed on the anthropometric variables (BMI, WHR and body fat percentage), VO_{2max} and tHcy concentrations at the different interventions (Figure 4.3).



BMI = body mass index; BOD POD = body fat percentage; tHcy = total plasma homocysteine concentration; VO_{2max} = maximal oxygen consumption; WHR = waist-to-hip ratio; a = significant difference between percentage change of exercise training and supplement intervention; b = significant difference between percentage change of exercise training and control intervention (significant difference = $p \leq 0.05$).

Figure 4.3: Means of the ANCOVA for the percentage change from baseline to end of the anthropometric variables, cardio respiratory fitness and tHcy concentrations during the different interventions, corrected for baseline and adjusted for age and BMI

The percentage change in VO_{2max} (%Δ VO_{2max}) and percentage change in tHcy (%Δ tHcy) concentrations with the different interventions resulted in the most pronounced changes. There was a 12.1 ± 26.8% increase in %Δ VO_{2max} after the exercise training intervention, but the vitamin supplementation intervention showed a greater, however, non-significant increase of 27.3 ± 134.3% in %Δ VO_{2max} (Figure 4.3). The lack of a significant increase in %Δ VO_{2max} after the 12-week exercise training intervention confirms that compliance to the exercise training intervention (58%) was inadequate. The %Δ tHcy concentrations increased by 12.9 ± 28.1% with the exercise training intervention, but decreased by 9.7 ± 27.5% and 5.9 ± 32.0% with the

vitamin supplementation and the control respectively (Figure 4.3). This increase in %Δ tHcy concentration after the exercise training intervention differed statistically ($p \leq .05$), according to the Tukey HSD *post hoc* test, from the vitamin supplementation and control interventions.

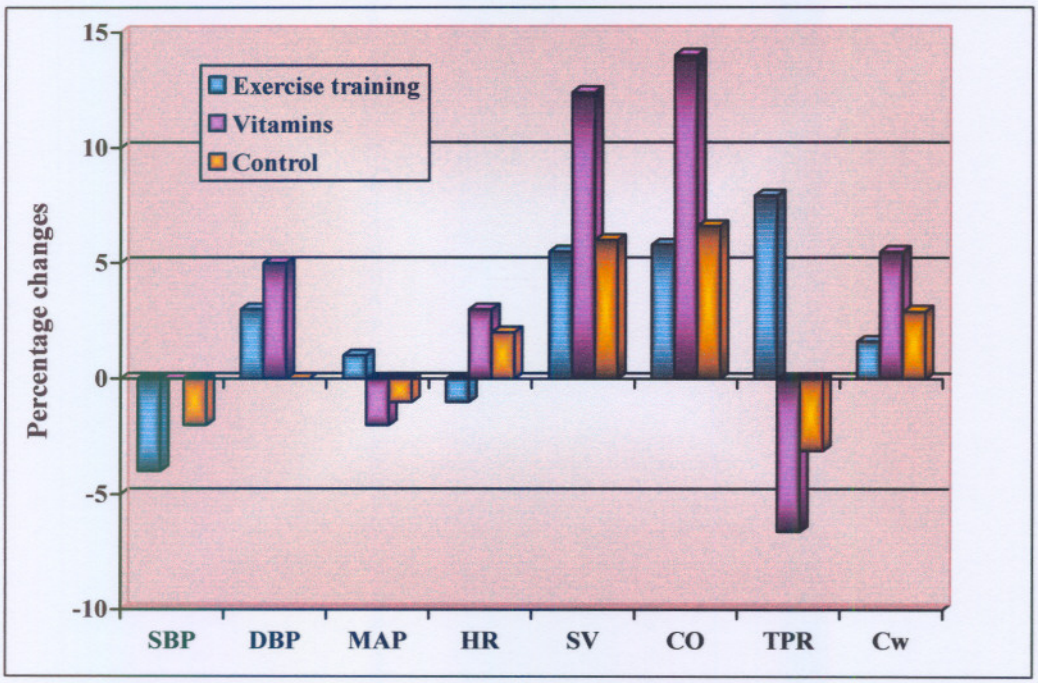
Figure 4.4 demonstrates the means of the ANCOVA for the %Δ of cardiovascular variables at the different interventions. Percentage change was corrected for baseline and adjusted for age and BMI, because of their influence on blood pressure, arterial stiffness and arterial compliance.

No significant differences were observed for the %Δ of any of the cardiovascular variables with the different interventions (Figure 4.4). Resting percentage change in SBP (%Δ SBP) decreased with the 12-week exercise training intervention by 4 ± 12% and remained unchanged with the vitamin supplementation intervention. However, resting percentage change of DBP (%Δ DBP) increased by 3 ± 11% and 5 ± 12% with the exercise intervention and vitamin supplementation intervention respectively.

Resting percentage change of HR (%Δ HR) showed more positive effects with the exercise training intervention as with the vitamin supplementation intervention because of the lowering effect of the exercise training intervention on resting %Δ HR (1 ± 10%) in comparison to the increasing effect of the vitamin supplementation intervention on resting %Δ HR (3 ± 15%). However, resting percentage change of SV (%Δ SV) as well as resting percentage change of CO (%Δ CO) showed greater improvements with the vitamin supplementation intervention as with the exercise training interventions (Figure 4.4). There was only a 5.5 ± 34.6% increase in resting %Δ SV with the exercise training intervention, compared to the 12.4 ± 39.8% increase in resting %Δ SV with the vitamin supplementation intervention. Similar results were observed with resting %Δ CO where exercise training indicated only a 5.8 ± 39.8% increase in resting %Δ CO compared to the 14.0 ± 41.3% increase with the vitamin supplementation intervention.

The 12-week exercise training intervention caused the resting percentage change of TPR (%Δ TPR) to increase by 7.90 ± 40.03% and only caused a small 1.6 ± 15.9% increase in resting percentage change of C_w (%Δ C_w) (Figure 4.4). The 12-week vitamin supplementation

intervention, however, indicated improvements in cardiovascular function with the 6.62 ± 24.66% decrease in resting %Δ TPR and the 5.5 ± 16.5% increase in resting %Δ C_w.



CO = cardiac output; C_w = Windkessel arterial compliance; DBP = diastolic blood pressure; HR = heart rate; MAP = mean arterial pressure; SBP = systolic blood pressure; SV = stroke volume; TPR = total peripheral resistance.

Figure 4.4: Means of the ANCOVA for the percentage change from baseline to end of cardiovascular variables during the different interventions, corrected for baseline and adjusted for age and BMI

4.2.3 Relationship between changes in Hcy and changes in cardiovascular function

Before investigating significant (p≤ .05) two-tailed partial correlations between %Δ tHcy concentrations in relationship to %Δ of cardiovascular function within each of the different interventions, two-tailed partial correlations at baseline were performed (Table 4.4), adjusted for age, BMI and VO_{2max}. No significant correlations between %Δ tHcy concentration and any of the cardiovascular variables were observed at baseline. There was, however, a number of significant (p≤ .05) correlations between different cardiovascular variables at baseline (Table 4.4). This included a significant (p≤ .05) negative association between resting %Δ C_w and

%Δ DBP (r = .96). There were also significant (p≤ .05) negative correlations between resting %Δ C_w and %Δ MAP (r = .89), and between resting %Δ TPR and %Δ CO (r = .83).

Table 4.4: Partial correlations between baseline tHcy concentration and cardiovascular variables adjusted for age, BMI and VO_{2max}

Variable	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	SV (ml)	CO (l/min)	TPR (mmHg/ml)
tHcy (µmol/l)						
SV (ml)		r = - .64 p = .00	r = - .36 p = .02			
CO (l/min)		r = - .61 p = .00	r = - .37 p = .01			
TPR (mmHg/ml)		r = .69 p = .00	r = .46 p = .00	r = - .73 p = .00	r = - .83 p = .00	
C_w (ml/mmHg)	r = - .60 p = .00	r = - .96 p = .00	r = - .89 p = .00	r = .65 p = .00	r = .58 p = .00	r = - .64 p = .00

CO = cardiac output; C_w = Windkessel arterial compliance; DBP = diastolic blood pressure; MAP = mean arterial pressure; p = significant difference (p≤ .05); r = correlation; SBP = systolic blood pressure; SV = stroke volume; TPR = total peripheral resistance; – = negative correlation.

No significant associations between %Δ tHcy concentration and %Δ of any of the cardiovascular variables were observed with the exercise training intervention (Table 4.5). The significant (p≤ .05) correlations that did exist were the same that were observed at baseline, e.g. significant (p≤ .05) negative associations between resting %Δ C_w and %Δ MAP (r = .80), between resting %Δ TPR and %Δ SV (r = .89) and between resting %Δ TPR and %Δ CO (r = .89).

Table 4.5: Partial correlations between percentage change of tHcy concentration and percentage change of cardiovascular variables adjusted for age, BMI and VO_{2max} with the exercise training intervention

Variable	MAP (mmHg)	HR (beats/min)	SV (ml)	CO (l/min)	TPR (mmHg/ml)
tHcy (μmol/l)					
TPR (mmHg/ml)		**r** = - .39 **p** = .03	**r** = - .89 **p** = .00	**r** = - .89 **p** = .00	
C_w (ml/mmHg)	**r** = - .80 **p** = .00		**r** = .68 **p** = .00	**r** = .64 **p** = .00	**r** = - .78 **p** = .00

CO = cardiac output; C_w = Windkessel arterial compliance; HR = heart rate; MAP = mean arterial pressure; p = significant difference (p≤.05); r = correlation; SV = stroke volume; TPR = total peripheral resistance; – = negative correlation.

Significant (p≤ .05) associations were observed between %Δ tHcy concentration and %Δ of three cardiovascular variables with the vitamin supplementation intervention (Table 4.6). Decreased %Δ tHcy concentration was significantly (p≤ .05) associated with increased resting %Δ SV (r = .42), as well as a significantly (p≤ .05) increased resting %Δ CO (r = .49). The decreased %Δ tHcy concentration was also significantly (p≤ .05) associated with decreased resting %Δ TPR (r = .59). Similar significant (p≤ .05) correlations existed between the %Δ of the same cardiovascular variables within the vitamin supplementation intervention as at baseline and with the exercise training intervention (Table 4.6). A significant (p≤ .05) negative correlation existed between resting %Δ C_w and %Δ MAP (r = .93). Significant (p≤ .05) negative correlations were also observed between resting %Δ TPR and %Δ SV (r = .89) and between resting %Δ TPR and %Δ CO (r = .90). Increased resting %Δ C_w was also significantly (p≤ .05) associated with reduced resting %Δ TPR (r = .87) with the vitamin supplementation intervention that did not occur with the exercise training intervention.

Table 4.6: Partial correlations between percentage change of tHcy concentration and percentage change of cardiovascular variables adjusted for age, BMI and VO_{2max} with the vitamin supplementation intervention

Variable	SBP (mmHg)	MAP (mmHg)	SV (ml)	CO (l/min)	TPR (mmHg/ml)
tHcy (µmol/l)			**r** = - .42 **p** = .05	**r** = - .49 **p** = .02	**r** = .59 **p** = .00
HR (beats/min)	**r** = .47 **p** = .03	**r** = .42 **p** = .05			
SV (ml)	**r** = - .51 **p** = .02	**r** = - .66 **p** = .00			
CO (l/min)		**r** = - .52 **p** = .01			
TPR (mmHg/ml)	**r** = .47 **p** = .03	**r** = .70 **p** = .00	**r** = - .89 **p** = .00	**r** = -.90 **p** = .00	
C_w (ml/mmHg)	**r** = - .51 **p** = .02	**r** = - .93 **p** = .00	**r** = .84 **p** = .00	**r** = .74 **p** = .00	**r** = - .87 **p** = .00

CO = cardiac output; C_w = Windkessel arterial compliance; HR = heart rate; MAP = mean arterial pressure; p = significant difference (p≤ .05); r = correlation; SBP = systolic blood pressure; SV = stroke volume; tHcy = total plasma homocysteine concentrations; TPR = total peripheral resistance; – = negative correlation.

In the control group, decreased %Δ tHcy concentration was significantly (p≤ .05) associated with decreased resting %Δ SBP (r = .59) (Table 4.7). Two significant (p≤ .05) negative correlations also occurred, e.g. between resting %Δ TPR and %Δ CO (r = .89) and resting %Δ TPR and %Δ C_w (r = .84).

Table 4.7: Partial correlations between percentage change of tHcy concentration and percentage change of cardiovascular variables adjusted for age, BMI and VO_{2max} in the control group

Variable	SBP (mmHg)	MAP (mmHg)	SV (ml)	CO (l/min)	TPR (mmHg/ml)
tHcy (µmol/l)	r = .59 p = .02				
HR (beats/min)	r = .64 p = .01				
TPR (mmHg/ml)			r = - .73 p = .00	r = - .89 p = .00	
C_w (ml/mmHg)		r = - .75 p = .00	r = .68 p = .00	r = .71 p = .00	r = - .84 p = .00

CO = cardiac output; C_w = Windkessel arterial compliance; HR = heart rate; MAP = mean arterial pressure; p = significant difference (p≤ .05); r = correlation; SBP = systolic blood pressure; SV = stroke volume; tHcy = total plasma homocysteine concentrations; TPR = total peripheral resistance; – = negative correlation.

In Table 4.8 a summary is given between the significant (p≤ .05) partial correlations of percentage change of tHcy concentrations and percentage change of cardiovascular variables within the different interventions. In the control group, a significant (p≤ .05) positive correlation was reported for the %Δ tHcy concentration with one cardiovascular variable, e.g. resting %Δ SBP (r = .59). Exercise training did not generate significant partial correlations between %Δ tHcy concentration and %Δ of any cardiovascular variable and can probably again be ascribed to the poor compliance of the exercise training intervention (58%). However, with the vitamin supplementation intervention the %Δ tHcy concentration correlated significantly (p≤ .05) with the %Δ of three cardiovascular variables, e.g. negative correlations with resting %Δ SV (r = .43) and %Δ CO (r = .53) and a positive correlation with resting %Δ TPR (r = .61). The appearance of significant (p≤ .05) correlations between %Δ tHcy concentration and %Δ of cardiovascular variables within the vitamin supplementation intervention may be ascribed to the higher compliance (79%) of this intervention.

Table 4.8: Partial correlations between percentage change of tHcy concentrations and percentage change of cardiovascular variables adjusted for age, BMI and VO_{2max} within the different interventions

Variable	Exercise tHcy (µmol/l)	Vitamins tHcy (µmol/l)	Control tHcy (µmol/l)
SBP (mmHg)			**r** = .59 **p** = .02
SV (ml)		**r** = .43 **p** = .05	
CO (l/min)		**r** = .53 **p** = .01	
TPR (mmHg/ml)		**r** = - .61 **p** = .00	

CO = cardiac output; p = significant difference (p≤ .05); r = correlation; SBP = systolic blood pressure; SV = stroke volume; tHcy = total plasma homocysteine concentrations; TPR = total peripheral resistance; – = negative correlation.

4.3 DISCUSSION

The objectives of this study were to determine the effect of a 12-week exercise training and a 12-week vitamin supplementation intervention respectively on tHcy concentrations and cardiovascular function, and whether the change in tHcy concentrations during the different interventions revealed a relation with the change in cardiovascular function.

In comparing the exercise training intervention, vitamin supplementation intervention and the control group, it was found that the baseline characteristics of the subjects in the different interventions were similar with no significant differences (Table 4.2). This is an indication that the different interventions were matched for age, anthropometric variables, cardio-respiratory fitness, cardiovascular risk factors, tHcy concentrations and cardiovascular function.

The reason why the subjects had normal baseline tHcy concentrations (Table 4.2), although they had a mean of 3.9 cardiovascular risk factors, may be due to the fact that the dietary vitamin B_{12}

intake of the subjects was more than the RDI (Institute of Medicine, 1998). This confirms a statement by O'Grady *et al.* (2002:839) that vitamin B_{12} protects against the build-up of tHcy in the blood by converting Hcy to methionine and that a deficiency of B-vitamins including vitamin B_{12} is associated with elevated tHcy levels (Selhub *et al.*, 1993:2696; Eikelboom *et al.*, 1999:365).

The reason for the slightly higher resting SBP and MAP at baseline may be because all of the subjects were obese at baseline (Table 4.2). Similar results were found in a study by Danias *et al.* (2003:198) where the resting SBP of the obese subjects was higher than the non-obese control group. This confirms the inclusion criteria of this study, e.g. subject with cardiovascular risk factors.

The 12-week exercise training intervention lowered the anthropometric variables (BMI, WHR and body fat percentage) (Table 4.3), although these changes were not significant and the subjects remained obese. It is a well known fact that exercise has significant lowering effects on anthropometric variables (Boileau *et al.*, 1999:379-381; Savage *et al.*, 2003:319). The reason why the anthropometric variables in this study did not lower significantly may be due to the fact that the compliance to the exercise training was only 58%. The 12-week vitamin supplementation intervention, however, had no effect on BMI and WHR and an increased body fat percentage was observed (Table 4.3).

The %Δ VO_{2max} showed non-significant increases in the 2 different interventions as well as in the control group (Figure 4.3). The vitamin supplementation intervention, however, showed greater increases in %Δ VO_{2max} in comparison to the exercise training intervention and control group. These results are in contrast with studies that showed significant increases in VO_{2max} with exercise (Wilmore, 2003:48-49) compared to the control group (Boileau *et al.*, 1999:379-381). These changes cannot be explained and other factors than the interventions were probably responsible for the changes that were seen. The lack of compliance to the exercise training intervention may in part be a reason for the above mentioned result. Another possible reason for the increases in %Δ VO_{2max} with the supplementation intervention and control group may be due

to the fact that the data collection stretched over a season-alteration from winter to summer and people are probably more active in the summer compared to winter. The study objectives also might have caused the subjects to be more aware of their eating patterns as well as their fitness levels for a healthier lifestyle.

Although the subjects had normal baseline tHcy concentrations, the 12-week vitamin supplementation intervention significantly ($p \leq .05$) reduced tHcy concentrations by 9.7% (Figure 4.3). The decrease in %Δ tHcy after the vitamin supplementation intervention can be ascribed to the vitamin supplementation intervention because of the 79% compliance with this intervention. This correlates with other studies where reductions in tHcy levels were found with a combined supplementation of folic acid, vitamin B_6 and vitamin B_{12} (den Heijer *et al.*, 1998:359 & Clarke; Armitage, 2000:342).

In this study tHcy concentrations increased significantly ($p \leq .05$) by 12.9% with the 12-week exercise training intervention (Figure 4.3). The reason for this increase is unknown and can probably not be ascribed to the exercise training intervention, since only 58% of subjects complied with this intervention and only a 12.1% non-significant increase in VO_{2max} resulted from the exercise training intervention (Figure 4.3). Furthermore, a possible reason for the increased %Δ tHcy concentration from the exercise training might be due to the fact that the subjects were unfit at baseline, not accustomed to exercise and did not train regularly. Consequently their bodies were not able to show physiological adaptations due to the health improvements with physical conditioning. This causes oxidative stress with each acute exercise session they participated in, which is in correlation with a study by Chen *et al.* (2005:36). Due to the fact that tHcy enhances reactive oxygen species (ROS) formation (Signorello *et al.*, 2002:283; Moselhy & Demerdash, 2003,2004:29), and the hemoconcentration effects (plasma volume shifts) that are known to occur with exercise (Wright *et al.*, 1998:265), each exercise session in physically unconditioned subjects exerts an increased turnover of serine (Weiss *et al.*, 1998:52; Bailey *et al.*, 2000:1064), creatine and protein (De Crée *et al.*, 1999:276; König *et al.*, 2003:117). Because of this increased turnover, regeneration of these substrates is accompanied by a considerable stimulation of the methionine metabolism (Herrmann *et al.*, 2003:1521). If

this stimulation exceeds a distinct level, the steady state between Hcy production and its degradation by remethylation and transsulfuration may be disturbed in favour of the production, thus increasing Hcy levels (Bailey *et al.*, 2000:1064; Herrmann *et al.*, 2003:1521). Opposite effects occurred, in other words, tHcy concentrations decrease in trained subjects (Gaume *et al.*, 2005:129). Another study found that tHcy increases with exercise training (Duncan *et al.*, 2004:898). However, Ali *et al.* (1998:1543-1544) showed that an exercise training programme of the same length of this study (e.g. 36 exercise sessions in 12 weeks) resulted in a 12% reduction in Hcy levels. Thus, due to inadequate compliance to the exercise training intervention no conclusion can be drawn with regard to the effect of exercise training on tHcy concentrations.

Only a few studies measured VO_{2max} or any other form of compliance to the exercise intervention (De Crée *et al.*, 1999:274; Bailey *et al.*, 2000:1061; Duncan *et al.*, 2004:898). Hence, the inconsistent effects of exercise training on tHcy concentrations reported in the literature may, therefore, also be ascribed to inadequate compliance to the exercise intervention in some studies, or may even be due to an inadequate study design especially the lack of a control group. In studies examining the influence of exercise training on tHcy concentrations only two studies, e.g. de Jong *et al.* (2001:339) and Volek *et al.* (2002:586) made use of control groups. In this study, opposite changes in tHcy concentrations between the exercise training intervention and control group were found. Statistics in this study were based on the "intension-to-treat" method, where the outcome to prescribing exercise for health reasons is determined (Lang & Secic, 1997:19). It, therefore, still remains to be established what the effect of exercise training on tHcy concentrations is in well designed randomised control trials where extreme care is taken to ensure adequate compliance to the exercise interventions.

After adjusting for age and BMI, the exercise training intervention resulted in a small non-significant decrease in resting %Δ SBP and non-significant increases in resting %Δ DBP and %Δ MAP (Figure 4.4). These results are in contrast with other studies where aerobic exercise showed significant decreases in SBP (Cameron & Dart, 1994:H693-H696) as well as in DBP (Dunn *et al.*, 1999:332). The reason for the increased blood pressure values may be due to the fact that the subjects remained obese after the exercise training intervention. This tendency

was also illustrated in a study by Bella *et al.* (1999:788) where the DBP was higher in overweight subjects. The non-significant increased resting %Δ TPR and small, non-significant increased resting %Δ C_w after the exercise training intervention in this study (Figure 4.4) correlates with a study by Ferrier *et al.* (2001:225) where aerobic exercise training failed to improve resting large-arterial mechanical properties or blood pressure. The fact that resting %Δ TPR increased and the resting %Δ C_w only increased by a small percentage with the exercise training intervention, confirms the above mentioned fact that the patients were still obese after the exercise training intervention, because obesity and mild hypertension are associated with increased arterial stiffness and decreased compliance (Nichols & O'Rourke, 1998:330-342; Stepniakowski & Egan, 1995:R567).

After adjusting for age and BMI, the vitamin supplementation intervention resulted in improvements in %Δ of cardiovascular variables in comparison to the exercise training intervention. The resting %Δ SBP did not change and a non-significant increased resting %Δ DBP and decreased resting %Δ MAP resulted from the vitamin supplementation intervention (Figure 4.4). Other studies, however, showed more positive results in blood pressure values from vitamin supplementation interventions, where folic acid and vitamin B_6 were associated with significant improvements in SBP, DBP and MAP (van Dijk *et al.*, 2001:2076; Mangoni *et al.*, 2002:500). In contrast to the exercise training intervention, the resting %Δ TPR decreased and the %Δ with which the resting C_w increased was greater, although non-significant with the vitamin supplementation intervention (Figure 4.4). Williams *et al.* (2005:29) also found that supplementation with folic acid reduced arterial stiffness in men with normal to mildly elevated SBP.

The %Δ increases in resting SV and CO, although non-significant, were the greatest improvements in cardiovascular function in this study and were observed in the vitamin supplementation intervention (Figure 4.4). The resting %Δ HR, however, increased non-significantly with the vitamin supplementation intervention. The exercise training intervention resulted in smaller non-significant increases in resting %Δ SV and %Δ CO and non-significant decreases in resting %Δ HR (Figure 4.4). According to Hambrecht *et al.* (2000:3098-3099)

6 months of exercise training resulted in significantly decreased HR and significantly increased SV and CO. The resulting smaller non-significant improvements in the resting %Δ SV and %Δ CO with the exercise training intervention in this study may be due to the fact that the intervention period was only 12 weeks and, furthermore, the compliance of exercise training was only 58%.

The greater improvements in cardiovascular function with the vitamin supplementation intervention compared to the less positive or even negative influence of the exercise training intervention on cardiovascular function may be due to the fact that the compliance of the exercise training intervention was only 58% in comparison to the 79% compliance of the vitamin supplementation intervention. Furthermore, it may be that an exercise training period longer than 12 weeks is necessary to cause significant improvements in cardiovascular function.

At baseline there were a number of significant ($p \leq 0.05$) correlations between %Δ of different cardiovascular variables (Table 4.4). The correlations that occurred at baseline also occurred in the 2 different interventions as well as in the control group (Table 4.4 to Table 4.7). This confirms what the literature has already proven: 1) a decrease in MAP correlates with an increase in C_w (Oren *et al.*, 1996:666; Benetos *et al.*, 1998:560,563; Joyner, 2000:1214; Beltran *et al.*, 2001:1009); 2) an increase in TPR correlates with a decrease in SV as well as CO (Plowman & Smith, 1997:87,97,101; Tanaka *et al.*, 1998:1154; McArdle *et al.*, 2001:345; Parnell *et al.*, 2002:1); and 3) an increase in TRP correlates with a decrease in C_w (Oren *et al.*, 1996:666; Tanaka *et al.*, 1998:1154; Leibovitz *et al.*, 2003:716).

Studies have already indicated the relationship between tHcy concentration and increased pulse pressure (PP), a marker of large artery stiffness (Davis *et al.*, 2001:331) and increased arterial stiffness (Smilde *et al.*, 1998:1960; Bortolotto *et al.*, 1999:840; Nestel *et al.*, 2003:85) that also led to a reduction in arterial compliance (Davis *et al.*, 2001:331; Nestel *et al.*, 2003:85). High Hcy concentrations are also independently associated with isolated systolic hypertension (Sutton-Tyrell *et al.*, 1997:1748), increased DBP, SBP and HR (Nygård *et al.*, 1995:1529).

To our knowledge, this is the first study examining the relationship between %Δ tHcy concentration and %Δ of cardiovascular variables with the intervention of an exercise training and a vitamin supplementation programme respectively. Only the vitamin supplementation intervention and in the control group did significant ($p \leq .05$) associations occur between %Δ tHcy concentration and %Δ in cardiovascular variables (Table 4.8). With the vitamin supplementation intervention decreased %Δ tHcy concentration was associated with increased resting %Δ SV and %Δ CO and decreased %Δ TPR (Table 4.8). This is an indication that tHcy is a marker of decreased cardiovascular function. The reason these correlations only occurred with the vitamin supplementation intervention is due to the fact that the %Δ tHcy concentration and %Δ of cardiovascular variables were greater with the vitamin supplementation intervention compared to the exercise training intervention (Figure 4.3 and Figure 4.4). This, again, may be due to the fact that the compliance with the vitamin supplementation intervention (79%) was far better than the compliance of the subjects with the exercise training intervention (58%).

Subjects did not train enough to obtain health improvements, but maybe participated in just enough exercise sessions to increase the oxidative stress on unconditioned physiological systems.

4.4 CONCLUSIONS

In conclusion, a 12-week exercise training intervention significantly increased tHcy concentrations by 12.9% and a 12-week vitamin supplementation intervention significantly reduced tHcy concentrations by 9.7%. The vitamin supplementation intervention showed greater improvements in cardiovascular function in comparison to the exercise training intervention. Therefore, only with the vitamin supplementation intervention significant correlations occurred between the %Δ tHcy concentration and the %Δ of some cardiovascular variables. Due to inadequate compliance to the exercise training intervention, no conclusion can be drawn with regard to the effect of exercise training on tHcy concentrations as well as on cardiovascular function. If the compliance of the exercise training intervention (58%) was higher or maybe similar to the compliance of the vitamin supplementation intervention (79%), the same results or even greater improvements may have occurred. This emphasizes the importance of further

research regarding the influence of exercise training on tHcy concentrations and its correlation with cardiovascular function in well designed randomised control trials where extreme care is taken to ensure adequate compliance to the exercise intervention.

Chapter 5

Summary, conclusions and recommendations

5.1 SUMMARY

The objectives of this study were firstly to determine the tHcy concentrations and cardiovascular function of untrained men aged 45 to 60 years with three or more cardiovascular risk factors. Secondly, to determine the effect of a 12-week exercise training and a 12-week vitamin supplementation intervention respectively on tHcy concentrations and cardiovascular function in men aged 45 to 60 years with three or more cardiovascular risk factors. Thirdly, the relationship between the change in tHcy concentration and the change in cardiovascular function with the 12-week exercise training and 12-week vitamin supplementation intervention respectively were determined in men aged 45 to 60 years with three or more cardiovascular risk factors. In Chapter 1 the outline of the problem statement indicated the importance of research on the relationship between exercise, Hcy, cardiovascular function and vitamin supplementation that underlies the research question, objectives and hypothesis of this study.

In Chapter 2, the literature review on mediators for changes in Hcy and cardiovascular function explained that Hcy, the amino acid bound with a disulphide bond exists in several forms and is metabolised through the remethylation and transsulfuration pathways of mehionine. Factors contributing to elevated tHcy concentrations include genetic defects of enzymes or their cofactors or co-substrates involved in the metabolism of Hcy, vitamin deficiency, lifestyle factors, diseases and medications. The chapter further demonstrated the detrimental influences of elevated tHcy concentrations on CVD and higher mortality and morbidity rates and the endothelial injury/dysfunction through which Hcy exerts these damaging effects. Therefore, a positive relationship exists between an increased Hcy concentration and an impaired cardiovascular function. Factors influencing cardiovascular function, e.g. age, cardiovascular risk factors and certain biochemical markers of CVD also lead to the impairment of cardiovascular function that ultimately leads to CVD and higher mortality rates. Extensive research on the influence of vitamin supplementation leading to the lowering of tHcy concentrations as well as an improvement in cardiovascular function has been done. Exercise training results in improvement in functional capacity, reduction of CVD risk and improves cardiovascular function, but because of the equivocal results on exercise training and Hcy, more

randomised, controlled studies focusing on efficacy, mechanism and effectiveness of various modes, intensities, durations and frequencies of exercise training on tHcy are recommended for healthy individuals as well as CVD patients.

The research methods (Chapter 3) investigating the effect of an exercise training and a vitamin supplementation intervention respectively on tHcy concentrations and cardiovascular function used a randomised controlled cross-over study design. In 52 subjects, different variables were measured according to standard protocols and recognized statistical analyses. The statistical methods used were descriptive statistics, ANOVA, followed by the Tukey HSD *post hoc* test for significance ($p \leq .05$), ANCOVA, adjusted for age and BMI, and two-tailed partial correlations, adjusted for age, BMI and VO_{2max}.

The results of the outcome of this study are presented and discussed in Chapter 4. The general conclusion that can be drawn is that a 12-week vitamin supplementation intervention accepted the hypothesis stated, e.g. a significant reduction in tHcy concentration, an improvement in cardiovascular function and a significant positive relationship between these two factors in comparison to the 12-week exercise training intervention that rejected the hypotheses.

5.2 CONCLUSIONS

The conclusions that are drawn from this study are based on the hypotheses stated in Chapter 1.

Hypothesis 1:

The tHcy concentrations will be elevated and cardiovascular function will be impaired in untrained men aged 45 to 60 years with three or more cardiovascular risk factors.

The first part of the hypothesis, "the tHcy concentrations will be elevated in untrained men aged 45 to 60 years with three or more cardiovascular risk factors" is rejected, based on the fact that tHcy concentrations (between 8.5 and 9.2 µmol/l) were within the normal range of tHcy (5 to 15 µmol/l).

The second part of the hypothesis, "cardiovascular function will be impaired in untrained men aged 45 to 60 years with three or more cardiovascular risk factors" is partially accepted, as the resting SBP (between 137 and 139 mmHg) and MAP (104 mmHg) were slightly above the normal resting range of 134 mmHg and 97 mmHg respectively. The other cardiovascular function variables, e.g. resting DBP, HR, SV, CO, TPR and C_w were within the normal range for this age group.

Hypothesis 2:

A significant reduced tHcy concentration and significant improvement in cardiovascular function will result from a 12-week exercise training and a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors.

The first part of the hypothesis, "a significant reduced tHcy concentration and significant improvement in cardiovascular function will result from a 12-week exercise training intervention in men aged 45 to 60 years with three or more cardiovascular risk factors" is rejected, because the tHcy concentration significantly increased by 12.9% with the exercise training intervention and, furthermore, resting DBP, MAP and TPR showed increases with the exercise training intervention. Furthermore, SV, CO and C_w resulted in small, non-significant increases and the resting SBP and HR showed small, non-significant reductions with the exercise training intervention.

The second part of the hypothesis, "a significant reduced tHcy concentration and significant improvement in cardiovascular function will result from a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors" is accepted, based on the research finding that the tHcy concentration significantly reduced by 9.7% with the vitamin supplementation intervention. Furthermore, resting MAP and TPR decreased and resting SV, CO and C_w increased with the vitamin supplementation intervention. No changes were found in resting SBP and resting DBP and HR showed small, non-significant increases with the vitamin supplementation intervention.

Hypothesis 3:

A reduction in tHcy concentration will be positively related with an improvement in cardiovascular function after a 12-week exercise training and a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors.

The first part of the hypothesis, "a reduction in tHcy concentration will be positively related with an improvement in cardiovascular function after a 12-week exercise training intervention in men aged 45 to 60 years with three or more cardiovascular risk factors" is rejected, because no significant relationship between the percentage change in tHcy concentration and the percentage change of any cardiovascular variable was observed with the exercise training intervention.

The second part of the hypothesis, "A reduction in tHcy concentration will be positively related with an improvement in cardiovascular function after a 12-week vitamin supplementation intervention respectively in men aged 45 to 60 years with three or more cardiovascular risk factors" is accepted, based on the research finding that the decreased percentage change in tHcy concentration is significantly related to the increased percentage change in resting SV as well as the percentage change in resting CO with the vitamin supplementation intervention. The decreased percentage change in tHcy concentration is also significantly related with the decreased percentage change in resting TPR.

5.3 LIMITATIONS

The limitations experienced during this study are:

- Although the exercise training was supervised, the compliance was not high enough to ensure conclusive results.

- ❖ The subjects in the study were not hyperhomocysteinemic, therefore, the inclusion of 3 or more cardiovascular risk factors was not sufficient to include hyperhomocysteinemic subjects.

- ❖ Dietary folic acid and vitamin B_{12} intake was only determined at baseline and by means of a questionnaire and was not quantified by means of blood analyses due to cost constraints.

- ● The duration of the exercise training intervention might have been to short for significant improvements on large artery wall properties.

- ❖ Changes in specific amino acids or enzymatic activity related to Hcy metabolism could not be measured directly, therefore, the mechanism responsible for increased Hcy concentrations following the exercise training intervention is unknown.

5.4 RECOMMENDATIONS

The following recommendations are made in order to obtain more conclusive results with regards to the role of exercise training and vitamin supplementation in tHcy and cardiovascular function relationship.

- ❖ The results from this study emphasises the importance of further research regarding the influence of exercise training on tHcy concentrations and its relationship with cardiovascular function in well designed, randomised control trials where extreme care is taken to ensure adequate compliance to the exercise intensity and duration. Compliance should be improved through more motivational supervised exercise training.

- ❖ More significant results might be obtained if hyperhomocysteinemic patients are the inclusion criteria of future studies.

- ❖ It is well recognised that folic acid, vitamin B_{12} and vitamin B_6 influence Hcy metabolisms and, therefore, the results would be more significant if future studies determined dietary intake by means of blood analyses throughout the whole study at the pre and post-test measurements of different interventions.

- ❖ There is also a need for longitudinal studies to investigate the effect of long term exercise training on Hcy and CVD to explore the possible mechanisms to conclude whether Hcy is a cause or a marker of CVD.

- ❖ The duration of the exercise training intervention can be extended because research suggests that it may take more than 12 weeks before the effects of exercise on large artery wall properties become clear.

- ❖ In future studies, changes in specific amino acids or enzymatic activity related to Hcy metabolism can be measured directly, therefore, a more conclusive reason for the mechanism responsible for increased Hcy concentrations following exercise can be obtained.

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CONGRESS PRESENTATIONS

The following presentations, based on this dissertation, have been delivered:

HERBST, S.J., NEL, R., MOSS, S.J. & OOSTHUIZEN, W. Total plasma homocysteine, vitamin supplementation and physical conditioning in men with coronary risk factors. *Poster presented at the International 18th Puijo Symposium in Koupio, Finland, June 29 – July 2, 2005.*

MOSS, S.J., HERBST, S.J., NEL, R., VAN ROOYEN, J.M., SCHUTTE, A.E. & HUISMAN, H.W.G. Changes in cardiovascular function with physical activity and multivitamins. *Oral presentation at the International 18th Puijo Symposium in Koupio, Finland, June 29 – July 2, 2005-10-07.*

MOSS, S.J., VAN DER WESTHUIZEN, F.H., HERBST, S.J. & NEL, R. Changes in antioxidant and free radical capacity of middle aged men after a physical conditioning program and vitamin supplementation intervention. *Poster presented at the Fourth annual conference of the International society of behavioral nutrition and physical activity (ISBNPA) in Amsterdam, The Netherlands, June 6-18, 2005.*

Appendix A: Informed consent

IS: NAVORSINGSPROJEK – EFFEK VAN 'N OEFENPROGRAM EN 'N VITAMIEN AANVULLING OP HOMOSISTEÏENVLAKKE EN KARDIOVASKULÊRE FUNKSIE

INGELIGTE TOESTEMMINGVORM: (Etiekkomitee nommer: 04M08)

Ek, die ondergetekende (Volle name) _____

_____

het die voorafgaande gegewens in verband met die projek gelees en ook die mondelinge weergawe daarvan aangehoor en ek verklaar dat ek dit verstaan. Ek was die geleentheid gegun om tersaaklike aspekte van die projek met die projekleier te bespreek en ek verklaar hiermee dat ek vrywillig aan die projek deelneem. Ek gee my toestemming om as proefpersoon in projek op te tree. Ek vrywaar hiermee die Universiteit asook enige werknemer of student van die Universiteit, teen enige aanspreeklikheid wat teenoor my, in die loop van die projek mag ontstaan. Ek onderneem verder om geen eise teen die Universitiet in te stel weens skade of persoonlikheidsnadeel wat ek weens die projek mag ly, hetsy dit aan die nalatigheid van die Universiteit, sy werknemers of studente, of ander proefpersone mag ontstaan nie.

_____ _____

Handtekening **Datum**

Appendix B: Demographic information

INSTITUUT VIR BIOKINETIKA
POTCHEFSTROOMKAMPUS

PERSOONLIKE INLIGTING:

Naam: _____

Posadres: _____

_____

_____

Woonadres: _____

_____

_____

Tel: _____**(H)**_____**(W)**

Sel: _____**Fax:**_____

Geboorte datum:___/___/___ **Ouderdom:**_____

Geneesheer:_____

Geslag: **M**

Appendix C: Medical history and cardiovascular risk factor determination

MEDIESE GESKIEDENIS

1. Kan u 2 – 3km stap sonder om 'n pyn op die bors te ervaar of kort asem te word?

   Ja **Nee** **Onseker**

2. Indien Nee, gee die rede.

   _____

3. Het u geneesheer al aangetoon dat u 'n hart probleem het?

   Ja **Nee**

4. Indien Ja, noem die probleem.

   _____

5. Neem u enige medikasie op 'n gereelde basis?

   Ja **Nee**

6. Indien Ja, noem die medikasie en die rede vir gebruik.

   _____

7. Het u enige las van lae rugpyn?

   Ja **Nee**

8. Indien ja, spesifiseer.

   _____

9. Het u enige breuke?

   Ja **Nee**

10. Indien ja, spesifiseer.

    _____

11. Lei u aan enige van die volgende siektetoestande?

    a) Oefenings geinduseerde asma **J** **N**

    b) Gereelde hoofpyn of megraine aanvalle **J** **N**

    c) Gereelde moegheid **J** **N**

    d) Depressie **J** **N**

    e) Nier probleme **J** **N**

f) Krampe in die bene **J** **N**

g) Verkoue, griep of brongitis **J** **N**

h) Gewrigs of skelet probleme **J** **N**

i) Epilepsie **J** **N**

j) Spatare in die bene **J** **N**

k) Longsiektes **J** **N**

l) Duiseligheid of floutes **J** **N**

m) Bloed sirkulasie probleme **J** **N**

12. Koronêre arteriële siekte risiko faktore

a) Familie geskiedenis:

Koronêre hartsiektes of skielike dood:

*Voor 55 jarige ouderdom van manlike bloedverwante (vader, broer)? **J** **N**

*Voor 65 jarige ouderdom van vroulike bloedverwante (ma, suster)? **J** **N**

b) Rook u? **J** **N**

Indien *ja,* aantal per dag?_____

Indien *nee*, het u voorheen gerook en wanneer het u gestop?

_____

c) Ly u aan hoë bloeddruk? **J** **N**

(SBD ≥ 140mm Hg

DBD ≥ 90mm Hg)

d) Hoë serum cholesterol vlakke? **J** **N**

(> 200mg/dL; > 5.2mmol/L)

e) Diabetes mellitus **J** **N**

f) Oorgewig **J** **N**

(BMI ≥ 30ka/m² of middel omtrek ≥ 100cm)

g) Fisieke onaktiwiteit **J** **N**

(3 maande laas geoefen)

h) Merk die mees gepaste stelling

Nooit ooit gespanne	
Selde gespanne of angstig	
Soms gespanne	
Dikwels	
Gewoonlik gespanne	
Uitermatig gespanne, gebruik medikasie	

13. Het u geneesheer enige ander mediese probleme gediagnoseer wat u oefenprogram mag beïnvloed?

Ja **Nee**

14. Indien ja, spesifiseer?

_____

15. VO_{2max} toets

Weerstand	**Hart tempo**	**SBD**	**DBD**	**Borgskaal**
Rus				
50 W				
75 W				
100 W				
125 W				
150 W				
175 W				
200 W				
Herstel				

Appendix D: Physical activity index (PAI)

Physical activity index (PAI)

Mark the appropriate box

Do you participate in sport or any other kind of physical activity or physical recreation on a regular basis?

Yes* 1 No 2

* **If yes,** please complete the table below. Please indicate how tired you get from participating (**intensity**), for how long you participate (**duration**) and how many times you participate per week (**frequency**).

Example:

Walking		3					3					3			

Summer															
Activity	**Intensity**					**Duration**				**Frequency**					
	1 = Not tired	2 = Slightly tired	3 = Tired	4 = Very tired	5 = Exhausted	1 = < 10 min	2 = 10 - 19 min	3 = 20 - 30 min	4 = > 30 min	1 = 1 x month	2 = few times/month	3 = 1 - 2 x week	4 = 3 - 5 x week	5 = Almost daily	
	1	2	3	4	5	1	2	3	4	1	2	3	4	5	
Winter															

(Sharkey, 1997:7,8)

Appendix E: Coronary risk profile (CRP)

Coronary risk profile (CRP)

Risk factors for coronary heart disease

Complete the table below by marking the appropriate space. Read from left to right.

Age	**10 – 20 years**	1	**21 – 30 years**	2	**31 – 40 years**	3	**41 – 50 years**	4	**51 – 60 years**	6	**61+ years**	8	
Hereditary*: Parents and family	No family history of CVD	1	1 with CVD over 60 yrs	2	2 with CVD over 60 yrs	3	1 death from CVD under 60 yrs	4	2 deaths from CVD under 60 yrs	6	3 deaths from CVD under 60 yrs	7	
Weight	5 kg under standard weight	0	Standard weight	1	5 – 10 kg overweight	2	11 – 15 kg overweight	3	16 – 20 kg overweight	5	21+ kg overweight	7	
Smoking	No smoking	0	Occasional cigar/pipe	1	< 10 cigarettes per day	2	11 – 20 cigarettes per day	4	21 – 30 cigarettes per day	6	> 30 cigarettes per day	10	
Exercise	Intensive occupational and recreational exercise	0	Moderate occupational and recreational exercise	1	Sedentary occupational and intensive recreation	2	Sedentary occupation and moderate recreation	4	Sedentary occupation and light recreation	6	Sedentary occupation and no exercise or recreation	8	
Cholesterol	< 5.2 mmol.l^{-1}	1	Don't know	2	5.2 – 6.0 mmol.l^{-1}	3	6.1 – 6.6 mmol.l^{-1}	4	6.7 – 7.3 mmol.l^{-1}	5	7.4+ mmol.l^{-1}	7	
Systolic blood pressure	111 – 130 mm Hg.	0	131 – 140 mm Hg.	1	Don't know	2	141 – 160 mm Hg.	3	161 – 180 mm Hg.	5	> 180 mm Hg.	7	
Diastolic blood pressure	80 – 85 mm Hg.	0	86 – 90 mm Hg.	1	Don't know	2	91 – 95 mm Hg.	4	96 – 100 mm Hg.	7	> 101 mm Hg.	9	
Gender	Female	1	Female over 45 yrs	2	Male	4	Bald male	5	Bald, short male	6	Bald, short, stocky male	7	
Stress	No stress	1	Occasional mild stress	2	Frequent mild stress	3	Frequent moderate stress	4	Frequent high stress	5	Constant high stress	7	
Present CVD symptoms	None	0	Occasional tachycardia and/or irregular rhythm	2	Frequent tachycardia and /or irregular rhythm	4	Dyspnea on exertion	6	Occasional angina	8	Frequent angina****	10	
Past personal history of CVD	Completely benign	0	CVD symptoms not medically confirmed	2	History of CVD symptoms, examined by doctor	4	Mild CVD, no present symptoms	6	CVD under symptoms	8	Hospitalised for CVD	10	
Diabetes	No family history	0	Positive family history	1	Diagnosed pre-diabetic	3	Diabetes: dietary control	5	Diabetes: oral control	7	Diabetes: insulin control	9	
Gout	No family history	0	Family history	1	Elevated uric acid. No symptoms.	2	New onset gout: early detected	3	Repeated chronic gouty attacks	5	Gout with renal and ostea complications	8	

(Bjurstrom & Alexiou, 1978:524)

Appendix F: Quantitive food frequency questionnaire

QAUNTITATIVE FOOD FREQUENCY QUESTIONNAIRE

SUBJECT NO: ....

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

Do you eat maize meal porridge? YES 1 NO2

If YES, what type do you have at home now?

Brand name: .....

Don't know..... 2

Grind self..... 3

If brand name given, do you usually use this brand?

YES 1 NO2 DON'T KNOW 3

Where do you get your maize meal from? (May answer more than one)

Shop	1
Employer	2
Harvest and grind self	3
Other - specify	4
Don't know	5

FOR OFFICE USE

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Maize meal porridge	Stiff ('pap')						e4225 4250	
Maize meal porridge	Soft ('slap pap')						e4225 4250	
Do you pour milk on your soft porridge? YES 1 NO2								
If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? ..... INSTRUCTION: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your soft porridge? YES 1 NO2								
If YES, how much sugar?							9012	
Maize meal porridge	Crumbly (phutu)						e4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella Coarse Fine Rice	Soft						4082	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Do you pour milk on your mabella porridge YES 1 NO2								
If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)?								
INSTRUCTION: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your mabella? YES 1 NO2								
If YES, how much sugar?							9012	
Oats							4032	
Do you pour milk on your oats? YES 1 NO2								
If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)? .....".....								
INSTRUCTION: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your oats? YES 1 NO2								
If YES, how much sugar?							9012	
Breakfast cereals	Brand names of cereals at home now: Don't know.....						4036	
Do you pour milk on your cereal? YES 1 NO2								
If YES, what type of milk (whole fresh, sour, 2 %, fat free, milk blend)?								
INSTRUCTION: Show subject examples.								
If YES, how much milk?								
Do you pour sugar on your cereal? YES 1 NO2								
If YES, how much sugar?							9012	
Stamp	Bought Self ground with fat without fat						4043	
Stamp and beans							A014	
	Are the amounts of stamp and beans the same as in the picture? YES NO If NO, do you use more beans than in the Picture or less? MORE LESS							
Stamp and peanuts							A013	
	Are the amount of stamp and peanuts the same as in the picture? YES NO If NO, do you use more peanuts than in the Picture or less? MORE LESS							
Rice	White Brown Maize rice						4040 4134 4043	
Pastas	Macaroni Spaghetti Other						4062	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
You are being very helpful. Can I now ask you about meat? CHICKEN, MEAT, FISH How many times per day/week do you eat meat, fish or chicken? .....*Xlday* .....Xlweek								
Chicken:	Boiled, nothing added						1521	
	Fried: in butter/crumbs Not coated						1634 1520	
	Roasted, grilled						1520	
	Stewed						1520	
	What vegetables are in the stew?							
	Don't know							
Do you eat chicken skin? ALWAYS 1 SOMETIMES 2NEVER 3								
Chicken bones stew							A003	
Chicken feet	How do you cook it						A004	
							1609	
Chicken offal	How do you cook it?						1610	
Where do you get your MEAT from? (May answer more than 1) Shop, supermarket, plaza *Employer* Slaughter own Gift Other specify: Do not eat red meat								1 2 3 4 5 6
Red meat:	How do you like meat? With fat Fat trimmed							
Beef	Fried - with bone							
	Fried - without bone							
	Stewed - with bone						AO01	
	Stewed - without bone						AO01	
	Grilled - with bone							
	Grilled - without bone							
	Minced						1585	
Mutton	Fried - with bone						1522	
	Fried - without bone						1571	
	Stewed - with bone						1511	
	Stewed - without bone						1511	
	Grilled - with bone							
	Grilled - without bone							
	Minced						1662	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Pork	Fried - with bone							
	Fried - without bone							
	Stewed - with bone							
	Stewed - without bone							
	Grilled - with bone							
	Grilled - without bone							
Beef Offal	Intestines: boiled, nothing added						161	
	Heart						1565	
	Lungs							
	Liver						1515	
	Kidneys						1518	
	Other specify:							
Wors sausage	Fried Grilled						1526	
Bacon							1501	
Cold meats	Polony						1514	
	Ham						1564	
	Vienna's						1531	
	Other specify:							
Canned meat	Bully beef						1535	
	Other specify:							
Meat pie	Home made Bought						1548	
Hamburger	Home made Bought						A015	
Dried beans, peas, lentils (10)	How do you prepare them?							
Soya products e.g. Toppers	Brands at home now Don't know..... Show examples						3527	
Pilchards in tomato chili brine	Whole						2557	
	Mashed with fried onion						AO05	
Fried fish	With batter/ crumbs						2523	
	Without batter/crumbs						2509	
Other canned fish	Tuna							
	Pickled fish Other:						2562	
Fish cakes	Home made (describe) Frozen Bought						2531	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Eggs	Boiled poached Scrambled Fried						1001 1025 1003	
WE NOW COME TO VEGETABLES AND FRUIT How many times per day/week do you eat vegetables? .....Xlday .....Xlweek								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added							8066
	Boiled with potato and onion and fat							AO06
	Fried, nothing added							AO07
	Boiled, then fried with potato, onion							AO06
	Other:							
	Don't know							
Spinach / Other green/ leaf	How do you cook spinach?							
	Boiled, nothing added						8071	
	Boiled fat added						8209	
	Boiled with - onion, tomato & fat						A011	
	-onion, tomato & Potato						8212	
	- with Peanuts							
	Other:							
	Don't know							
Tomato and onion 'gravy'	Home made - with fat - without fat						 A012 A016	
	Canned (Is this the amount of pap you eat? How much more or less						8221	
Pumpkin	How do you cook Pumpkin?							
	Cooked in fat & sugar						A010	
	Boiled, little sugar and fat						AO09	
	Other:							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Carrots	How do you cook carrots?							
	Boiled, sugar & fat						8129	
	With potato/ onion						AO08	
	Raw, salad						8015	
	Other:							
	Don't know							
Mealies / Sweet corn	How do you eat mealies? On cob -with fat -without fat						8033	
	Off cob -with fat -without fat						8261	
Beetroot salad	Home made Bought						8005	
Potatoes	How do you cook potatoes?							
	Boiled/baked - with skin						8046	
	- without skin						8045	
	Mashed						8187	
	Roasted						8189	
	French fries						8048	
	Salad						8236	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked - with skin						8057	
	- without skin						8214	
	Mashed						8058	
	Other:							
	Oon/t know							
Salad vegetables	Raw tomato						8059	
	Lettuce Cucumber						8031 8025	
Other vegetables specify:								
FRUIT: Do you like fruit? YES NO How many times per day/week do you eat fruit? .....xlday .....xlweek								

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Apples/Pears	Fresh						7001	
Pears	Fresh Canned						7053 7054	
Bananas							7009	
Oranges / naartjies							7031	
Grapes							7020	
Peaches	Fresh Canned						7036 7038	
Apricots	Fresh Canned						7003 7004	
Mangoes	Fresh						7026	
Guavas	Fresh Canned						7021 7023	
If subject eats canned fruit: Do you have custard with canned fruit? YES 1 NO2								
Custard	Home made Ultramel						0004	
Wild fruit / berries	Stamvrugte Noen-noem Klappers Maroelas Nastergals Other - specify						7070	
Dried fruit:	Types:							
Other fruit:								
BREAD AND BREAD SPREADS								
Bread Bread rolls	White						4001	
	Brown						4002	
	Whole wheat						4003	
Do you spread anything on the bread? ALWAYS 1 SOMETIMES 2 NEVER 3 If YES, what do you spread?								
Margarine	What brand do you have at home now? ..... Don't know Show examples						6508 6521	
Butter	What brand do you have at home now? ..... Home made Don't know						6502	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Peanut butter							6509	
Jam/syrup/ honey							9008	
Marmite/Fray Bentos etc.							9501	
Fish/meat paste							1512	
Cheese	Type:						0010	
Atchar							3004	
Polony							1514	
Other spreads: specify								
Dumplings							4001	
Vetkoek							4057	
Provita, crackers etc.								
FATS: What fats do YOU use and where do you use them?								
Margarine	Where used: on bread							
	with vegetables.. Number of spoons ..... /number in family							
Butter	on bread with vegetables.. Number of spoons ..... Inumber in family							
Holsum / vegetable fat	Where used: Number of spoons ..... Number in family						6508	
Oil	Where used: Number of spoons ..... Number in family						6510	
Dripping	Where used: Number of spoons ..... Number in family							
Mixed fat (makhuru)	Where used: Number of spoons ..... Number in family							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
Mayonnaise salad dressing	Number of spoons ..... *Number* in family						6573	
Cream	Fresh/Long life *canned* Orlev whip						6503	
DRINKS:								
Tea							9514	
Sugar/cup tea							9012	
Milk/ cup tea	What type of milk do you use in tea?							
	Fresh/ long life whole						0006	
	Fresh/ long life 2%							
	Fresh/ long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	
	Evaporated milk						0003	
	None							
Coffee								
Sugar / cup coffee							9012	
Milk / cup coffee	What type of milk do you use in coffee?							
	Fresh/long life whole						0006	
	Fresh/ long life 2 %							
	Fresh/ long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
	Condensed milk						0002	
	Evaporated milk						0003	
	None							
Milk as such	What type of milk do you drink as such?							
	Fresh/ long life whole						0006	
	Fresh / long life 2 %							
	Fresh/long life fat free						0072	
	Sour / Maas						0006	
	Buttermilk						0001	
	Whole milk powder Brand						0006	
	Skimmed milk powder Brand						0072	
	Milk blend Brand						0068	
Milk drinks Brand	Nestle Milo Other						0023	
Yoghurt	Drinking yoghurt Thick yogurt						0044 0020	
Squash	Sweeto Sixo Oros/Lecol - with sugar - artificial sweetener Kool Aid Other						9013 9013 9002 9013 9002	
Fruit juice	Fresh/Liquifrui/Ceres Tropica Concentrates e.g. Halls Nectars Flavour							
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Home brew							9516	
Beer							9506	
Spirits							9510	
Wine red							9508	
Wine white							9518	
liqueur							9517	
Other: specify								

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/ DAY
			Per day	Per week	Per month	Seldom Never		
SNACKS AND SWEETS:								
Potato crisps							4275	
Cheese curls Niknaks etc.							4067	
Peanuts	Raw Roasted						6001 6007	
Raisins							7022	
Peanuts and raisins								
Chocolates	Name						9024	
Candies	Sugars, gums, hard sweets						9009	
Sweets	Toffees, fudge, caramels						9014	
Biscuits	Type							
Cakes & tarts	Type							
Scones							4029	
Rusks							4160	
Savouries	Sausage rolls Samoosas Biscuits e.g. Bacon kips Other						1534 4196 4162	
PUDDINGS:								
Canned fruit	Type							
Jelly							9004	
Custard	Homemade Ultramel						0004	
Baked pudding							4181	
Instant pudding							4066	
Ice cream Sorbet							6507 6516	
Other: specify								
SAUCES/ GRAVIES/ CONDIMENTS:								
Atchar							3004	
Tomato sauce Worcester sauce							3027	
Chutney							9524	
Pickles							8176	
Packets of soups							3046	
Others:								

(MacIntyre et al., 2001:63-71)