Total plasma homocysteine, vitamin supplementation and physical conditioning in men with coronary risk factors

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2 Timothy 1:7

God did not give me a spirit of timidity, but a spirit of power, of love and of self-discipline. Thanks be to God! He gives me the victory through my Lord Jesus Christ (1 Cor. 15:57).

DECLARATION

AUTHORS' CONTRIBUTION

The study reported in this dissertation was planned and executed by a team of researchers. The contribution of each of the researchers is depicted in the table hereafter. Also included in this section is a statement from the co-authors confirming their individual roles in the study and giving their permission that the articles may be part of the dissertation.

NAME	ROLE IN THE STUDY
Ms. S.J. Herbst B.Sc., Hons. (Biokineticist)	Together with S.J. Moss and R. Nel were responsible for the execution of the total study, data collection, exercise prescription, data management and statistical analyses. Main author of the paper.
Dr. S.J. Moss Ph.D. (Biokineticist)	Project co-ordinator and scientist; responsible for all aspects of the study. Significant contribution towards writing of the paper. Supervisor of S.J. Herbst and R. Nel.
Prof. W Oosthuizen Ph.D. (Nutritionist)	Co-supervisor of S.J. Herbst. Significant contribution toward writing of the paper.
Ms. R. Nel B.Sc., Hons (Biokineticist)	Together with S.J. Moss and S.J. Herbst responsible for the execution of the total study, data collection, exercise prescription, data management and statistical analyses. Fellow M.Sc student.

The dissertation is submitted in article format, as approved by the Senate of North-West University (Potchefstroom Campus) and, therefore, includes a review (Chapter 2) and research article (Chapter 3) which will later be presented to appropriate and accredited journals. Chapter 2, a literature review, in the form of a review article on homocysteine and physical activity by Herbst, S.J., Moss, S.J. & Oosthuizen W. will be presented for publication in Sports Medicine journal. The chapter firstly

introduced the reader to the literature format of this article by means of an introduction. The introduction is followed by a section comprising of homocysteine as a cardiovascular heart disease risk factor and different treatments, especially physical activity, focussing on men in particular. This is followed by a summary of studies investigating the association of physical activity and homocysteine concentrations. This chapter was concluded by focussing on physical activity as a possible preventive and therapeutic measure to reduce and remedy the occurrence of elevated homocysteine concentrations.

The research article entitled "Total plasma homocysteine, vitamin supplementation and physical conditioning in men with coronary risk factors" by Herbst, S.J., Moss, S.J., Nel R. & Oosthuizen W. wil be presented for publication to the American Journal of Preventive Medicine. The main purpose was to determine whether significant changes in homocysteine concentrations will be observed when men aged 45-50 years are submitted to a physical conditioning programme and vitamin intervention. The above mentioned article will be written according to the authors instructions, consisting of an introduction, problemstatement with the resulting research questions and purposes of the study. Research methods (subjects, measurement procedures and data analysis) were described, after which the results were presented and discussed with a conclusions and implications.

I declare that I have approved the above mentioned articles and that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc. dissertation of S.J. Herbst.

_____ _____

Dr. S.J. Moss Prof. W. Oosthuizen

_____

Ms. R. Nel

SUMMARY

Motivation:

Cardiovascular disease (CVD) is one of the leading causes of mortality and morbidity in South Africa and worldwide. Various investigations have confirmed the hypothesis that elevated plasma homocysteine (Hcy) levels may be linked to vascular disease, and it has become clear that hyperhomocysteinemia is an independent risk factor for atherosclerosis and atherothrombosis. Extensive research on the influence of vitamin supplementation leading to the lowering of homocysteine levels has been done, but extensive research on the effect of physical activity on high homocysteine levels is lacking. The interaction of vitamin supplementation in combination with physical activity has also not been investigated. If a conditioning exercise programme can demonstrate a lowering effect on elevated homocysteine levels, it will confirm the importance of physical activity as a less expensive alternative for a better lifestyle that can also continue to lower morbidity and mortality rates.

Objective:

This study examined the effect of a conditioning program, vitamin supplement and a combination of both on Hcy levels in men with coronary heart disease risk factors.

Methods:

In a randomized, placebo-controlled, blinded crossover study, 84 men matched for physical activity (PA) levels, age and risk factors were randomly assigned to one of 4 groups [A = physical conditioning, 20-30 min; 70-80% (THR), B = physical conditioning + supplement, C = supplement (12,5 µg vitamin B_{12} ; 200 µg folic acid) or D = control]. Groups A, B, and C were crossed over according to the Latin square design. Total plasma homocysteine, maximal oxygen consumption (VO_{2max}) and body composition (BMI & Fat %) were measured before and after each 12-week intervention period. A 6-week washout period separated the crossovers.

Results:

The experimental and control groups presented similar baseline characteristics and the profile analysis of the VO_2max values and Hcy concentrations indicated positive results (multivariate p-value <0.0001), due to the fact that the four groups repeated measurements, presented different patterns. A phase effect for the VO_2max values and a phase and interaction effect for the Hcy concentrations were indicated, though all the subjects were requested to maintain their normal daily routine (eating pattern, PA levels and alcohol consumption) for the duration of the study. The lack of compliance to the conditioning programme makes it impossible to draw conclusions for VO_2max values. The poor compliance lead to a small sample size that eventually leads to less statistical power.

Conclusion:

This study found that a 12-week conditioning programme had no effect on Hcy concentrations. The results of this study make it impossible, due to poor compliance, to suggest that the effect of increased PA on homocysteine may play an important role in the prevention and treatment of CVD. It is, therefore, recommended that more studies should be conducted to further investigate the effect of PA and vitamin supplements on tHcy levels.

Keywords:

Homocysteine, Physical conditioning, Coronary risk factors, VO_2max, cholesterol profile and Vitamins

OPSOMMING

Motivering:

Kardiovaskulêre siektes (KVS) in een van die hoofoorsake van sterftes in Suid-Afrika, asook wêreldwyd. Verskeie intervensies het bewys, dat verhoogde plasma Homocysteine (Hcy) vlakke geassosieer word met vaskulêre siektes, en dat Hiperhomocysteinemia 'n onafhanklike risiko faktor is vir aterosklerose and aterotrombose. Daar bestaan reeds baie navorsing oor die effek van vitamien supplementasie op Hcy, maar beperkte navorsing is beskikbaar oor die effek van oefening op Hcy. Die interaksie van vitamien supplementasie in kombinasie met oefening op Hcy is ook nog nie nagevors nie. Indien 'n kondisionering oefen program 'n verlagende effek op Hcy het, bevestig dit die belangrikheid van fisieke aktiwiteit as 'n goedkoper alternatief vir 'n beter leefstyl en kan dit selfs bydrae tot verlaagde morbiditeit en mortaliteit syfers

Doel:

Om die effek van 'n kondisionerings program, vitamien supplementasie en 'n kombinasie van beide op Hcy vlakke in mans met koronêre risiko faktore te bepaal.

Metodes:

In 'n ewekansige, plasebo gekontroleerde, blinde oorkruis studie van 84 mans met dieselfde fisieke aktiwiteit vlakke, ouderdom en risiko faktore is ewekansig verdeel in 4 groepe [A = fisieke kondisionering, 20-30 min 70-80% THT, B = fisieke kondisionering + supplement, C = supplement (12,5 µg vitamien B_{12} , 200 µg folien suur) of D = kontrole]. Groep A, B, C en D is oorkruis omgeruil t.o.v die Latynse Vierkant ontwerp. Totale plasma Hcy, maksimale suurstof verbruik (VO_2max) en liggaam samestelling (LMI & Vet %) was gemeet voor en na elke 12-week intervensie periode. n' 6-weke uitwas periode het na elke oorkruis gevolg.

Resultate:

Die eksperimentele en kontrole groepe het dieselfde basislyn karakteristieke getoon en die profiel analises vir VO_2maks waardes en Hcy konsentrasies het positiewe resultate aangedui (multivariate p-value <0.0001), omrede die vier groepe se herhaalde metings, verskillend patrone getoon het. Die VO_2maks waardes het 'n fase effek en interaksie effek is gevind vir Hcy konsentrasies, alhoewel al die proefpersone gevra is om met hul normale daaglikse roetine voort te gaan (eet patroon, fisieke aktiwiteit en alkohol inname) vir die duur van die studie. Die gebrek aan deelname aan die kondisionering program maak dit onmoontlik om tot 'n gevolgtrekking te kom ten opsigte van VO_2maks waardes. Die gebrek aan deelname lei tot 'n verlaging in statistiese betekenisvolheid as gevolg van 'n kleiner hoeveelheid proefpersone.

Gevolgtrekking:

Die studie het gevind dat 'n 12-week kondisionering program geen effek gehad het op Hcy konsentrasies nie. Die resultate maak dit onmoontlik om voor te stel dat die effek van verhoogde funksionele kapasiteit (VO_2maks) op Hcy konsentrasies 'n belangrike rol speel in voorkoming en behandeling van KVS. Daarom word voorgestel dat meer studies gedoen word om die effek van fisieke aktiwiteit en vitamien supplementasie op Hcy konsentrasies na te vors.

Sleutelwoorde:

Homocysteine, Fisieke kondisionering, Koronêre risiko faktor, VO_2maks, cholesterol profiel and Vitamiene

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LIST OF ABREVIATIONS

A

A = Aerobic

B

b = Beginning

BMI = Body mass index (kg/m^2)

C

CAD = Cardio Arterial disease

CVD = Cardio vascular disease

CBS = Cystathionine β-synthase

CRP = Coronary Risk Profile

E

EC = Endothelial cell

EDRF = Endothelial derived relaxant factor

Enos = endothelial nitric oxide synthase

E = End

En = Endurance

Exe = Exercise

G

GLUT-1 = glucose transporter protein

H

Hcy = Homocysteine

HyperHcy = Hyperhomocysteinemia

HIHF = High intensity- high frequency

HILF = High intensity- low frequency

h = hour

HTG = High-training group

I

INS = plasma insulin

Inc = Included

K

Kg = kilogram

L

LDL = Low-density lipoprotein

Lp(a) = Lipoprotein (a)

LDL-C = Low-density lipoprotein cholesterol

LTG = Low-training group

M

MTHFR = methylentetrahydrofolate reductase

MILF = Moderate intensity - low frequency

MIMF = Moderate intensity – moderate frequency

Min = minute

m^2 = square meter

N

NO = Nitric Oxide

NR = Not reported

P

PAI = Physical Activity Index

PA = Physical Activity

PC = Physical Conditioning

R

R = Randomized

R/C = Randomized controlled

S

SMC = Smooth muscle cell

Signf = Significant

Supp = Supplements

T

TAS = total antioxidant status

THcy = Total plasma homocysteine

TC = Total cholesterol

THR = Target Heart Rate

V

VCAM-1 = vascular cell adhesion molecule

$VB_{12/6}$ = Vitamin B_{12} or Vitamin B_6

VO_{2max} = Maximal oxygen consumption

W

W = Watt

X

X = Excluded

LIST OF SYMBOLS

[ ] = Concentration

↑ = Increase

↓ = Decrease

↔ = Unchanged

μ = micro

β = Beta

Δ = Delta (Change from baseline to end, adjusted for baseline)

CHAPTER 1: PROBLEM STATEMENT AND AIM OF THE STUDY

INTRODUCTION

McCully (1969:118) made a clinical observation linking elevated plasma homocysteine (Hcy) concentrations with cardio vascular disease (CVD). Subsequent investigation has confirmed McCully's hypothesis and it has become clear that hyperhomocysteinemia may be an independent risk factor for atherosclerosis and atherothrombosis (Ueland & Refsum, 1989:489). According to Stampfer *et al.* (1992:878), men with plasma Hcy concentrations that were 12% above the desirable upper limit had approximately a threefold increase in the risk of myocardial infarction, as compared with those with lower concentrations , even after correction for other risk factors like hypertension, smoking, hyperlipidemia and diabetes.

An increase of 5 μmol/L in the plasma Hcy concentration raises the risk of CAD by as much as an increase of 0.52 mmol/L in the cholesterol concentration (Boushey *et al.*, 1995:1050). Atherosclerosis is a condition that, among others, is associated with hyperlipidemia, because it is characterized by the formation of lipid-enriched lesions in the early phase, and eventually by fibrosis in the walls of arteries.

Atherosclerosis is a multi-factorial condition and the complex interaction between these factors modifies the atherogenic effects of lipids (Delport, 1998:6). The process of atherosclerosis is summarised in terms of the "response to injury" and the "lipid infiltration" hypothesis (Thompson & Smith, 1989:90). According to the "response to injury" hypothesis, morphologic changes are observed in endothelial and sub-endothelial layers of arterial walls. These changes are ascribed to an inflammatory response to certain stimuli, i.e. changes in blood flow as observed with turbulence or stagnation and other conditions such as anoxia, hypertension, hypercholesterolemia (Schwartz *et al.*, 1991:14) and hyperhomocysteinemia (Harker *et al.*, 1974:540).

Low-density lipoprotein (LDL) cholesterol accumulates in the intima due to influx of LDL-cholesterol through the injured endothelium (Thompson & Smith, 1989:89). The lipid-infiltration hypothesis (Vasile *et al.*, 1983:1677) postulates that increased circulating levels of LDL-cholesterol lead to an increase in transcytosis of LDL-cholesterol to the intima and the endothelial injury is secondary to sub-endothelial events (Simionescu *et al.,* 1990:13). Lipoprotein (a) [Lp(a)] is a circulating lipoprotein that is structurally related to LDL-cholesterol (Gaubatz *et al.*, 1987:70), may also cross the vascular endothelial layer (Beisigel *et al.*, 1990:178) and may contribute to the disease process. Hcy has prothrombotic and atherogenic properties that may explain the increased risk of CVD (Zamani, 2002).

Experimental evidence suggests that endothelial dysfunction is the major mechanism by which Hcy exerts its deleterious effect. Although the exact mechanism of this endothelial dysfunction is unknown, Hcy probably leads to oxidative damage of the endothelial cell by the reactive oxygen radicals that are produced during auto-oxidization of Hcy in plasma (Welch & Loscalzo, 1998:1042). Hcy is an amino acid intermediate formed during the metabolism of methionine, an essential amino acid derived from dietary protein. It is metabolized by one of two pathways: remethylation and transsulfuration. In the remethylation cycle, acquiring a methyl group in a reaction catalyzed by the vitamin B_{12}-dependent enzyme methionine synthase salvages homocysteine (Ueland *et al.,* 1993:1778).

Under conditions in which an excess of methionine is present or cysteine synthesis is required, Hcy enters the transsulfuration pathway. In this pathway, Hcy condenses with serine to form cystathionine in a reaction catalyzed by the vitamin B_6-dependent enzyme cystathionine β-synthase (Ueland *et al.,* 1993:1778). Cystathionine is subsequently hydrolyzed to form cysteine, which may in turn be incorporated into glutathione or further metabolized to sulfate and excreted in the urine (Finkelstein *et al.,* 1988:11752).

Causes of hyperhomocysteinemia include: enzymatic defects in the metabolic pathway, dietary deficiency of folic acid, vitamin B_{12} and vitamin B_6, renal failure (Huhberg *et al.*, 1993:234), liver disorders (Ueland & Refsum, 1989:479), hormonal factors such as hypothyroidism (McCully, 1996:389), malignancy including breast, ovarian or pancreatic cancers, drugs, toxins (methotrexate, phenytoin and theophyline) and smoking (Welch & Losculzo, 1998:1050). Recommended normal plasma Hcy concentrations are 5-15 μmol/L (Zamani, 2002). The treatment of hyperhomocysteinemia varies with the underlying cause but generally involves supplementation with folic acid, vitamin B_{12} and pyridoxine (vitamin B_6) (Den Heijer *et al.*, 1998:359).

Folic acid supplementation decreases Hcy concentration, but co-administration of vitamin B_{12} may be needed to prevent irreversible neurological damage (Moustapha *et al.*, 1999). Among the vitamin studies, folic acid has the dominant Hcy-lowering effect and an inverse relation between serum Hcy and folic acid has been recognized (Clarke & Armitage, 2000:342). A diet rich in fruits, vegetables and low fat dairy products and reduced in saturated and total fat can also lower serum Hcy concentrations (Apple *et al.,* 2000:852). Extensive research on the influence of vitamin supplementation leading to the lowering of Hcy concentrations has been done, but extensive research on the effect of physical activity on high Hcy concentrations is lacking.

A meta-analysis of epidemiological studies has documented the role of physical activity in moderating the risk of CVD (Berlin *et al.*, 1990). A limited number of studies have investigated the effect of physical activity on plasma Hcy concentrations (Ali et al., 1998; Duncan *et al.*, 2004) with contradicting results. These results varied from acute PA interventions to training interventions (Ali et al., 1998). Wright *et al.* (1998) concluded that acute exercise had no effect on Hcy in young healthy men aged 24 to 39 years.

Research on the effect of physical activity on Hcy concentrations are lacking together with the interaction of vitamin supplementation in combination with physical activity. A recently performed meta-analysis of the published controlled clinical trials indicated (Clarke *et al.*, 2000) that vitamin supplementation reduces Hcy concentrations. The question to be answered in this research project is whether a 12 week conditioning programme, a vitamin B_{12} and folic acid supplement or a combination of physical activity, vitamin B_{12} and folic acid supplementation will result in a decrease of plasma Hcy concentrations in men between the ages of 45 and 60 years with three or more coronary heart disease (CHD) risk factors.

AIM AND OBJECTIVES

Main aim

The main aim of this dissertation is to investigate the effect of a conditioning programme, a vitamin supplement and a combination of both on plasma Hcy concentrations in men aged 45 to 60 years with three or more CHD risk factors in a randomised, placebo-controlled, blinded crossover study.

Objectives

The objectives of this study are:

- To determine the effect of a PA conditioning programme on Hcy concentrations in men aged 45-60 years with three or more CHD risk factors.

- To determine the effect of vitamin B_{12} and folate supplementation on Hcy concentrations in men aged 45-60 years with three or more CHD risk factors.
- To determine the effect of a combination of a PA conditioning programme in combination with vitamin supplementation on Hcy concentrations in men aged 45-60 years with three or more CHD risk factors.

HYPOTHESIS

The following hypotheses were postulated:

- A PA conditioning programme leads to a decrease in plasma Hcy concentrations in men aged 45-60 years with three or more CHD risk factors.
- Vitamin B_{12} and folate supplementation leads to a decrease in plasma Hcy concentrations in men aged 45-60 years with three or more CHD risk factors.
- The combination of a PA intervention and vitamin supplementation will decrease Hcy significantly more than each intervention individually.

STRUCTURE OF THIS DISSERTATION

This dissertation is presented in article format and consists of three chapters, namely an introduction (Chapter 1), a review article (Chapter 2) and a research article (Chapter 3). In the introduction, the motivation, objectives and hypotheses are presented. The review article (Chapter 2) is based on Hcy as a CVD risk factor and physical activity as a treatment of elevated Hcy concentrations. The research article (Chapter 3) investigates the effect of a conditioning programme, a vitamin supplement and a combination of both on plasma Hcy concentrations in men with CHD risk factors. From the literature study it became clear that more information on Hcy as a CVD risk factor and physical activity as a treatment of elevated Hcy concentrations is needed. A schematic representation of the structure of the dissertation is shown in Figure 1.

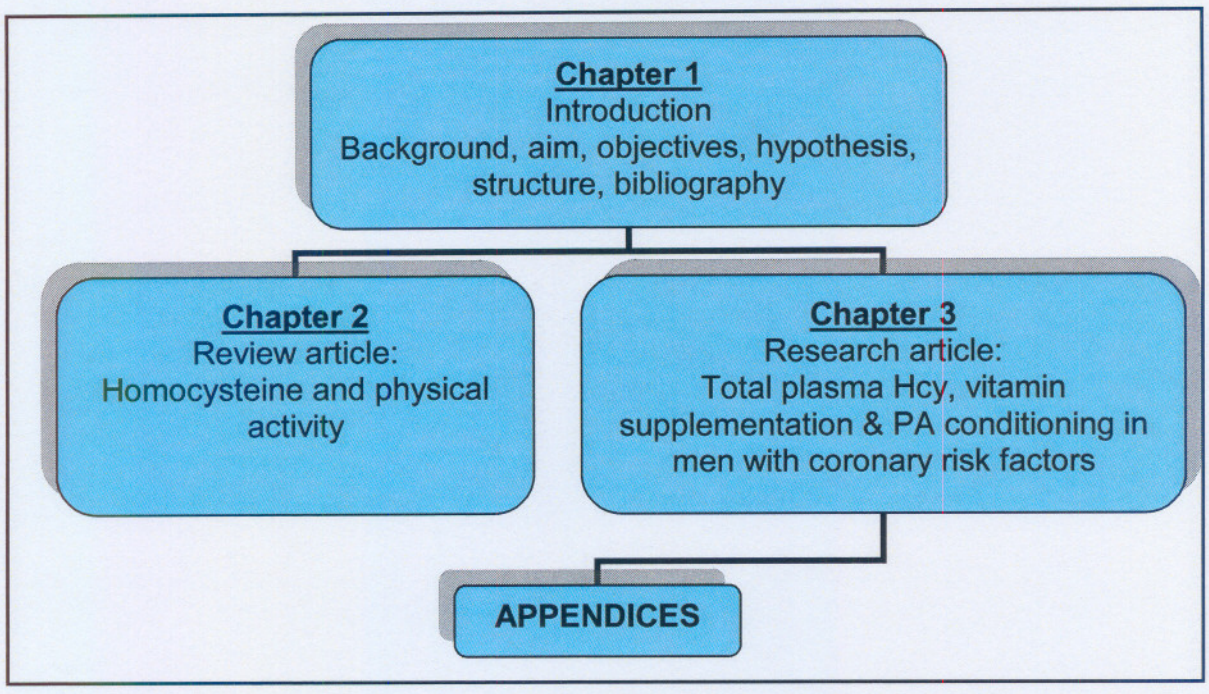

Figure 1: A schematic representation or the structure of this dissertation

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CHAPTER 2: Homocysteine: Food or Physical Activity?

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ABSTRACT

Research hypothesizes that elevated homocysteine (Hcy) concentrations may be linked to serebo- and cardiovascular disease. This foreshadows an increase in the rates of morbidity and mortality from vascular diseases. Elevation in tHcy are due to rare enzymatic defects and severe genetic alterations in enzymes which are important in the metabolism of Hcy. The treatment of hyper-Hcy varies with the underlying causes, but generally involves supplementation with folic acid, vitamin B_{12} and pyridoxine. Published data on Hcy and physical activity are rare and the existing data are equivocal and do not permit general conclusions. This article aims to provide a review on the possible role physical activity may play in reducing Hcy and the risks associated with cardiovascular disease (CVD), one of the most common health problems world wide.

Key words: Homocysteine, Physical activity, Cardiovascular disease, VO_2max and nutrition, vitamin supplementation.

Introduction

According to McCully (1969), high concentrations of homocysteine (Hcy) have been associated with cardio vascular disease (CVD). Not all the studies supports a link between elevated Hcy concentrations and arthrosclerosis (Nygard *et al.*, 1997; Choy *et al.*, 2000 & Thomas *et al.*, 2003). Taylor *et al.* (1999) reported that an increase of 1 µmol/L total plasma homocysteine (tHcy) concentration can be associated with a 5.6% increased possibility of death from vascular disease. Hcy has prothrombotic and artherogenic properties that may explain the increased risk of vascular diseases.

Factors contributing to elevated Hcy can be categorized into three classes: (1) enzymatic defects in the metabolic pathway (Carson *et al.,* 1962), (2) dietary deficiency (Lee *et al.* (2004), and (3) other causes of hyperhomocysteinemia. Other causes includes, physiological determinants(Mudd *et al.,* 1995), lifestyle (Stolzenberg-Solomon *et al.* (1999), renal failure (Norlund *et al.*, 1998), hormonal factors (hypothyroidism) (Welch *et al.* (1998), breast, ovarian or pancreatic cancer (McCulley *et al.* (1996) and drugs and toxins (Ubbink *et al.* (1996),

The treatment of hyper-Hcy varies with the underlying causes but generally involves supplementation with folic acid, vitamin B_{12} and pyridoxine (vitamin B_6) (den Heijer *et al.*, 1998). A recently performed meta-analysis of the published controlled clinical trials (Weiss *et al.*, 2002) indicated that folic acid supplementation reduces Hcy concentrations by 25% with similar effects in a daily dose range of 0.5-5 mg. Hyperhomocysteinemia may also be treated through dietary intervention or physical activity. Physical activity is known to modify risk factors and an epidemiological study (Nygard *et al.,* 1995) found an inverse relationship with Hcy.

According to Franklin *et al.* (1999), regular exercise has favorable effects on the progression of coronary atherosclerosis and mortality in CVD prevention. Increased physical activity modifies known risk factors for CVD, as it decreases blood pressure, serum cholesterol levels, plasma insulin (INS), and increases

insulin action (Franklin *et al.*, 1999) and cardiorespiratory fitness (VO_2max) (Blair *et al.*, 1995). These alterations are associated with reduction in CVD morbidity and mortality (Paffenbarger *et al.*, 1993). Although habitual physical activity levels were inversely related to Hcy in an epidemiological study (Nygard *et al.*, 1995), equivocal results have been obtained from studies examining the effects of acute and chronic exercise on Hcy (Ali *et al.*, 1998).

The mechanisms underlying the protective effect of physical activity on CVD are still unclear. Physical activity has a direct effect on the heart, it increases myocardial oxygen supply that decreases oxygen demand and improves myocardial contraction and electrical impulse stability (Thompson *et al.*, 2001). Physical activity also increases the diameter and dilatory capacity of coronary arteries, which increases collateral artery formation and reduces rates of progression of atherosclerosis (Thompson *et al.*, 2001). The objective of this review article is to evaluate data on exercise training with reference to improving health by lowering Hcy concentrations. Hypothesis 1, an active lifestyle leads to decrease in Hcy concentrations and hypothesis 2, exercise interventions acute/chronic decreased Hcy concentrations.

The effect of exercise on homocysteine concentrations

The ultimate purpose of applied health research is to improve health care. In this review all the evidence of acute and chronic interventions studies regarding exercise and Hcy, are presented. Published data on Hcy and physical activity are rare and the existing data are equivocal and do not permit general conclusions. A literature search were conducted on Medline and Sport Discus by using the keywords like 'homocysteine, exercise, exercise therapy, physical fitness and physical activity' from 1998 to December 2005. In addition references were hand-searched from original reviews and contents pages from journals.

Acute exercise

The scientific evidence for the effect of physical activity on Hcy concentrations will now be discussed in more detail. Main findings from seven studies that have examined the relationship between acute physical activity and Hcy concentrations in males are summarized in Table 1. None of the studies compared the exercise intervention with a no-exercise control group. The sample size of the studies ranged from 20-100 and three of the studies included less than 25 subjects, which leads to reduced statistical power. According to power calculations, 100 subjects are needed to obtain 80% power with 5% significance in detecting a change of 1 µmol/L in Hcy concentration. An increase of 1 µmol/L is associated with a 5.6% increased possibility of death from vascular disease (Taylor *et al.*, 1999). Comparability between the studies is also limited due to the fact that three of the studies used subjects between the ages of 21-45 years and one of the studies used subjects between the ages of 55-57 years. It is known that age has an influence on Hcy concentrations. All of the studies used aerobic exercise interventions, where three used cycle ergo meters (Wright *et al.*, 1998 & DeCrée *et al.*, 2000) and the other four used running (Herrmann *et al.*, 2003 & König *et al.*, 2003).

The duration of the interventions was 30 minutes (Wright *et al.*, 1998), 60 minutes (De Crée *et al.*, 2000), 67 minutes (König *et al.*, 2003) and until exhaustion (Gaume *et al.* (2005) respectively. A major problem regarding acute exercise is that the studies have to indicate that the values presented were corrected for exercise induced shifts in plasma volume. The blood samples have to be analyzed for haematocrit, haemoglobin and albumin. According to Wright *et al.* (1998) and DeCrée *et al.* (2000), exercise had no effect on Hcy concentrations after an acute exercise session. A decrease in Hcy concentrations after exercise was found by Gaume *et al.* (2005). Herrmann *et al.* (2003) indicated an increase in Hcy concentrations for up to twenty-four hours after acute endurance exercise. It is evident that further research is necessary to determine the exact contribution of acute exercise on Hcy concentrations.

Table 1: A summary of studies investigating the effect of acute exercise on Hcy

Author(s) and year	Subject (n)	Study design	Age (yr)	Subject characteristics	Intervention	Outcomes (Major results)	Authors' conclusion(s)
Wright *et al.*, 1998	20	Intervention with no control	24-39	Active normal healthy males, exe 3 times weekly for 30 minutes. Initial Hcy [] = 10.4±3.2 µmol/L	A exe, cycling, 30 minutes at 70% MHR	↔ (corrected for plasma volume)	Moderate acute exe have no effect on Hcy in young healthy men
De Crée *et al.*, 2000	7	R/C cross over (NO inhalation controlled compared to no-control of NO inhalation)	21.6 ± 1.3	Moderately A trained males. X cyclists, no supp, family history of CVD, hypertension or diabetes. No control for caloric intake 1) Control NO intake 2) No NO control. Initial Hcy [] = 10.89±2.05 µmol/L	A; Warm-up on cycling for 1 hour at 60% VO_{2max}:	↔ when not controlled for NO (corrected for plasma volume)	Plasma Hcy is not affected by acute sub maximal exe
Herrmann *et al.*, 2003	100	Intervention with no control	37(33 - 45)	Male (87), female (13), 100 km run & marathon runners; mountain biking. Initial Hcy [] overall = 9.7±2.6 µmol/L; marathon = 9.8±2.4 µmol/L; 100 km run = 10.2±3.6 µmol/L; mountain biking = 9.1±2.2 µmol/L.	A En training; Marathon running (n=46), 100 km run (n=12) and mountain biking (n=42). Blood samples taken before, 15min, 3h and 24h after race.	**Overall**: ↑23% (15min), ↑19% (3h), ↑33% (24h). **Marathon**: ↑3h (15min), ↑ (3h), ↑ (24h). **100km run**:↔ **Mountain biking**: ↔ Change in Hcy correlated negatively with duration of exercise. (not corrected for plasma volume)	Acute End exe ↑Hcy

Table 1: continued

Author(s) and year	Subject (n)	Study design	Age (yr)	Subject characteristics	Intervention	Outcomes (Major results)	Authors' conclusion(s)
König *et al.*, 2003	39	Intervention with no control	27.1 ± 5.3	Well-trained male tri-athletes (VO_{2max} > 55 ml/kg/min), no supp. HTG: 14.9h training/wk (n= 9) LTG: 9.1h training/wk (n=9). Initial Hcy [] = 12.4±2.00 µmol/L	28 day En training regimen preceded acute exe intervention Sprint triathlon=; Swimming (400m), bicycle (25km), long-distance run 4km, mean duration=67.1 ± 4.6 min.	A signf Hcy ↑ 1h (12%) and 24h (11%) after training, largest ↑ Hcy in the LTG. (corrected for plasma volume)	Acute exe ↑ Hcy
Gaume *et al.*, 2005	24	Intervention with no control	Group A 56.23 ± 0.9 Group B 52.33 ± 2.45	Inc; male, nonsmokers & medication free. Group A: Untrained (n=12) Group B: Trained (En) (n=12). Initial Hcy [] trained = 9.79±0.4 µmol/L; initial Hcy [] untrained = 7.48±0.4 µmol/L	Direct VO_{2max} protocol Only trained men performed the intervention.	Baseline: Trained < untrained Group B: A ↓ Hcy (10.7%) (Return to baseline after 15min recovery)	A exe ↓ Hcy

A = Aerobic; CVD = Cardio vascular disease; En = Endurance; Exe = Exercise; HIHF = High intensity- high frequency; HILF = High intensity- low frequency; h = hour; HTG = High-training group; Inc = Included; LTG = Low-training group; MILF = Moderate intensity - low frequency; MIMF = Moderate intensity – moderate frequency; min = minute; NO = Nitric Oxide; Hcy = Plasma Homocysteine; R/C = Randomized controlled; Signf = Significant; supp = supplements; VO_{2max} = Maximal oxygen consumption; wk = weekly; X = Excluded; yr = years; ↑ = increase; ↓ = decrease; ↔ = unchanged.

The effect of chronic exercise training interventions on Hcy concentrations is summarized in Table 2. Similarly to the report of the acute studies, the included studies in Table 2 also had problems with comparability due to the study designs used. Two of the four studies were randomized controlled trails (De Jong *et al.*, 2001 & Duncan *et al.*, 2004). Two of the studies included less than 85 subjects or the studies that included a larger sample size divided them into smaller groups, which led to reduced statistical power. The high drop-out rate of the subjects plays a important role in the results obtained by the studies (Ali et al. 1998; De Jong *et al.*, 2001; König *et al.*, 2003 & Duncan *et al.*, 2004). Three studies included subjects over the age of 60 years, one study included subjects of the ages 48.9 ± 8.4 years and the other one included subjects of the ages 27.1 ± 5.3 years, this enhances the comparability problem.

According to the inclusion criteria of the studies, three of the studies used unhealthy, sedentary subjects and the other two studies used normal healthy subjects. Training prescription regarding the type of exercise included aerobic and endurance training and the duration of the exercise sessions ranged from 12 weeks (Ali et al. 1998) to 12 months (König *et al.*, 2003), respectively. The frequency varied between 2/week (moderate) and 5-7/week (high).

According to Ali *et al.* (1998) cardiac rehabilitation and exercise training may significantly decrease Hcy concentrations in normolipidemic CAD subjects with hyperhomocysteinemia. Although Duncan *et al.* (2004) found a decrease in Hcy concentrations after six months of brisk walking, the conclusion that was drawn from the study indicated that higher intensity exercise leads to elevated serum Hcy concentrations. According to de Jong *et al.* (2001) no significant change was found in Hcy concentrations on any of the indexes of extremely low intensity, all-round exercise. No conclusion can be drawn from these studies due to controversy that exists, the poor quality and the limited number of studies that have been published. More randomized, controlled studies focusing on efficacy, mechanism and effectiveness are recommended on Hcy.

Table 2: A summary of studies investigating the effect of chronic exercise interventions on Hcy

Author(s) and year	Subject (n)	Study design	Age (yr)	Subject characteristics	Intervention	Outcomes (Major results)	Authors' conclusion(s)
Ali *et al.*, 1998	65	Intervention with no control	63 ± 10	Patients with CAD with normal lipid levels. Men (n=65), women (n=11). Nonsmokers for ≥ 3 months, serum creatinine ≤ 2 mg/dl, no lipid medication & supp. Initial Hcy [] = 11.4±4.3 µmol/L	12 weeks, 36 exe sessions, aerobic exe	Total group: ↔ Subject with hyper-Hcy (n=11): ↓ (12%)	The 12% ↓ in Hcy, may lead to a ↓ of 20-30% in CAD risk Cardiac rehabilitation and exe training may signf reduce Hcy levels in normolipidemic CAD subjects with hyper-Hcy
de Jong *et al.*, 2001	165 (exe group = 31)	R/C	≥ 70	Inc; males & females, requirement of health care, no regular exe, BMI ≤ 25, ability to understand the procedures, no vitamin supp, mean subjective health score 6.9 as rated on 10-point scale. Initial Hcy [] = 16.8±7.0 µmol/L	A; Skills training, muscle strength, coordination, flexibility, speed, En. 2X weekly (45 min), moderate, gradually ↑ intensity, supervised, 17 weeks	↔ Hcy compared to the control group. No signf change in Hcy [] in any of the indexes of all-round exe	The exe ↔
König et al., 2003	39	Intervention with no control	27.1 ± 5.3	Well-trained male tri-athletes (VO_{2max} > 55 ml/kg/min), no supp HTG: 14.9h training/wk (n=9) LTG: 9.1h training/wk (n=9). Initial Hcy [] = 12.3±2.0 µmol/L	End training	Total group: ↔ Hcy HTG: ↓8 % LTG: ↔	↓ Hcy after 28 days in HTG but not in LTG

Table 2: continued

Author(s) and year	Subject (n)	Study design	Age (yr)	Subject characteristics	Intervention	Outcomes (Major results)	Authors' conclusion(s)
Duncan et al., 2004	324	R/C	48.9 ± 8.4	X individuals (Female = 47% & Male = 53%) with known chronic disease, engaged in structured physical activity not more than twice weekly (30 min) for the previous 12 months. Initial Hcy []; HIHF = 7.73±2.02 µmol/L HILF = 7.20±1.83 µmol/L MIHF = 7.62±4.25 µmol/L MILF = 8.27±5.13 µmol/L	A; Walking HIHF: 65-75% HRR, 5-7×/wk (N=83) HILF: : 65-75% HRR, 3-4×/wk (N=77) MIHF: : 45-55% HRR, 5-7×/wk (N=92) MILF: : 45-55% HRR, 3-4×/wk (N=72) for 6 months	HIHF: ↑12 % HILF: ↑14 % MIHF: ↔ MILF: ↔ Hcy ↓ after 6 months of brisk walking. No significance between groups	High intensity chronic exe ↑ serum Hcy

A = Aerobic;; BMI = Body mass index (kg/m^2); CAD = Cardio artery dieaseCVD = Cardio vascular disease; En = Endurance; Exe = Exercise; HIHF = High intensity- high frequency; HILF = High intensity- low frequency; h = hour; HTG = High-training group; Inc = Included; LTG = Low-training group; MILF = Moderate intensity - low frequency; MIMF = Moderate intensity – moderate frequency; min = minute; Hcy = Plasma Homocysteine; R/C = Randomized controlled; Signf = Significant; VO_{2max} = Maximal oxygen consumption; wk = weekly; X = Excluded; yr = years; [ ] = Concentration; ↑ = Indicates increase; ↓ = Indicates decrease; ↔ = no significant change.

Conclusion

In conclusion Hcy has recently been identified as a potential CVD risk factor (Choy *et al.* 2000). Considerable evidence supports a link between elevated Hcy concentrations and arthrosclerosis (Nygard *et al.*, 1997). The treatment of hyperHcy varies according to the underlying causes but generally involves supplementation with folic acid, vitamin B_{12}, and pyridoxine (vitamin B_6) (den Heijer *et al.*, 1998). Extensive research has been done on the influence of vitamin supplementation, but no extensive research on the effect of physical activity on high Hcy levels exist. Although it remains controversial, recent data suggest that physical activity may be associated with decreased Hcy concentrations (Ali et al., 1998; König et al., 2003 & Gaume *et al.,* 2005).

No conclusions regarding the effect of exercise on Hcy concentrations can be made from the existing published literature at this stage. The results of the studies that examined the effect of acute exercise on Hcy concentrations are inconsistent and most of the studies were poorly designed, more randomized controlled trails is needed in this regard. It is furthermore extremely important that Hcy concentrations are corrected for exercise induced shifts in plasma volume. The health benefits of regular exercise not only include the decrease in CVD risk factors, but it also lead to a feeling of well being and a higher self-esteem. The fact that exercise includes more health benefits and is cost effective contributes to the fact that physical activity is a better choice of treatment.

Recommendations

More randomized, controlled exercise interventions focusing on mechanisms and effectiveness of increased physical activity on Hcy are recommended. Control over the subject and compliance to the exercise programmes may be improved by including supervised exercise sessions. There is also a need for longitudinal studies to investigate the effect of long term PA on Hcy and CVD.

The studies should include a larger sample size and try to improve adherence by understanding the social environments of the participants. Power calculations and methods of randomization should be reported. Access to a variety of exercise equipment is necessary to measure more variables. Different modes of exercise should also be studied, including lifestyle activity. Reporting of the results should be simple but substantial. It is important to indicate that the values presented were corrected for exercise induced shifts in plasma volume.

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CHAPTER 3: TOTAL PLASMA HOMOCYSTEINE, VITAMIN SUPPLEMENTATION AND PHYSICAL CONDITIONING IN MEN WITH CORONARY RISK FACTORS

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ABSTRACT

The purpose of this randomised, placebo-controlled blind cross-over study on 84 free-living male volunteers was to examine the effect of a conditioning programme, vitamin supplement and a combination of both on total plasma homocysteine (tHcy) concentrations in men with coronary heart disease risk factors. The 84 subjects, matched for physical activity (PA) levels, age and risk factors, were randomly assigned to one of four groups [A = physical conditioning (PC), cycling 20-30 min, 70-80% THR (Target heart rate) and placebo, B = PC + supplement (25 µg vitamin B_{12}; 400 µg folic acid/day), C = supplement and D = control]. Groups A, B, and C were crossed over according to a Latin square design with group D continuing with their usual inactive lifestyle. Body composition, tHcy and VO_2max were measured before and after each 12-week intervention period that was separated by a 6-week washout period. The results of this study was analysed by repeated measures ANOVA, which indicated a phase effect for VO_2max ($p<0.001$) and a phase and interaction effect for tHcy ($p<0.0001$). Inadequate compliance to the PC programme and high dropout rates probably partly explain the lack of effects. The results indicated no significant differences between the groups and interventions.

Key words: Homocysteine, Physical conditioning, Coronary risk factors, VO_2max and Vitamins.

INTRODUCTION

Since the first observation by McCulley (1969) many observational trials have confirmed the association between tHcy concentrations and vascular disease (Ueland *et al.*, 1989 and Mayer *et al.*, 1996). The increased risk for vascular disease may be explained through the prothrombotic and atherogenic properties of Hcy (Zamani *et al.*, 2002). Hcy, an amino acid intermediate formed during the metabolism of methionine which is an essential amino acid derived from dietary protein, is metabolized by one of two pathways: remethylation and transsulfuration.

Experimental evidence suggests that endothelial dysfunction may be the major mechanism by which Hcy exerts its deleterious effect (Welch *et al.*, 1998). Recommended normal Hcy concentrations are 5-15 μmol/L (Zamani *et al.*, 2002). The treatment of hyper-Hcy varies with the underlying causes but generally involves supplementation with folic acid, vitamin B_{12}, and pyridoxine (vitamin B_6) (Den Heijer *et al.*, 1998). A meta analysis on the influence of vitamin supplementation on Hcy indicated that folate (0.5-5 mg/day) and vitamin B_{12} (0.5 mg/day) reduced Hcy concentrations by one quarter to one third (Clarke *et al.*, 2000).

The prescription of physical activity (PA) for the lowering of risk factors for coronary heart disease (CHD) is widely advocated (Blair *et al.*, 1995; Ali *et al.,* 1998*).* A limited number of studies have investigated the effect of PA on plasma Hcy concentrations (Ali *et al.,* 1998; Duncan *et al.*, 2004; Herbst *et al.*, 2003; Wright *et al.*, 1998) with contradicting results. The studies varied from acute PA interventions lasting 30 minutes to training interventions of six months. Wright *et al.* (1998) concluded that acute exercise had no effect on Hcy concentrations in young healthy men and according to Ali *et al.* (1998), chronic exercise training resulted in a significant reduction of 12% in Hcy concentrations in normolipidemic cardio arterial disease (CAD) subjects with hyper-Hcy. On the contrary high intensity chronic exercise produced an increase in Hcy concentrations (Duncan *et al.*, 2004) in individuals with known chronic diseases.

These inconclusive results indicate that research on the effect of physical activity on Hcy concentrations is lacking. No research on the interaction of vitamin supplementation in combination with physical activity could be found in the published literature. The objective of this study is, therefore, to determine whether a 12-week PA conditioning programme, vitamin supplement or a combination of both will decrease Hcy concentrations in white males with three or more risk factors for CHD.

METHODS AND PROCEDURES

Subjects

One hundred men aged 45 to 60 years were recruited to participate voluntarily in this study. Only 82 subjects complied with the inclusion criteria. The number of subjects required was determined by a power calculation, to provide 80% power and 5% significance for a clinical significant change of 2 µmol/L (Margetts *et al.*, 1997). All the subjects completed an informed consent form. The study was also approved by the Ethics committee of the North-West University with code 04M08. The following criteria were used for inclusion and exclusion of the subjects.

Inclusion criteria:

- Men
- 45 – 60 years of age
- BMI (Body mass index) > 25 kg/m^2
- Inactive for the last 3 months
- More than 3 risk factors for CHD.

Exclusion criteria:

- Kidney and liver disease
- Hypothyroidism
- Diabetes mellitus
- Chronic medication that has an influence on homocysteine metabolism
- Allergies to any of the compounds in the vitamin supplement
- Serious medical conditions as determined by the principal investigator.

Study design

A randomised, placebo-controlled, blind cross-over study design was used (Table 1). The study was conducted under free-living conditions with volunteers meeting the selection criteria randomly assigned to one of the following groups, A, B, C or D. Group D was the control group and received no treatment throughout the study. Table 1 provides a schematic representation of the various interventions that were performed. The subjects followed the respective treatments for 12 weeks, followed by a 6-week washout period. The groups were then crossed over for another 12 weeks according to the Latin square design (Neter *et al.*, 1996:1408). Measurements were taken for all the described variables at baseline and end of each phase.

Table 1: A schematic representation of the study design

Group	A	B	C	D
Baseline	Pre-test	Pre-test	Pre-test	Pre-test
Phase 1	**PC & P**	**PC & S**	**S**	**C**
End	Post-test	Post-test	Post-test	Post-test
Washout	(6 weeks)	(6 weeks)	(6 weeks)	(6 weeks)
Baseline	Pre-test	Pre-test	Pre-test	Pre-test
Phase 2	**PC & S**	**S**	**PC & P**	**C**
End	Post-test	Post-test	Post-test	Post-test
Washout	(6 weeks)	(6 weeks)	(6 weeks)	(6 weeks)
Baseline	Pre-test	Pre-test	Pre-test	Pre-test
Phase 3	**S**	**PC & P**	**PC & S**	**C**
End	Post-test	Post-test	Post-test	Post-test

PC & P = Physical conditioning and placebo; PC & S = Physical conditioning and supplementation (vitamin B_{12} and folic acid); S = supplementation; C = control.

Measure instruments

The percentage body fat was determined by means of air displacement plethysmography instrument; the BOD-POD according to Boyle's law (Life Measurement, Inc, CA, USA). The height (cm) was measured with a vertical stadiometer to the nearest 0.1 cm and the body mass with the calibrated electronic scale of the BOD-POD to the nearest 0.1 kg. The body mass index (BMI) was calculated as kg/m^2. All the subjects completed a coronary risk profile (CRP) questionnaire (Bjurstrom *et al.,* 1978) and a risk determination questionnaire (ACSM, 2000). All the subjects completed a quantitative food frequency questionnaire to determine their individual baseline daily vitamin intake (Macintyre *et al.,* 2001). The vitamin intake was analyzed by the Food-Finder 3 (Medical Research Council, Tygerberg, South Africa) computer programme.

VO_2max protocol

Functional capacity was determined by means of direct maximal oxygen consumption (VO_{2max}) ($mLO_2/kg/min$) on a Monark cycle ergometer (Model 834 E; Monark Exercise AB, Vansbru, Sweden), with the Mijnhardt Para-Magnetic Analyzer (Model UG-61D, Gebr. Mijnhardt B.V., Odijk, Holland) that continuously sampled expired air and rate of oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (Ve) and the respiratory exchange ratio (R), calculated every 30 sec by an on-line computer system. An ECG was connected as a safety precaution.

The resting blood pressure (mmHg) and heart rate (beats/minute) were measured with a mercury blood pressure monitor (Baumanometer®) and stethoscope. The subjects completed a warm-up of one minute at 50 revolutions per minute (rpm) and 50 Watt (W). The resistance was progressively increased every minute (50 W) and the test terminated when the subjects reached exhaustion or the clinical exercise test endpoints (ACSM, 2005). Recovery was then initiated at a resistance of 50 W and 50 rpm for five minutes for safety reasons.

Blood samples and Hcy

The subjects were required to take part in a 10-hour overnight fast. A registered nurse collected venous blood samples from the antebrachail vein using a sterile winged infusing set and syringes. The EDTA blood samples were immediately placed on ice and centrifuged for 15 min within one hour of sampling to yield plasma for the analyses of total plasma Hcy. Aliquots were stored at -82°C until the analyses were performed. Hcy concentrations were measured by means of competitive Immunoassay (Immulite 2000®, Homocysteine: DPC, USA, Catalog number L2KH02).

Interventions:

Conditioning programme

The programme consisted of aerobic exercises at 70-80% THR, for 20-30min, resistance exercises for large muscle groups, performing 2 sets of 15 repetitions, that were increased every 4 weeks and 4 stretch exercises (gastrocnemius, hampese, quadricep and lower back) with each exercise performed in 3 sets of 30 seconds. The subjects were requested to perform the exercises at least 3 days per week, for a 12 week period. The researchers that conducted the statistical analysis were blind to the order of the PA intervention.

Supplementation

One months' supply of the multivitamin [Pharma Natura (Pty) Ltd] containing 12.5 µg vitamin B_{12} and 200 µg folic acid per tablet was provided to the subjects. Two tablets had to be taken daily, before breakfast. The tablets were in unmarked bottles. The placebo consisted of corn starch and appeared to be similar to the vitamin supplement. The researchers and the subjects were blinded for both supplement and placebo.

Compliance

Compliance to the multivitamin intervention was determined by pill counting. Compliance to the physical conditioning was done by reporting the maximal heart rate attained during every exercise session and by keeping record of the attendance of exercise sessions.

Statistical procedure

Statistical analyses were performed using the computer software package Statistica® 7 (Statsoft, Inc. Tulsa, Oklahama, USA) and Statistical Consultation Services of the North-West University. Demographic characteristics of the subjects were determined by means of descriptive statistics. Between group differences for baseline characteristics were determined by means of ANOVA ($P<0.05$). The results were analyzed on the basis of intention-to-treat, in which the outcomes of all patients were analyzed with the group to which they were originally assigned, whether or not they completed the protocol. Repeated measures ANOVA were used to determine statistical significance for percentage changes in VO_2max and Hcy in the groups over time to determine the effect of changes in each phase multivariately. Additional repeated measures ANOVA were preformed only on those subjects who complied with the interventions.

RESULTS

The trial profile (Figure 1) indicates that 82 subjects were recruited for the study with 16 drop-outs. The subjects terminated their participation for various reasons. These were: refusing to stop exercising during the wash-out period (n=1), transferred out of the city (n=1), medically unfit (n=1) and the researchers were unable to re-establish contact with the subjects (n=13). Only 26% of the subjects attended more than 75% of the requested 36 exercise sessions. The average of 62% of the subjects complied to the supplement intervention.

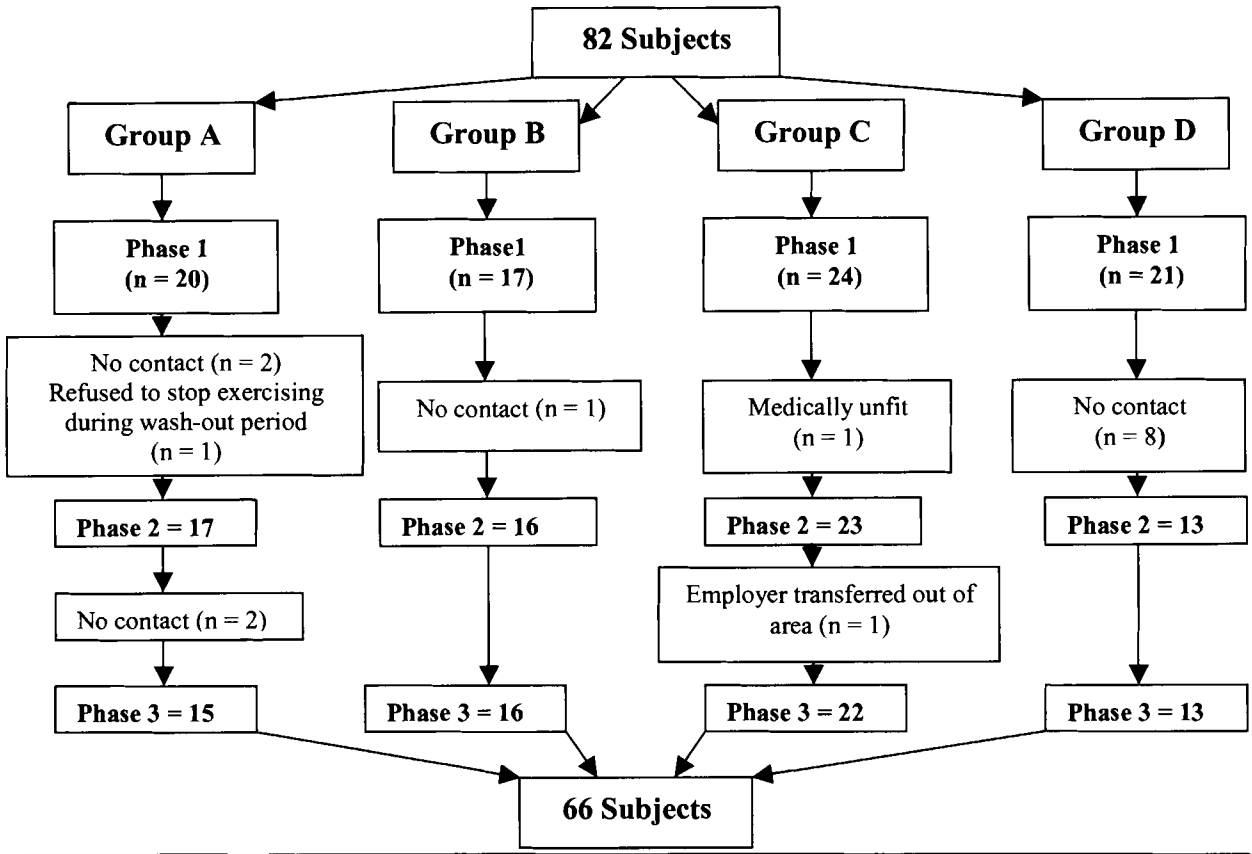

Figure 1: The trial profile of the subjects of the study

There were no statistically significant differences between the experimental and control groups for all characteristics at baseline (phase 1) (Table 2). The mean BMI of all the groups were > 30 kg/m^2 and the mean fat % > 30% as measured with the BOD POD. This indicated that all the subjects were obese. The mean VO_2max values indicate that the groups had a low cardiorespiratory fitness level, as was required. The results of the dietary questionnaire indicate that the subjects had low intake of dietary folate (213.3 ± 110.9 - 306.5 ± 175.0 µg/day), but ingested more than the indicated recommended daily allowance of vitamin B_{12} (5.4 ± 2.5 - 6.9 ± 3.1 µg/day). The RDA of folate and vitamin B_{12} respectively are 400 µg/day and 2.4 µg/day. The reason for the high intake of vitamin B_{12} may be due to the fact that al the subjects had a high red meat intake.

Table 2: **Means and standard deviations of the baseline characteristics of the subjects as randomised to the different groups**

Variable	Group A n=20		Group B n=17		Group C n=24		Group D n=21	
	M	SD	M	SD	M	SD	M	SD
Age (years)	51.0 ± 5.7		51.5 ± 4.4		52.8 ± 5.8		49.3 ± 4.8	
Body mass (kg)	94.9 ± 20.4		98.9 ± 17.4		101.9 ± 14.5		98.1 ± 18.3	
Height (m)	1.8 ± 0.1		1.8 ± 0.16		1.8 ± 0.2		1.8 ± 0.1	
BMI (kg/m^2)	30.3± 6.2		30.6 ± 6.3		31.8 ± 5.0		31.1 ± 5.5	
BOD POD (fat %)	32.9 ± 9.1		30.3 ± 11.6		34.5 ± 10.4		30.6 ± 7.2	
VO_2max (mLO_2/kg/min)	24.9 ± 6.5		28.4 ± 7.1		22.6 ± 6.4		25.8 ± 6.7	
Total Hcy (µmol/L)	11.7 ± 8.2		10.2 ± 2.5		10.4 ± 4.3		8.6 ± 2.3	
Dietary VB_{12} (µg/d)	6.6 ± 3.9		5.4 ± 2.5		6.9 ± 3.1		6.4 ± 2.7	
Dietary F (µg/d)	255.3 ± 149.2		253.4 ± 119.4		306.5 ± 175.0		213.3 ± 110.9	

F = Folate; M = means; SD = standard deviation; VB_{12} = vitamin B_{12}; Hcy = homocysteine; VO_2 max = functional capacity.

The distribution of the risk factors (Table 3) indicates that apart from all subjects being inactive, the most prevalent risk factors were hypertension and obesity.

Table 3: A distribution of the different coronary risk factors of the subjects

Risk Factors	Group A n=20	Group B n=17	Group C n=24	Group D n=21
Family History	5	8	4	8
Smoking	10	8	9	11
Blood pressure	15	12	22	14
Cholesterol	7	3	7	9
Diabetes	2	2	1	0
Obesity	12	11	18	15
Inactive	20	17	24	21
Stress	12	3	11	11

The repeated measures ANOVA indicated a phase effect for VO_2max (Wilks-lambda $p<0.001$) (Figure 2) and a phase and interaction effect for Hcy (Wilks-lambda $p<0.0001$) (Figure 3) as indicated with the profile analysis of the changes in VO_2max values and the change in Hcy concentrations for the consecutive three phases. An interaction effect is when one group differed statistically significant from another group over time. No statistically significant differences were found between the groups and no statistically significant changes within groups with different interventions were found, except for the changes in Hcy concentrations in group A during the first phase (Figure 3).

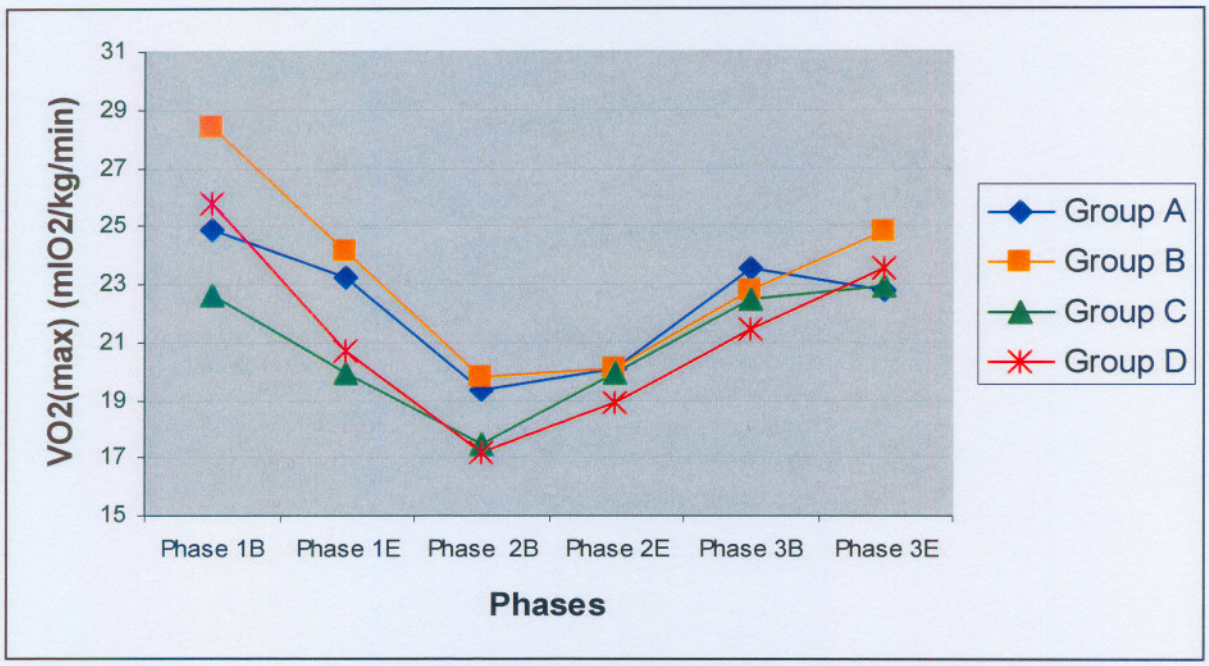

Figure 2: Profile analysis of repeated measures ANOVA of VO_2max for all the groups indicating means and 95% CI (CI = Confidence intervals) (B = beginning; E = end)

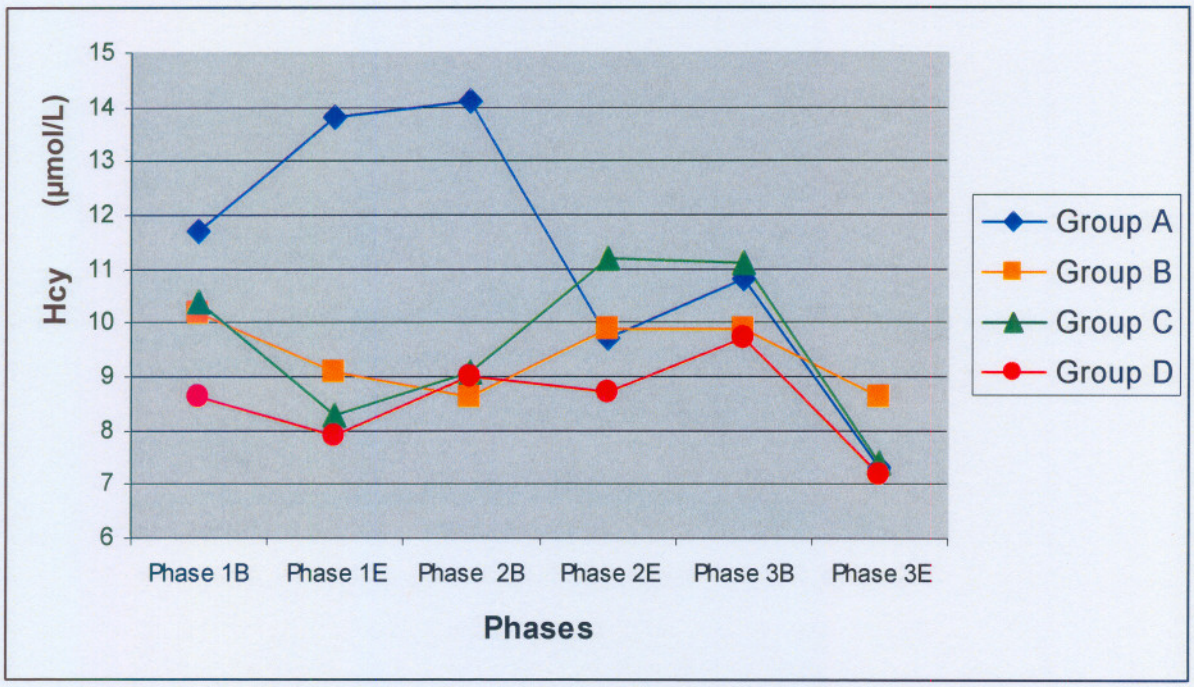

Figure 3: **Profile analysis of repeated measures ANOVA of total plasma Hcy for all the groups indicating means and 95% CI** (Vertical bars denote 0.95 Confidence intervals) (B = beginning; E = end)

Due to the phase effect the results of the interventions in the different phases can not be grouped. The interaction effect indicated that the groups reacted differently over time, therefore the results of each group will be discussed separately. The lack of compliance to the three times weekly PA conditioning intervention resulted in a drastic decrease in the sample size with a consequent lack in statistical power. The results for VO_2max are summarized in Table 4. In group A, the VO_2max values (Table 4) decreased (11.0%) during the PA conditioning intervention (phase 1) and decreased during the wash-out period (Figure 2). The reason for this may be due to the fact that only 10% of the subjects trained the requested three times per week (Figure 2). During the combination intervention (PA conditioning and supplementation, phase 2) the VO_2max values increased (7.1%) with a increase during the wash-out period (Figure 2). With the supplement intervention (phase 3) the VO_2max decreased (4.2%).

Table 4: Mean [95%CI] baseline to end changes in of VO_2max (mLO_2/kg/min) of the different interventions

	PC & P	PC & S	S
Group A	n = 20	n = 17	n = 14
B	24.9 (21.9 – 27.9)	19.3 (16.2 – 22.5)	23.5 (17.9 – 29.1)
E	23.2 (19.5 – 26.9)	20.1 (16.9 – 23.2)	22.8 (16.9 – 28.5)
Δ (%)	-11.0 (-19.1 – 2.9)	7.1 (1.2 – 15.4)	-4.2 (-32.2 – 23.8)
Group B	n = 17	n = 17	n = 16
B	22.8 (19.6 – 26.0)	28.4 (24.7 – 32.0)	19.8 (17.2 – 22.4)
E	24.8 (21.5 – 28.0)	24.1 (20.2 – 27.9)	20.1 (17.9 – 23.1)
Δ (%)	7.9 (0.4 – 16.2)	-15.4 (-22.1 – 8.7)	3.4 (4.9 – 11.6)
Group C	n = 23	n = 21	n = 24
B	17.5 (15.4 – 19.7)	22.5 (20.3 – 24.7)	22.6 (19.8 – 25.3)
E	19.9 (17.8 – 22.0)	22.9 (20.5 – 25.4)	19.9 (18.1 – 21.8)
Δ (%)	17.4 (1.6 – 33.2)	5.3 (3.3 – 13.9)	-5.7 (-21.9 – 10.5)
Group D	No treatment n = 20	No treatment n = 11	No treatment n = 11
B	25.8 (22.7 – 28.9)	17.2 (14.1 – 20.3)	21.4 (19.7 – 23.2)
E	20.7 (18.7 – 22.7)	18.9 (16.3 – 21.5)	23.5 (20.3 – 26.6)
Δ (%)	-21.9 (-31.4 – 12.4)	10.8 (6.4 – 27.9)	4.5 (10.2 – 19.1)

(B = beginning; E = end; PC & P = Physical conditioning and placebo; PC & S = Physical conditioning and Supplementation (vitamin B_{12} and folic acid); S = supplementation; C = control; Δ = changes from baseline to end)

In group B, the VO_2max values decreased (15.4%) during the first phase with the combination intervention (PC and supplementation) and a decrease was observed during the wash-out period (Figure 2). The reason for this may be due to the fact that only 35% of the subjects complied with the PA conditioning intervention. During phase 2, the VO_2max values increased (3.4%) with the supplementation intervention and an increase was observed in the wash-out period (Figure 2). The VO_2max values increased (7.9%) during phase 3 with the PA conditioning intervention.

In group C, the VO_2max values decreased (5.7%) during the first phase with the supplement intervention and a decrease was observed during the wash-out period. During the second phase, the VO_2max values increased (17.4%) with the PA intervention and a increase was observed in the wash-out period (Figure 2). The VO_2max values increased (5.3%) during phase 3 with the combination intervention (PA conditioning and supplementation).

In group D, the VO_2max values decreased (21.9%) during the first phase and a decrease was observed during the wash-out period (Figure 3). During phase 2 (including the wash-out period) and phase 3 the VO_2max values increased respectively with 10.8% and 4.5% (Figure 3). A definite trend can be observed during phase 1 regarding the decrease of the VO_2max values in all the groups. In phase 2, the VO_2max values of all the groups increased statistically significantly and during phase 3, the change did not differ statistically significantly from phase 2 for all the groups (Figure 2). No significant differences were found between the groups. The lack of changes in the VO_2max values according to the PA interventions, as is expected when PA increase or decrease, confirms that compliance to the PA conditioning programme was inadequate.

The results of the changes that were found with the Hcy will be discussed in a similar manner with the statistically significant phase and interaction effect for Hcy in all the groups summarized in Table 5.

Table 5: **Mean [95%CI] baseline to end changes adjusted for baseline in total plasma Hcy (µmol/L) concentrations of the different interventions**

	PC & P	PC & S	S
Group A	n = 20	n = 17	n = 14
B	11.7 (7.9 – 15.5)	14.1 (7.9 – 20.2)	10.8 (8.1 – 13.6)
E	13.8 (8.9 – 18.7)	9.7 (7.8 – 11.5)	7.3 (5.6 – 8.9)
Δ (%)	15.6 (2.7 – 28.4)	-6.4 (-23.0 – 10.3)	-29.3 (-39.0 – 19.5)
Group B	n = 17	n = 17	n = 16
B	9.9 (8.3 – 11.5)	10.2 (8.9 – 11.5)	8.6 (7.7 - 9.6)
E	8.6 (7.0 – 10.1)	9.1 (7.8 – 10.4)	9.9 (8.3 – 11.5)
Δ (%)	-9.8 (-21.6 – 2.0)	-10.9 (-18.8 – 2.9)	11.5 (0.2 – 22.9)
Group C	n = 23	n = 21	n = 24
B	9.1 (7.5 – 10.8)	11.1 (8.1 – 14.2)	10.4 (8.6 – 12.2)
E	11.2 (8.8 – 13.6)	7.4 (6.5 – 8.3)	8.3 (7.5 – 9.0)
Δ (%)	25.5 (14.0 – 36.9)	-21.8 (-30.4 – 13.2)	-13.3 (-22.5 – 4.0)
Group D	No treatment n = 20	No treatment n = 11	No treatment n = 11
B	8.6 (7.6 – 9.7)	9.0 (6.9 – 11.1)	9.7 (7.9 – 11.4)
E	7.9 (6.1 – 9.7)	8.7 (7.1 – 10.4)	7.2 (5.4 – 9.0)
Δ (%)	-6.2 (-16.2 – 3.9)	11.5 (8.8 – 31.9)	-26.4 (-42.5 – 10.2)

(B = beginning; E = end; PC & P = Physical conditioning and placebo; PC & S = Physical conditioning and Supplementation (vitamin B_{12} and folic acid); S = supplementation; C = control; Δ = delta value)

In group A, the Hcy concentrations increased (15.6%) with the PA conditioning intervention and a decrease was observed in the wash-out period (Figure 3). The increase differed statistically significantly ($p<0.017$) from the other groups in phase 1 (Figure 3). The reason for the increase is unknown and can probably not be ascribed to the PA conditioning programme since only 10% of the subjects complied to the intervention (Figure 3). During the second phase, the Hcy concentrations decreased

(6.4%) with the combination intervention (PC and supplementation) and an increase was observed during the wash-out period (Figure 3). The Hcy concentrations decreased during the third phase with the supplementation intervention. No significant differences were seen between interventions in group A (Table 5).

In group B, the Hcy concentrations decreased (10.9%) during the first phase with the combination intervention (PA conditioning and supplementation) and a further decrease was observed during the wash-out period (Figure 3). During phase 2, the Hcy concentrations increased (11.5%) with the supplementation intervention and during the wash-out period a plateau was reached (Figure 3). The Hcy concentrations decreased (9.8%) during phase 3, with the PA conditioning intervention (Figure 3). Only 35% of the subjects complied with the PA conditioning intervention and 88% of the subjects complied with the supplementation intervention. No significant differences were seen between interventions in group B (Table 5).

In group C, the Hcy concentrations decreased (13.2%) with the supplementation intervention and an increase was observed during the wash-out period that followed immediately afterwards (Figure 3). During the second phase, the Hcy concentrations increased (25.5%) with the exercise intervention and a further increase was observed in the wash-out period (Figure 3). The Hcy concentrations decreased (21.8%) during phase 3 with the combination intervention (PA conditioning and supplementation).

In group D, the Hcy concentrations decreased (6.2%) during the first phase and an increase was observed during the wash-out period (Figure 3). During the second phase, the Hcy concentrations increased (11.5%) and a further increase was observed during the wash-out period (Figure 3). The Hcy concentrations decreased (26.4%) during the third phase. A definite trend can be observed during phase 1 regarding the decrease of the Hcy concentrations in all the groups, except for group A.

Group A had a 16% increase in Hcy concentration, but a decrease of 6-13% in Hcy concentration was observed in the other groups (B, C) (Figure 3). During the second phase, the Hcy concentrations of all the groups increased, except for group A that decreased. The Hcy concentrations of all the groups decreased during the third phase. No significant differences were seen in group D (Table 5).

A repeated measures ANOVA was performed only on the subjects (n=16) who complied (>75%) with the three times weekly PA conditioning programme. Similar results to those of the total group for the change in VO_2max were obtained. Repeated measures ANOVA indicated no statistically significant effects for the change in Hcy concentrations. No conclusions can be drawn from these results due to the fact that poor compliance led to a small sample size that had inadequate statistical power.

Discussion

The objective of this study was to determine the effect of a 12 week PA conditioning programme, vitamin supplementation and a combination of both on Hcy concentrations. VO_2max values were determined to control for compliance to PA intervention. The main results of the study indicated a statistically significant phase effect for VO_2max values and a statistically significant phase and interaction effect for the Hcy concentrations. No significant differences were found between the groups over time, regarding the repeated measures ANOVA for VO_2max.

All the groups including the control followed the same pattern that makes it very difficult to explain these results. Other factors than the interventions were probably responsible for the changes that were seen. The lack of compliance to the PA conditioning programme may in part be a reason for the above mentioned. Repeated measure ANOVA for Hcy concentrations illustrated that the Hcy concentrations of group A differed significantly from the other three groups.

This difference can probably not be ascribed to the interventions as discussed earlier. Thus, due to inadequate compliance to the PA conditioning programme no conclusions can be drawn with regard to the effect of PA on Hcy concentrations. Furthermore intake of folic acid and vitamin B_{12} did not affect Hcy concentrations in this study compared to the other interventions. So far, there have been few literature data available on the effects of PA on Hcy concentrations. The current study was well designed compared to other studies in the literature that investigated the effect of PA on Hcy concentrations (De Jong *et al.,* 2001 & Duncan *et al.,* 2004).

A randomised, placebo controlled, cross-over, blinded design were used for this study. Other studies in the literature also reported difficulties with compliance to a PA conditioning programme (Ali *et al.,* 1998, De Jong *et al.,* 2001, König *et al.*, 2003; Duncan *et al.,* 2004) and had high dropout rates of the subjects (Ali *et al.,* 1998, De Jong *et al.,* 2001; Duncan *et al.,* 2004). None of the reported studies measured VO_2max or any other form of compliance to the PA intervention. The inconsistent effects of PA on Hcy concentrations reported in the literature may therefore also be ascribed to inadequate compliance to the PA intervention in some studies or may be due to inadequate study designs especially the lack of a control group. In this study similar changes in the experimental group and control group were found. It therefore still remains to be established what the effect of PA on Hcy concentrations are in well designed randomised control trials where extreme care is taken to ensure adequate compliance and to prevent dropout of the subjects.

The lack of effect of the folate and vitamin B_{12} supplementation on Hcy concentrations, in spite of the relatively good compliance, is in contrast to what is reported in the literature (Clarke *et al.*, 2000). A meta analysis was performed on 12 randomised controlled studies that had a minimum of three weeks supplementation intervention (Clarke *et al.*, 2000). The reason may be the lost of statistical power as a result of the decreased sample size. It was also observed that Hcy concentrations decreased more in people with higher baseline Hcy concentrations and low plasma folate status (Clarke *et al.*, 2000).

After standardization of baseline Hcy concentrations and plasma folate concentrations, Hcy concentrations decreased by 25% [95%CI 23-28%] with a 0.5-5 mg/d intake of folate and an additional decrease of 7% with a 0.5 mg/d vitamin B_{12} intake Clarke *et al.*, 2000). The reason why no changes in Hcy concentrations were observed in this study may be due to the fact that the subjects had low Hcy concentrations at baseline. Another reason may be low statistical power due to the lack of compliance to the PA conditioning programme that led to smaller groups.

Conclusion

In conclusion, the data indicated that a 12-week PA conditioning programme, vitamin supplementation or the combination did not alter Hcy concentrations. These results emphasize the importance of further research regarding homocysteine concentrations as a CVD risk factor and physical conditioning, as there is clearly a shortage of literature that focuses on this research theme and age group (Ali *et al.*, 1998, De Jong *et al.*, 2001, König *et al.*, 2003; Duncan *et al.*, 2004). The need for more research to investigate the influence of physical conditioning programme interventions and combination (PA conditioning and supplementation) interventions is strongly emphasized in subjects with hyper-Hcy.

Limitations and recommendations

Certain shortcomings regarding this study are as follows:

- The subjects were not all hyperhomocysteinemic, future studies should include hyperhomocysteinemic subjects.
- Control over the subjects and smaller drop-out rates may lead to more conclusive results.
- Compliance should be improved through more aggressive supervised training sessions.

- The duration of the intervention must be kept to a minimum of 12 weeks, except if the exercise sessions are increased to obtain an increase in physical fitness.
- Inclusion of a control group in the study is very important and should also form part of the cross over process.

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CONGRESS PRESENTATIONS

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

HERBST SJ, NEL R, MOSS SJ & 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 SJ; HERBST SJ, NEL R, VAN ROOYEN JM, SCHUTTE AE & HUISMAN HWG. 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 SJ, VAN DER WESTHUIZEN FH, HERBST SJ & 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 16-18, 2005.*

APPENDICES

- Instructions for Authors: Sports Medicine Journal
- Instructions for Authors: American journal of preventive medicine

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American Journal of Preventive Medicine

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