PHYSICAL ACTIVITY AND HOMOCYSTEINE IN TSWANA ADOLESCENTS:

The Play - Study

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"I can do all things through Christ which strengthenth me"

(Philip 4:13)

Thank you Jesus for not giving up on me to complete this study...and a special thanks to all the people you've worked through to never give up on your strength.

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ABSTRACT

PHYSICAL ACTIVITY AND HOMOCYSTEINE IN TSWANA ADOLESCENTS

Plasma homocysteine, a thiol containing amino acid, has been indicated to possibly be a risk factor for various cardiovascular diseases and strokes. Investigators reported normal plasma homocysteiene concentration values of 5 μmol/L - 15 μmol/L for adults and a 4 μmol/L – 8 μmol/L for children younger than 12 years. Plasma homocysteine can be influenced by age, gender, ethnicity and lifestyle. Age, gender and ethnicity are factors that can increase plasma homocysteine concentrations. Lifestyle factors such as physical activity, diet, smoking and alcohol seems to affect plasma homocysteine concentrations. Physical activity however, may change plasma homocysteine concentrations but research is needed, to determine the change in plasma homocysteine concentrations. A diet rich in Vitamin B_6, B_{12} and folic acid has been indicated to decrease plasma homocysteine concentrations. Smoking and alcohol consumption contribute to plasma homocysteine concentrations increases but the exact mechanism by which homocysteine concentrations are influenced needs further investigation.

The purpose of this study was to examine the homocysteine concentrations for black adolescents and to determine the effect a physical activity intervention programme may have on the plasma homocysteine concentrations of the black adolescents.

A intervention study was done on 148 girls and 114 boys from a similar socio – economic status area. Fasting blood samples were taken to determine the plasma homocysteine concentrations. Anthropometric measurements were performed to determine the percentage body fat and muscle mass. A 20 m shuttle-run, was performed on the experimental and control group to establish the fitness level of the subjects. A 10-week physical activity intervention programme was followed, which include muscle endurance and cardio respiratory training. The subjects were retested after the intervention.

Descriptive statistics indicated that the experimental and control group presented similar baseline characteristics with regard to the BMI and WHR. Plasma homocysteine

concentrations ranged between 5.93 (± 0.92) μmol/L and 7.03 (± 1.67) μmol/L. A significant relation was found between muscle mass and plasma homocysteine concentration ($r = 0.25$; $p = 0.00$). Plasma homocysteine increased in the experimental group with 1 % during the 10-week intervention period and with 15 % in the control group.

An ANOVA of the changes for the various percentages of compliance to the intervention program indicated that subjects of the experimental group that attended < 33 % and > 66 % of the intervention programme had a significant increase in plasma homocysteine concentration of 7 %. Subjects attending between 33 % and 66 % of the intervention programme reported a 4 % decrease in plasma homocysteine.

Plasma homocysteine concentrations were within the recommended range for these adolescents according to the literature. Plasma homocyteine concentrations did not decrease significantly in the experimental and control groups with the physical activity intervention in this study.

Key Words: Adolescents, black population, age, gender, ethnicity, physical activity, plasma homocysteine

OPSOMMING

FISIEKE AKTIWITEIT EN HOMOSISTEÏEN IN TSWANA ADOLESENTE

Plasma homosisteïen 'n tiol bevattende aminosuur word beskryf as 'n risiko faktor vir verskeie kardiovakulêre siektes en beroertes. Ondersoeke dui daarop dat normale plasma homosisteïen konsentrasie tussen 5 - 15 µmol/L vir volwassenes en 4 – 8 µmol/L vir kinders onder 12 jaar is. Plasma homosisteïen word beïnvloed deur ouderdom, geslag, etnisiteit en lewenstyl. Ouderdom, geslag en etnisiteit van persone kan plasma homosisteïen verhoog. Leefstyl faktore soos fisieke aktiwiteit, dieët, rook gewoontes en alkohol gebruik beïnvloed plasma homosisteïen. Fisieke aktiwiteit kan moontlik plasma homosisteïen verander maar verdere navorsing is nodig. Dieët kan as 'n behandelings - modaliteit beskou word veral ten opsigte van vitamien en mineraal supplementasie, en kan plasma homosisteïen verlaag. Plasma homosisteïen kan verhoog word deur rook en alkohol gewoontes van mense. Die meganisme wat betrokke is by plasma homosisteïen konsentrasie veranderinge by hierdie faktore is onduidelik.

Die doel van hierdie studie is egter om die normale waardes vir plasma homosisteïen - konsentrasie te ondersoek vir swart adolesente en te bepaal of 'n fisieke aktwiteits intervensieprogram 'n invloed het op die plasma homosisteïen konsentrasie van swart adolesente.

'n Intervensie studie is gedoen op 148 dogters en 114 seuns van dieselfde sosio -ekonomiese area. Bloed analises is gedoen in 'n vastende toestand om die plasma homosisteïen - konsentrasie te bepaal. Die persentasie liggaamsvet en spiermassa was bepaal deur antropometriese metings. Die "bleep" toets was gebruik om die kardio respiratoriese funksie te bepaal van die kontrole en eksperimentele groepe. Die fisieke aktiwiteits-intervensieprogram is gevolg vir 10–weke. Die intervensieprogram het die spieruithouvermoë en kardiorespiratoriese fiksheidskomponente ingesluit. Die proefpersone is weer getoets na die intervensie - periode.

Eksperimentele en kontrole groepe toon dieselfde eienskappe ten opsigte van die LMI (Liggaamsmassa Index en MHR (Middel-Heup ratio) volgens beskrywende statistiek. Plasma homosisteïen - konsentrasie wissel tussen 5.93 (± 0.92) µmol/L en 7.03 (± 1.67) µmol/L vir al die groepe. 'n Statistiese betekenisvolle verband is gevind tussen spiermassa en plasma homosisteïen ($r = 0.25$; $p = 0.00$). Plasma homosisteïen konsentrasie het met 1 % gestyg in die eksperimentele groep en 15 % in die kontrole groep na afloop van die intervensie - periode.

'n ANOVA is gedoen om die persentasie deelname van die proefpersone te bepaal, tydens die intervensie program. Minder as 33 % en meer as 66 % deelname van proefpersone in die eksperimentele groep toon 'n betekenisvolle statistiese verhoging van 7 % in plasma - homosisteïen konsentrasie. Deelname van 33 % tot 66 % toon 'n 4 % verlaging in plasma - homosisteïen konsentrasie.

Die plasma homosisteïen konsentrasie vir die adolesente was binne die normal waarde soos gevind uit die literatuur. Die plasma homosisteïen konsentrasie het egter nie statisties betekenisvol verander in die eksperimentele groep en kontrole groep gedurende die intervensie periode nie.

Sleutel woorde: Adolesente, swart populasie, ouderdom, geslag, etnisiteit, fisieke aktiwiteit, plasma homosisteïen

DECLARATION

The co-authors of the article which form part of this disseration, Dr. S.J. Moss (supervisor), Dr. A. Boonstra (Co-supervisor) hereby give permission to the candidate, Ms. Lourien Snyman to include a literature review and a research article as part of a Masters dissertation, the contribution (advisory and supportive) of these co-authors was kept within reasonable limits, thereby enabling the candidate to submit this dissertation for examination purposes. This dissertation, therefore, serves as a fulfilment of the requirements for the M.A. degree within the school of Biokinetics, Recreation and Sport Science in the Faculty of Heath Sciences at the North – West Universit, Potchefstroom campus.

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Supervisor

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LIST OF ABRIVIATIONS

B	BMI	Body Mass Index
C	cm	centimeter
	CVD	Cardiovascular disease
E	En	Endurance
	Exe	Exercise
H	Hcy	Homocysteine
I	ISAK	The International society for the advancement of Kinanthropometry
K	Kg	Kilogram
M	m	mass
	MA	Mexican American
	Min	minute
	MTHFR	methylentetrahydrofolate reductase
N	n	subjects
	NHB	non Hispanic blacks
	NHW	non Hispanic whites
P	PDPAR	Previous Day Physical Activity Recall
V	VO_2max	Maximal oxygen consumption
W	WHR	Waist – Hip - ratio
Y	Yr	Year

LIST OF SYMBOLS

β	Beta
%	Percentage
μ	Micro
<	Greater than
>	Smaller than
L	Litre

CHAPTER 1

PROBLEM STATEMENT, OBJECTIVES AND HYPOTHESES

1.1 INTRODUCTION

A variety of occlusive cardiovascular diseases and strokes is the leading cause of a high mortality and morbidity rate (Dinavahi *et al.*, 2003:757). To prevent these diseases the associated risk factors have to be altered in the early stages of life (Reddy, 1997:153). These risk factors include: high blood pressure, circulating levels of serum cholesterol, plasma insulin and increases in insulin action (Duncan *et al.*, 2004:894) and an amino acid called homocysteine as an independent risk factor.

Plasma homocysteine as a risk factor for cardiovascular diseases might be influenced by certain perpetual factors such as age, gender, ethnicity and lifestyle factors. The purpose of this chapter is to present the problem statement that has lead to the research question posed in the dissertation. The objective and hypotheses that is set for this investigation is described with finally the structure of the dissertation.

1.2 PROBLEM STATEMENT

Homocysteine can be defined as a thiol-containing amino acid (Merouani *et al.*, 2001:805) derived from the metabolism of methionine. Methionine can be remethylated to methionine or metabolised to cysteine (Shai *et al.*, 2003; Thomas *et al.*, 2004; Ganji *et al.*, 2005) by cystathionine-β-synthase through a transsulfuration process and the reaction is dependent on vitamin B_{12} as a cofactor. In addition, a folate derivative must also be synthesized by methylenetetrahydrofolate reductase to provide for the methyl group, so that the methionine synthase reaction can take place (Merouani *et al.*, 2001:805). Elevated homocysteine, also termed hyperhomocysteinemia, has been linked to histopathological features of vessel injury, including proliferation of vascular smooth muscle cells, inhibition of fibrinolysis and homeostatic changes of the pro-thrombotic state (Dinavahi, 2003:767). Mild to moderate hyperhomocysteinemia and severe hyperhomocysteinemia respectively can be categorized between 16 to 100 μmol/L and >100 μmol/L (Ali *et al.*, 2000:49). A 5-μmol/L increment in total fasting homocysteine concentration has shown an associated higher risk for various occlusive cardiovascular diseases (Jacques *et al.*, 1999:482).

The recommended homocysteine concentration for adults is 5 to 15 μmol/L (Ali *et al.*, 2000:49; Zamani, 2002; Dinavahi *et al.*, 2003; Thomas *et al.*, 2003) with 4 to 8 μmol/L homocysteine as the reference for children younger than twelve years (Ueland, 2001:928). Furthermore, research states that plasma homocysteine concentrations could change as the aging process takes place. The Third National Health and Nutrition Examination survey (Jacques *et al.*, 1999:483) established plasma homocysteine concentrations for children ≤10 years at 5.8 μmol/L, for children between 11 and 15 years 6.6 μmol/L, and for adolescents between 16 and 18 years 8.1 μmol/L. No differences were found in plasma homocysteine concentrations between boys and girls of above-mentioned ages (Jacques *et al.*, 1999:483; Ganji *et al.*, 2005:2253). There is little overall evidence about age and race-ethnicity concerning homocysteine concentrations (Must *et al.*, 2003:2644) in young healthy black or white children and adolescents. The Bogalusa Heart Study also supported the fact that the plasma homocysteine concentrations may be similar for black and white children (Greenland *et al.*, 1999:2144).

Factors like age and gender, as well as reduced serum folate levels and low physical activity levels may contribute to elevated plasma homocysteine concentrations (Ali *et al.*, 2000:49). Currently elevated plasma homocysteine concentrations can be treated with supplements such as folate and Vitamins B_6 and B_{12}. These three vitamins are essential because they can prevent elevated plasma homocysteine concentrations, minimizing arterial damage and slowing or preventing formation of arteriosclerotic plaques (McCully, 1998:7). Physical activity has been conformed as a modifier of risk factors for cardiovascular heart diseases, but physical activity as a role player in changing total plasma homocysteine concentrations has resulted in controversial findings. There is, however, limited epidemiological evidence linking regular physical activity to lower plasma homocysteine concentrations (Ali *et al.*, 2000:49). The vast majority of epidemiological studies have found contradicting results. These results varied from acute activity to training interventions with a 12% reduction in homocysteine concentrations (Ali *et al.*, 2000:49). Gallistli *et al.* (2001:1220) stated that if the lean muscle mass in children and adolescents' increases and the fat mass decrease in a weight reduction program, the total plasma homocysteine concentration would be reduced. In obese, overweight women with polycystic ovary syndrome, regular moderate physical activity lowered elevated plasma homocysteine concentrations whether or not the women's fat percentages were reduced (Randeva *et al.*, 2006:4496). The intensity at which the physical activity has to be performed before having an effect on elevated plasma homocysteine is still controversial. Physical activity as a treatment modality has yet to be investigated.

The two main research questions raised in this study are, therefore, firstly to determine plasma homocysteine concentration in black adolescents and secondly, whether a physical activity intervention program will have an effect on plasma homocysteine concentrations in these adolescents.

The outcomes of this study will reflect the importance of physical activity on homocysteine in black adolescents originating from a low socio-economic background and the link to various occlusive cardiovascular diseases in later life. Homocysteine has been linked to stroke. Blacks have been identified as an at risk group for stroke. Investigating homocysteine in black adolescents may give an indication of the role a physical intervention may have in preventing stroke in the later life.

1.3 OBJECTIVES

The objectives of this study are:

- To determine the plasma homocysteine concentrations, in Tswana speaking adolescents.
- To determine the effect of a physical activity intervention programme on plasma homocysteine concentrations in Tswana adolescents from a low socio-economic area.

1.4 HYPOTHESES

This study will be based on the following hypotheses:

- The average plasma homocysteine concentration, for Tswana adolescents from a low socio-economic area is within the normal range as indicated by published data.
- A physical activity intervention will reduce homocysteine levels of Tswana adolescents from a low socio-economic area.

1.5 STRUCTURE OF THE DISSERTATION

The dissertation is presented in article format and consists of four chapters, namely an introduction (Chapter 1), and a review of the literature (Chapter 2). Chapter 3 the empirical research article and the summary, conclusion and recommendations (Chapter 4). In the introduction (Chpater 1), the problem statement, objectives and hypotheses are presented. The literature review (Chapter 2) investigates the influence of age, gender, and ethnicity on total plasma homocysteine as well as on the lifestyle factors that influence the total plasma homocysteine concentrations. The research article (Chapter 3) investigates the effect of a physical activity intervention on plasma homocysteine concentrations of black adolescents. Chapter 4 the summary and final conclusions of the study is presented. The referencing of Chapter 1,2 and 4 will be according to the Harvard style. Referencing of Chapter 3 will be according to the guidelines of the journal that the article has been prepared for.

Chapter 1: Problem Statement, Hypotheses and Objectives

Chapter 2: Literature Review: Influence of age, gender, ethnicity and lifestyle factors on homocysteine concentrations

Chapter 3: Research Article: Changes in homocysteine following a physical activity intervention

Chapter 4: Summary, conclusions and recommendations

Figure 1: **Presentation of the structure of the dissertation.**

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CHAPTER 2

INFLUENCE OF AGE, GENDER, ETHNICITY AND LIFESTYLE FACTORS ON HOMOCYSTEINE CONCENTRATIONS

2.1 INTRODUCTION

Many people can harbor high levels of plasma homocysteine, a sulphur containing amino acid, especially those who have a family history of arterial occlusive diseases, like atherosclerosis particularly in the presence of other risk factors (MaLlinow *et al.*, 1999:178).

In this chapter the metabolic pathways of homocysteine will be indicated. Consequently the influence of age, gender, ethnicity and lifestyle factors on homocysteine concentrations will be discussed.

2.2 METABOLISM OF HOMOCYSTEINE

In the 1990s the importance of plasma homocysteine was discovered and described as a possible independent risk factor for various vascular occlusive diseases (Reddy, 1997:153). According to McCully, children born with homocystinuria (high levels of homocysteine) died at a very young age with advanced atherosclerosis (McCully, 1969:111). Plasma homocysteine may have prothrombotic and artherogenic properties, which might explain the increased risk for vascular diseases. Taylor *et al.* (1999:8) showed that an increase of 1 μmol/L plasma homocysteine concentration could be associated with a 5.6 % increase in the possibility of death from vascular occlusive diseases.

The term plasma "Homocysteine", a sulphur-containing essential amino acid derived from dietary protein, is used to define the combined pool of mixed disulphides and thiolactone found in the plasma of people (Welch & Loscalzo. 1998:1042; Zamani, 2002:3). The nature and metabolism of homocysteine in the methionine process (MTHFR) can be explained as two divergent pathways, namely remethylation and transsulfuration (Welch & Loscalzo. 1998:1043). Both these pathways need vitamin B_6 for the methionine process to take place, as well as vitamin B_{12} and folic acid as cofactors. In the remethylation cycle homocysteine is salvaged through a methyl group that is catalyzed by the above-mentioned cofactors (B_{12}-dependant methione synthase) (Zamani, 2002:3). Two donors, N_5-methyltetrahydrofolate and the enzyme N_5, N_{10}-methyllenetetrahydrofolate reductase, also function in the pathway as catalysts. In the presence of excess methionine, homocysteine can enter the transsulphuration pathway, where homocysteine condenses with serine to form cysstathionine that is catalyzed by vitamin B_6-limiting enzyme and cystathionine β-synthase (Cortese & Motti. 2001:493). Cystathionine forms cysteine in a hydrolyzing action that is necessary for the syntheses of biological compounds like glutathione, which is an important intracellular thiol. Cystathionine and other sulf sulphur containing amino acids are then metabolized to water and sulphate and excreted in the urine thiol (Moustapha & Robinson. 1999:41). .

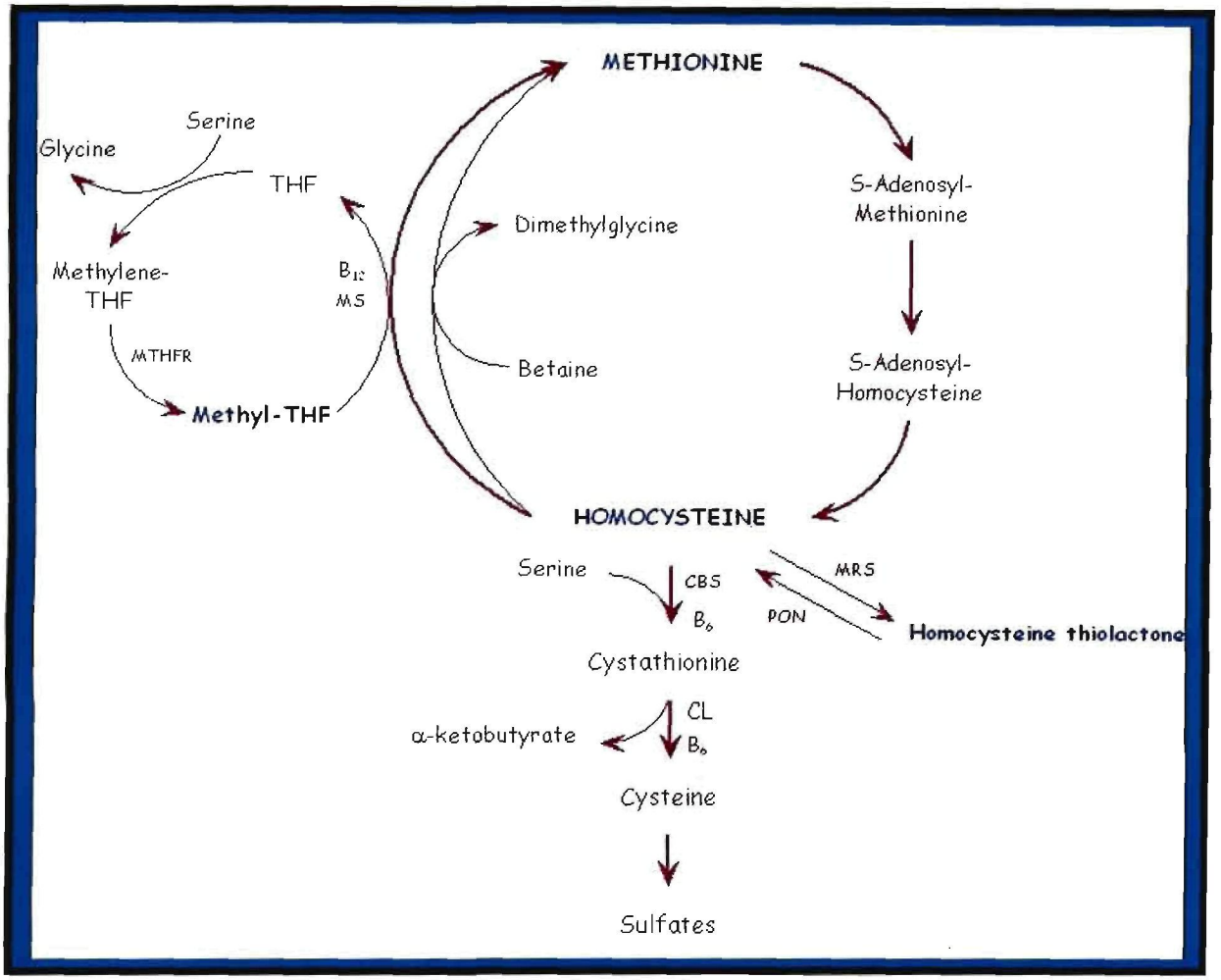

Figure 2.1. Metabolic pathway of Homocysteine (Zamani, 2002)

Normally plasma homocysteine concentrations are between 5-15 μmol/L but the mean value reported for plasma homocysteine is 10 μmol/L in the general population (Moustapha *et al.*, 1999:41; Dinavahi *et al.*, 2003:767). On the basis of fasting homocysteine values, moderate readings are (16-30 μmol/L), intermediate (31-100 μmol/L) and severe (>100 μmol/L), which is also characterized as hyperhomocysteinemia (Mallinow *et al.*, 1999:178)

Many researchers describe certain factors that may influence plasma homocysteine concentrations like genetic and non-genetic factors, specifically lifestyle factors (Welch & Loscalzo 1998:1043; Mousthapa & Robinson. 1999:41; Merouani *et al.*, 2001:805 & Zamani, 2002:3).

Genetic causes of hyperhomocysteinemia include homocysteine metabolism deficiencies, in the general population (Zamani, 2002:3). Metabolic deficiencies include conditions of: 1) enzymatic defects in the metabolic pathway (cystathione β-synthase deficiency), the most common enzymatic defect responsible for homocystinuria, and 2) MTHFR deficiency or its thermolabile variant, which is a form of genetic hyperhomocysteinemia, and methionine synthase deficiency or other rare enzymatic defects (Welch & Loscalzo. 1998:1042; Mousthapa & Robinson. 1999:41; Merouani *et al.*, 2001:805 & Zamani, 2002:3). These genetic mutations may lead to severe elevated plasma homocysteine concentrations and may cause hyperhomocysteinemia that may give an increased risk for various vascular diseases.

On the other hand, non-genetic causes, which elevate plasma homocysteine moderately include: dietary deficiencies of folate, Vitamin B_{12} and Vitamin B_6 deficiencies and lifestyle factors (Zamani, 2002:3). Markedly elevated plasma homocysteine concentrations have been observed in patients with nutritional deficiencies of the essential cofactor vitamin B_{12} and the co-substrate folate (Welch & Loscalzo. 1998:1043). Over the past decade studies have shown that dietary factors are of primary importance in the pathogenesis of arteriosclerosis, and homocysteine was only then described as a possible independent risk factor in the general population (Merouani *et al.*, 2001:805). This will, however, be discussed in more detail as part of the lifestyle factors.

Other causes of elevated homocysteine concentrations include factors like: renal failure, liver disorders and hypothyroidism, malignancies including breast, ovarian or pancreatic cancer in addition to all drugs interfering with the metabolic pathways deteriorating renal functions, and retarded and vitamin synthesis or reduced absorption of vitamins (Zamani, 2002:3). This state of hyperhomocysteinemia may be linked histopathologically with features of vessel injury, proliferation of vascular smooth muscle cells and inhibition of fibrinolysis and hemostatic changes of the prothrombotic state (Dinavahi *et al.*, 2003:767).

The focus of this literature review is to investigate the changes in total plasma homocysteine concentrations during the aging process of people from birth to adulthood and the influence of gender and ethnicity and lifestyle on the total plasma homocysteine concentrations.

2.3 INLUENCE OF AGE, GENDER AND ETHNICITY ON PLASMA HOMOCYSTEINE CONCENTRATION

2.3.1 Age

Plasma homocysteine concentrations can be influenced by certain determinants such as age, gender and ethnicity. As people age, plasma homocysteine concentrations can change and gender and ethnicity also seem to have an effect on plasma homocysteine concentrations (De Laet *et al.*, 1999:968; Jacques *et al.*, 1999:482 & Must *et al.*, 2003:2643 & Ganji & Kafai. 2005:2253).

A research study done on the cysteine concentration in older people, of three different age groups, 40-42 yr, 43-64 yr and 65-67 yr, has shown that the aging process influenced homocysteine concentrations and displayed higher levels. The cysteine distribution ranged between 72.2 μmol/L to 441.3 μmol/L with an overall mean value of 270.2 μmol/L and 268.0 μmol/L respectively for the groups (El-Khairy *et al.*, 1999:1018). These changes may be due to age related factors, such as a decrease in enzymatic activities, involved in cysteine and homocysteine metabolism as well as renal failure (Nordstrom & Kjellstrom 1992:213; Moustapha *et al.*, 1999:41). Another factor that influences homocysteine concentration levels in the elderly, is low blood folate concentrations and a vitamin B_{12} deficiency which results of malabsorption in the digestive system (Ganji & Kafai 2003:830).

2.3.2 Gender

When investigating the role of gender on homocysteine, some researchers reported higher plasma homocysteine concentrations in men than in women (14.5 μmol/L and 10.8 μmol/L) respectively. Plasma homocysteine differences $\geq$ 15 μmol/L was not significant (Chysohoou *et al.*, 2004:119 & Mildred *et al.*, 2004:305). Homocysteine concentrations might be affected by gender when hormonal effects are taken into account. Plasma homocysteine concentration may be higher in men than in women because of their increased muscle mass which is a source of homocysteine formation (creatine-creatinine synthesis) (Brauttstrom *et al.*, 1994:635). According to El-Khairy. (1999:1020), the hormonal effect on homocysteine vanishes as people get older, especially in men. Further research studies have also shown that

homocysteine concentrations are higher in men than in women because of differences in muscle mass and the hormone estradiol concentrations (Dierkes *et al.,* 2001:640 & Rasmussen & Moller 2001:627)

2.3.2 Ethnicity

Sacco *et al.,* (2004:105) suggested that homocysteine concentrations might vary between ethnic groups, because of different genetic profiles within ethnic groups. Plasma homocysteine concentrations of white hispanic subjects were found to be higher than normal values.

Plasma homocysteine concentration found in black women (8.80 μmol/L) were higher than in white women (7.8 μmol/L) by (≥ 1.0 μmol/L). Due to these racial differences black women may have an increased risk for cardiovascular diseases (Gerhard *et al.*, 1998:1043). In contrast to the above results, plasma homocysteine concentrations were higher in South-African white men (12.0 μmol/L) than in Venda men (9.7 μmol/L) respectively. Furthermore, Ubbink *et al.* (1996:1255) indicated that the plasma homocysteine concentration was lower (5.1 μmol/L) in 7 to 15 year old South-African white children (boys and girls), compared to homocysteine concentrations of Venda children (5.8 μmol/L) of the same age.

Published data on plasma homocysteine concentration and age, gender and ethnicity are very rare and the existing data are equivocal and do not permit general conclusions. Main findings from studies that established certain relationships between plasma homocysteine concentrations and variables such as age, gender and ethnicity are summarized in Table 1 and homocysteine concentration distribution is also presented graphically in Figures 2.2 – 2.4.

Table 2.1. Homocysteine concentrations for different ages, genders and ethnic groups

AUTHOR	SUBJECTS	SUBJECT CHARACTERISTICTS	STUDY LIMITATION	AGE	GENDER	ETHNICITY	HCY µmol/L
De Laet *et al.*, 1999	n = 647 353girls 294boys	Children Adolescents	Sample size too small for signif. diff between sexes	1. 5-9y 2. 10-14y 3. 5-19y	Girls Boys	Belgian pediatric population	1. All-6.21 G-6.11 B-6.30 2. All-7.09 G-7.07 B-7.12 3. All-8.84 G-8.33 B-9.78
Jacques *et al.*, 1999	n = 40000	Adolescents Adults	No Reference data	12-15y 16-19y 20-29y 30-39y 40-49y 50-59y 60-69y 70-79y >80y	Girls Boys Female Male	NHW NHB MA	See FIgure 3.1
Must *et al.*, 2003	n = 40000	Children Adolescents	No Reference data	4-5y 6-11y 12-15y 16-19y	Girls Boys	NHC NHAA MA	See Figure 3.2
Ganji *et al.*, 2005	n =6461	Children Adolescents	No Reference data	All <4y 4-7y 8-11y 12-15y 16-18y	Girls Boys	NHW NHB MA	See Figure 3.3

* NHW = non Hispanic whites; NHB = non Hispanic blacks; MA = Mexican Americans; NHAA = Non haspanic African American; MA = Mexican American

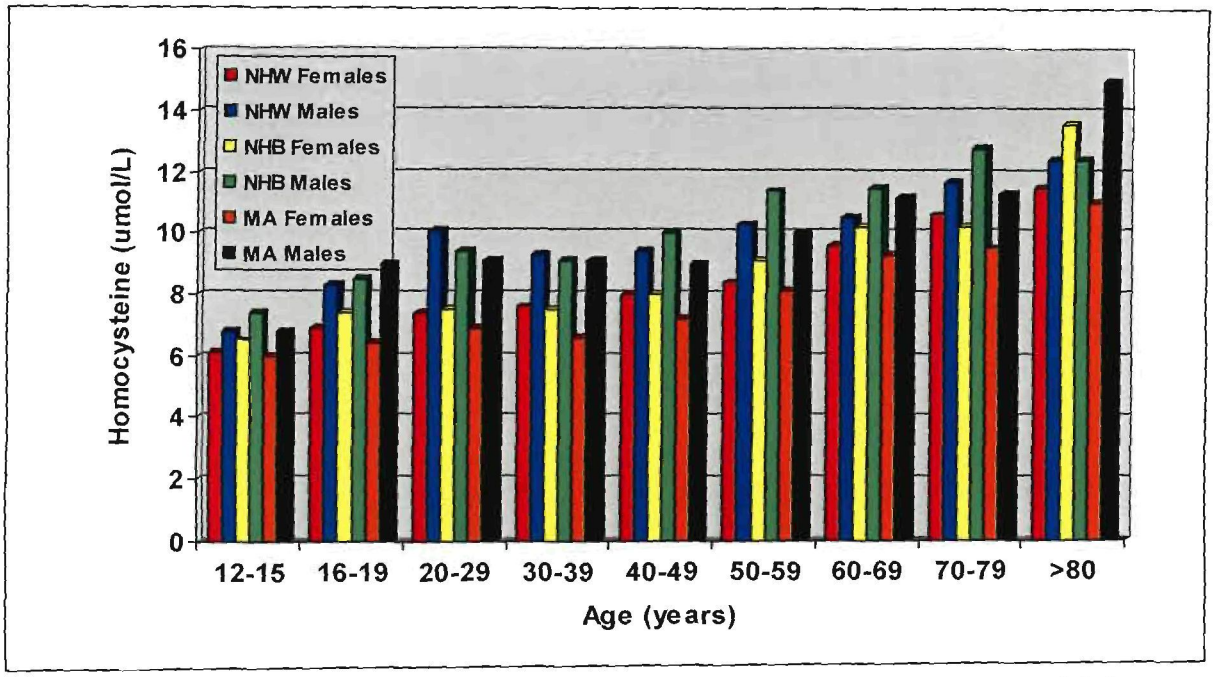

Figure 2.2: **Changes in Hcy concentrations between different ethnic groups with increase in age (Jacques *et al.*, 1999:484), NHW = non Hispanic whites; NHB = non Hispanic blacks; MA = Mexican Americans**

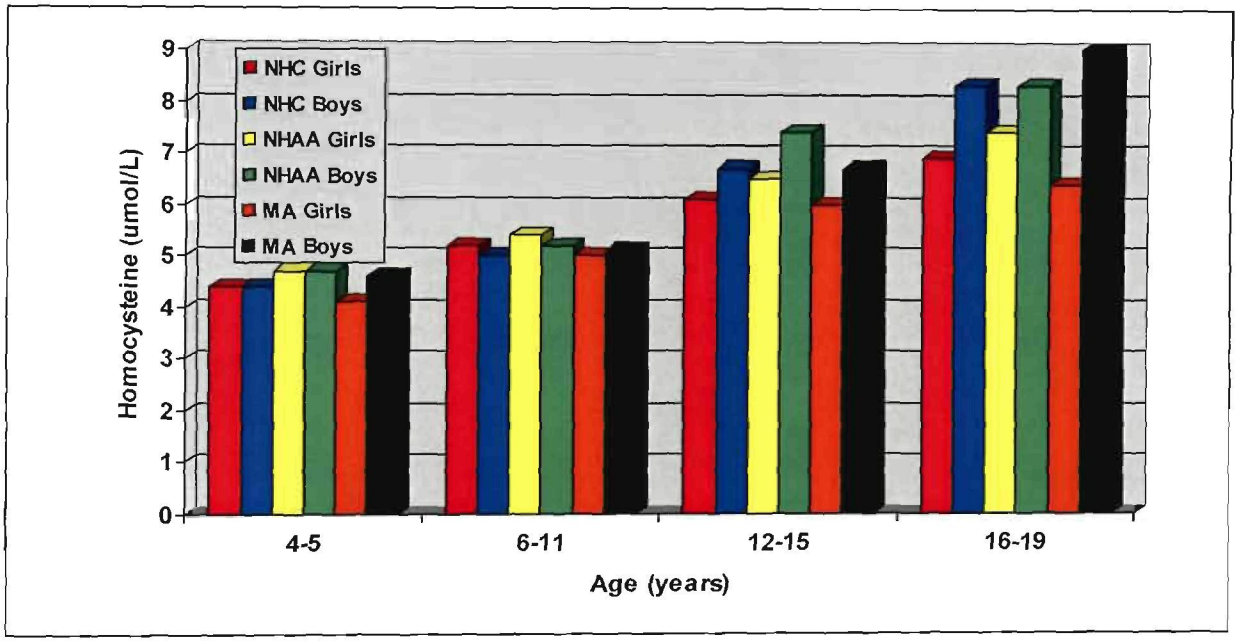

Figure 2.3: Changes in Hcy concentrations between different ethnic groups with increase in age (Must *et al.*, 2003:2645), NHC = non Hispanic Caucasian; NHAA = non Hispanic African American; MA = Mexican Americans

A comparison that was made between homocysteine concentration distribution over age, gender and ethnicity was limited due to the limited information available reporting on homocysteine concentration over life span. Sample sizes of the studies investigating homocysteine concentrations ranged from 600 - 40000 subjects. All studies included subjects ranging from the age of 4 years to over 60 years of age. Both males and female subjects were included, as well as ethnic different populations. Three of the studies reported on subjects between the ages of 5-19 years and one of the studies reported on subjects between the ages of 12-80 years. Three of the studies reported on children and adolescents and the other one used adolescents and adults for homocysteine concentration distribution (De Laet *et al.*, 1999:969; Must *et al.*, 2003:2644). Ganji & Kafai (2005:2253-2254) investigated the changes after food fortification with folic acid was introduced (Fig 2.4) homocysteine still increased with an increase in age but the baseline levels were lower.

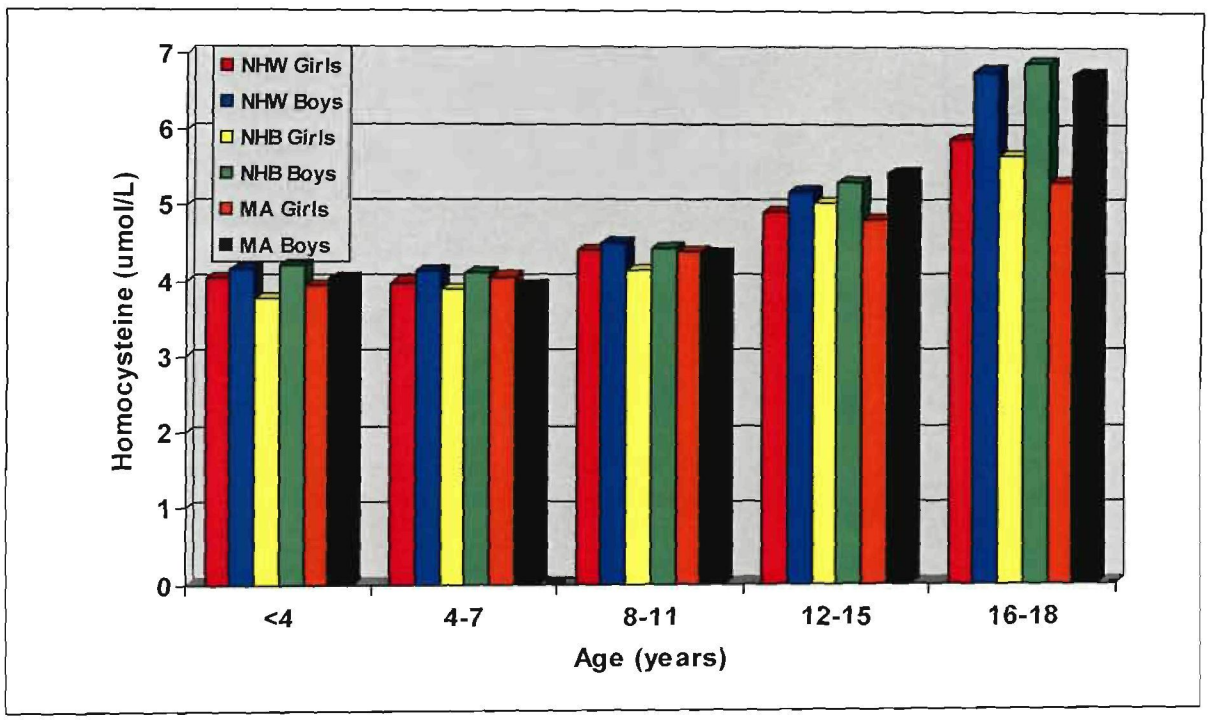

Figure 2.4: Changes in Hcy concentrations between different ethnic groups with increase in age (Ganji & Kafai. 2005:2254), NHW = non Hispanic Whites; NHB = non Hispanic Blacks; MA = Mexican Americans

An overview of the above-mentioned studies indicates homocysteine concentrations to be the lowest in young children 4–9 years, and that plasma homocysteine concentrations increase with age. A notion was supported that circulating plasma homocysteine concentrations increase between the ages of 8-11 years, and the age related increase is greater in the boys than in the girls (Ganji *et al.*, 2005:2255). De Laet *et al.* (1999:972) found no significant differences in plasma homocysteine concentrations between girls and boys in children aged <15 years; however, the concentrations were again overall higher in boys than in girls especially in post pubertal children. This appearance can be due to genetic, nutritional and endocrine factors that play a role in homocysteine concentration. According to Jacques *et al.*(1999:485) and Must *et al.* (2003:2655) no significant ethnic differences were found between the racial groups they have studied although there was an age and gender related difference.

Age and gender differences are evident and ethnicity seems to play a role between different racial groups. The exact mechanisms involved and manner in which homocysteine concentrations are influenced must still be investigated properly. Nevertheless, other factors like physical activity, dietary habits and significant lifestyle factors can also influence plasma homocysteine concentrations.

2.4 INFLUENCE OF LIFESTYLE FACTORS ON PLASMA HOMOCYSTEINE

Present lifestyle factors can have an elevating effect on plasma homocysteine concentrations. These factors include physical activity, fitness, diet, smoking and alcohol consumption, which are also factors that may lead to an increased risk for heart disease (Nygard *et al.*, 1997:239). To comprehend the influence of the above mentioned lifestyle factors on homocysteine concentrations, each lifestyle factor will be discussed individually in the following sections.

2.4.1 Physical activity

Physical activity has been conformed as a modifier of risk factors for cardiovascular heart diseases, but physical activity as a role player in changing total plasma homocysteine concentrations has resulted in controversial findings. There is, however, limited epidemiological evidence linking regular physical activity to lower plasma homocysteine concentrations (Ali *et al.*, 2000:49). These results vary for acute physical activity interventions to training interventions with a 12% reduction in homocysteine concentrations (Ali *et al.*, 2000:49). The impact of physical activity on plasma homocysteine concentrations appears to be based on fitness levels, nutritional status and other factors that are not accounted for in certain studies. Primary variables of physical activity that may influence homocysteine may be mode, intensity and duration of exercise, which might explain the inconsistencies in the plasma homocysteine concentrations of subjects (Joubert & Manore. 2006:355).

Different types of exercise intervention have been investigated. Main findings of certain studies suggest a link between acute and chronic exercise and changes in homocysteine concentrations (Wright *et al.*, 1998:264; Hermann *et al.*, 2003(a):1519; Herrmann *et al.*, 2003(b):1526; Konig *et al.*, 2003:115 & Joubert *et al.*, 2006:355). These investigations have been either acute exercise defined as an episode of physical activity lasting between 10 to 210 min, or chronic exercise defined as a physical activity program, lasting 10 days or more of regular physical activity (Joubert *et al.*, 2006:355).

Sample size of these studies ranged between 20 and 40 subjects with ages between 16 and 60 years. In all the studies, aerobic exercise interventions were investigated. Two studies supplied cycling (Wright *et al.*, 1998:264; Konig *et al.*, 2003:115), one swimming (Herrmann *et al.*, 2003(b):1526) and another running (Herrmann *et al.*, 2003(a):1519) as intervention modality. The duration of the intervention programme varied from 30 minutes (Wright *et al.*, 1998;264), to a 3 week orientated 30 km/week and a high intensity endurance 20 km/week training programme in preparation for a swimming competition (Herrmann *et al.*, 2003(b):1526). Another intervention consisted of a 28-day acute and endurance-training regimen (Konig *et al.*, 2003:115).

According to Wright *et al.* (1998:264), exercise had no effect on homocysteine concentrations after acute exercise, although hemoconcentration affected concentrations slightly but it was not a significant increase of homocysteine, whereas Konig *et al.* (2003:115) found an elevation in homocysteine concentrations after an acute physical exercise. Major problems regarding acute exercise are that the studies have to indicate that homocysteine concentrations presented are corrected for exercise-induced shifts of plasma volume. Acute exercise studies take the measurements for haemodilution or concentrations as a consequence of acute exercise into account. It is evident that further research investigating the exact influence of both acute and chronic exercise on homocysteine concentrations is lacking.

The effect of chronic exercise intervention on plasma homocysteine concentrations included aendurance training and the duration of the exercise sessions ranged from a 3 to a 4-week training regimen (Herrmann *et al.*, 2003:1523(a); Konig *et al.*, 2003:115). Herrmann *et al.*

(2003(b):1531) found a prolonged increase in plasma homocysteine concentrations after a strenuous endurance-training programme while Konig *et al.* (2003:115) showed that plasma homocysteine concentrations decrease after an extensive period of endurance training. This suggests that both higher training volume and increased plasma folate levels affect homocysteine concentrations, but then they attenuate the increase in homocysteine levels after acute physical exercise. Further investigations are still needed.

2.4.2 Diet

Dietary habits may alter plasma homocysteine concentrations. Chrysohoou *et al.* (2004:120) observed a significant inverse correlation between homocysteine concentrations with fruit ($r = -0.15$), vegetables ($r = -0.15$) and grain products ($r = -0.25$), but extensive research is still needed on population diets. In turn, supplementation plays a big part in people's diets, particularly vitamin supplementation. An significant correlation was found between multivitamin supplementation and plasma homocysteine concentrations (Giles *et a.,* 1999:310; Chrysohoou *et al.,* 2004:120). People who regularly consumed multivitamins were 60% less likely to have elevated plasma homocysteine concentrations than those who did not consume multivitamins containing folic acid, vitamin B_6 and vitamin B_{12} (Giles *et al.*, 1999:310). Folic acid had an inverse relationship with plasma homocysteine levels (Kalita *et al.*, 2007:118). But the reduction of plasma homocysteine levels can reach a plateau when folic acid intake approaches 400 μmol/L a day. Just 200 μmol/L/day results in a 4 μmol/L difference in total plasma homocysteine (Yajnik *et al.,* 2006:775). Folic acid is present in most food but especially in meats, vegetables and cereals (Yajnik *et al.*, 2006:775; Selhub & Jacques. 1993:2693).

Bonaa *et al.*, (2006:1586) reported that people who already had a cardiovascular incident would not have a lowering effect of plasma homocysteine concentrations with B vitamin and folic acid interventions. To use supplementation as secondary prevention treatment to cardiovascular incidents would not be recommended to lower high concentrations of plasma homocysteine, although it seems that plasma homocysteine is not the causative agent of vascular disease. Plasma homocysteine at higher levels may be an indicator of an unhealthy lifestyle and a epiphenomen reflecting atherogenic processes, or a consequence of vascular disease itself (De Craen *et al.*, 2006:210 & Tomlinson *et al.*, 2006:210). The HOPE-2 trail suggests that the use of

B vitamin supplementation may protect against the risk of strokes, but only after the introduction of folic acid fortification in food (Yang *et al.*, 2006:1335).

It seems that plasma homocysteine concentrations may change with a healthier diet as well as with increases in the intake of folate and vitamin B_{12}. Whether if plasma homocysteine is the agent to focus on for the prevention of cardiovascular diseases is a question that remains unanswered.

2.4.3 Smoking

Elevated plasma homocysteine concentrations were positively associated with cigarette smoking. Ganji *et al,* (2003:832) did the first study on people older than 17 years and found a positive association between serum cotinine (a metabolite of nicotine) concentration and serum homocysteine concentration. In another study by Chrysohoou *et al.* (2004:119), a significant dose response relation was observed between plasma homocysteine concentration and the daily number of cigarettes smoked. A 0.7 μmol/L elevation in plasma homocysteine concentration was observed for every 10 cigarettes smoked per day for men and a 0.3 μmol/L elevation for women per 10 cigarettes smoked per day (Chrysohoou *et al.*, 2004:119)

The mechanism by which cigarette smoking increases plasma homocysteine concentrations is unclear. It might be explained by low concentrations of blood folate, vitamin B_6 and vitamin B_{12} in smokers (De Bree *et al.*, 2001:152; Ganji *et al.,* 2003:831). In addition, smoking doesn't reduce the availability of folate for the remethylation of homocysteine to methione, but either induces local effects in cells exposed to cigarette smoke. This, however, changes the plasma thiol redox status meaning that homocysteine is an aminothiol, which inhibits the action of enzymes such as methione synthase (Piyathilake *et al.*, 1992:566; Pryor *et al.*, 1993:12; Mansoor *et al.*, 1995:232; Bergmark *et al.*, 1997:1997 & Blom. 1998:188). Extensive research is needed to determine the exact mechanism by which homocysteine levels are influenced by smoking habits of people.

2.4.4 Alcohol consumption

As with smoking, a positive association was reported between alcohol intake per day and total plasma homocysteine concentrations (Van der Gaag *et al.*, 2000). Gender differences were also found in the alcohol consumption habits of people, with men consuming more alcohol than women (Chysohoou *et al.*, 2004:120). The alcohol consumption of ≤1 drink/day may not adversely influence plasma homocysteine concentrations. Hard-liquor consumption can be a significant predictor of plasma homocysteine but the same was not true for wine and beer. Acute alcohol intoxication with acetaldehyde, a metabolite of the alcohol metabolism, exerts an inhibitory effect on methionine synthase, which is essential for the remethylation of plasma homocysteine to methionine. The presence of increased plasma homocysteine concentrations can be explained by low circulation concentrations of folate, vitamin B_6 and Vitamin B_{12} in chronic alcoholics (Ganji *et al.*, 2003:832). The influence in the adolescent population is not known and more research is needed.

2.5 SUMMARY

Plasma homocysteine concentrations change over the lifespan of people. If these changes take place it can lead to an increased risk for cardiovascular diseases. It was found that plasma homocysteine concentrations changes depend on factors like age, gender and ethnicity. The literature indicates age related changes in plasma homocysteine concentrations as well as in gender and ethnicity.

Lifestyle factors such as physical activity, diet, smoking and alcohol consumption influence plasma homocysteine concentrations. Physical activity as a role player on plasma homocysteine changes remains controversial due to the limited number of research studies that exist. Diet, smoking and alcohol consumption have an elevating effect on plasma homocysteine concentrations but further extensive research is still needed. Exact mechanisms by which these lifestyle factors influence plasma homocysteine concentration are still elusive.

The lack of knowledge on homocysteine in young persons may be complemented by further investigations in specific ethnic populations. It is also of importance to monitor smoking, alcohol consumption and physical activity habits to adjust for confounders.

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CHAPTER 3

CHANGES IN HOMOCYSTEINE FOLLOWING A PHYSICAL ACTIVITY INTERVENTION

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ABSTRACT

BACKGROUND: Plasma homocysteine may be an independent risk factor for cardiovascular diseases. Plasma homocysteine was also described as a risk factor for the prevalence of stroke especially in the black population. The purpose of the study is therefore to determine baseline concentrations of homocysteine in black adolescents from the North - West as well as the influence of a physical activity intervention on the homocysteine concentrations. **METHODS:** An intervention study was done on black adolescent boys and girls consisting of a control group and experimental group that were subjected to baseline testing as well as an intervention programme. The tests included homocysteine analysis, anthropometric measurements and cardio respiratory fitness. After baseline testing the experimental group followed a 10-week physical activity intervention programme of aerobic and resistance training wereafter the groups were retested. Statistical analysis consisted of descriptive characteristics of subjects, significant correlations and t-tests to describe the change of plasma homocysteine concentrations during the intervention. An Anova was also performed for the percentage change for plasma homocysteine and the compliance of the group. **RESULTS:** Descriptive statistics indicated the baseline homocysteine concentrations between 5.93 (± 0.92) μmol/L and 7.03 (± 1.67) μmol/L. A significant difference was found between plasma homocysteine and muscle mass ($r = 0.25$; $p = 0.00$). As a result of the intervention, cardio - respiratory fitness increased significantly in the experimental group. Plasma homocysteine increased in the experimental group by about 1% and in the control group by about 15%. **CONCLUSION:** The plasma homocysteine concentrations in black adolescents are within the normal range. Moderate attendance during the physical activity intervention indicated a significant decrease in the plasma homocysteine concentrations compared to very low levels and very high levels of physical activity.

Key words: Cardiovascular diseases, risk factor, decrease

INTRODUCTION

Plasma homocysteine and amino acid derived from dietary protein have been described as possible independent risk factors for various cardiovascular diseases and most recently the incidence of stroke (Reddy, 1997; Casas *et al.*, 2005). High concentrations of circulating plasma homocysteine can be related to cardiovascular diseases because of the prothrombotic and atherogenic properties (Zamani, 2002).

The recommended normal plasma homocysteine concentrations are 5 – 15 μmol/L (Dinavahi *et al.*, 2003). Plasma homocysteine is influenced by factors such as age, gender and ethnicity. As people age their plasma homocysteine concentration may increase according to investigative studies (Nordstrom & Kjellstrom. 1992; El-Khairy *et al.*, 1999 & Moustapha & Robinson. 1999). Gender differences also affect plasma homocysteine concentrations. Higher concentrations are reported in males compared to females due to hormonal effects and a larger muscle mass (Dierkes *et al.*, 2001; Rasmussen & Moller. 2000 & Chrysohoou *et al.*, 2004). Ethnicity plays an important role in plasma homocysteine concentrations. Blacks tend to have a higher concentration of plasma homocysteine than whites (Gerhard *et al.*, 1998). This may be because of different genetic profiles within ethnic groups but extensive research is still needed (Gerhard *et al.*, 1998; Ubbink *et al.*, 1996 & Sacco *et al.*, 2004:105).

Other factors that may determine the plasma homocysteine concentrations in people are lifestyle factors. These factors include physical inactivity, diet and smoking habits. Physical activity can be a possible modifier for plasma homocysteine concentrations. According to Joubert & Manor, (2006), the mode, intensity and duration of exercise is the primary determinant when physical activity is used as a modifier of plasma homocysteine concentrations. Limited research exists on physical activity intervention and plasma homocysteine concentrations. Study outcomes concluded that acute exercise leads to increases in plasma homocysteine (Wright *et al.*, 1998; Herrmann *et al.*, 2003a) whereas endurance exercise decreases plasma homocysteine, but over a extensive time period (Herrmann *et al.*, 2003b; Konig *et al.*, 2003).

A healthy diet can contribute to lower plasma homocysteine concentrations (Chryssohoou *et al.*, 2004). Supplementation of vitamin B_6 and B_{12} and folate (Giles *et al.*, 1999) leads to a reduction in plasma homocysteine concentrations (Clarke & Armitge. 2000). Higher intake of theses vitamins and folate can have a lowering effect on plasma homocysteine concentrations, which in turn may protect against the risk of cardiovascular diseases and stroke (De Crean *et al.*, 2006; Yang *et al.*, 2006 & Bonaa *et al.*, 2006).

Another lifestyle factor that influences plasma homocysteine is smoking. A dose - response relation was observed between plasma homocysteine and cigarette smoking, indicating a plasma homoocystiene increase of 0.7 μmol/L for men and 0.3 μmol/L for women for every 10 cigarettes smoked (Chrysohoou *et al.*, 2004). However, the nature by which these factors increase plasma homocysteine concentration is still unclear.

If these lifestyle factors can be modified, the risk for cardiovascular diseases and stroke can be lowered. Furthermore, a relationship was found between the prevalence of stroke and high plasma homocysteine, especially in black people. According to Giles *et al.* (1999), black women have a higher level of plasma homocysteine than white women that in turn increased the risk for stroke. Above-mentioned study was, however, done on an older population and not on adolescents.

The high association between stroke and black subjects is an indication that research is needed to determine the role of plasma homocysteine and its association of strokes. The purpose of this study is therefore to determine baseline concentrations of homocysteine in black adolescents from the North - West as well as the influence of a physical activity intervention on the plasma homocysteine concentrations. The results of this study will give an indication of the adolescent population risk for hyperhomocysteinemia, which may lead to stroke.

METHODS

Study design

The PLAY – Study (Physical Activity in the Young) investigates whether a physical activity intervention programme will reverse stunting in black adolescents longitudinal. This study will focus on the homocysteine concentrations in black adolescents in the North - West as well as the influence of a physical activity intervention on plasma homocysteine concentrations. The study is a parallel intervention study with a control group and an experimental group. Both groups were subjected to baseline testing. The experimental group followed a 10-week physical activity intervention programme, while the control group continued with normal daily activities and lifestyle. Both groups were retested after the physical activity intervention.

Subjects

About 183 black adolescents between the ages of 14 -18 years, who were apparently healthy and had similar socio-economic status, were recruited for tthis study. All subjects originated from two secondary schools in the Pothchefstroom area in the North-West province. Each pupil's guardian(s) or parent(s) signed an informed consent letter. The Ethics Committee of the North - West University approved the study and ethical codes for the handling of pupils and blood samples were strictly followed (code 04M01). Baseline measurements were obtained over a one-week period.

Procedures

All subjects were requested to fast for at least 10 hours before arrival at the test station. Blood samples were taken immediately upon arrival, followed by anthropometric measurements (length, weight, skin folds and circumference). The stature of the subjects was measured with a stadiometer to the nearest 0.1 cm and body mass was measured with an electronic weighing scale to the nearest 0.1 kg. The body mass index (BMI) was calculated with the height and weight measurements. The waist and hip circumference was used to determine the waist/hip ratio. Skin

folds that were measured were triceps, subscapula, medial calf, abdominal and supraspinale skin folds, according to the International Society for the Advancement of Kinanthropometry standards (ISAK, 2001) from which the body composition was calculated (Jackson *et al.*, 1978).

Cardio respiratory fitness was performed by means of the 20 m – shuttle run (Leger & Lambert, 1982). The objective of this multi-stage fitness test (MSFT) was to measure the maximum oxygen uptake (VO_2max) of each subject indirectly. The test consisted of 23 levels that lasted approximately one minute each. A 20 m section was measured and each end marked. The subject then placed one foot beyond the 20 m marker at the end of each shuttle. If the subject arrived at the end of a shuttle before the bleep, he/she had to wait for the bleep and then resume running. The subject kept on running until he/she was unable to keep up with the pace increase. If the subject failed to reach the end of the shuttle before the bleep the test was ended. The number of shuttles completed was then recorded from which the indirect VO_2max was calculated (www.topendsports.com/testing.beepcalc.htm) (Ramsbottom *et al.*, 1988). After the baseline measurements were taken a 10-week physical activity intervention programme was followed after which the baseline tests were repeated.

Physical activity intervention

The intervention programme consisted of aerobic type exercises for 40 minutes, targeting large muscle activity at 70% of the subject's age predicted maximal heart rate (Karvoven *et al.*, 1957). This was followed by a 10-minute period of stretch exercises to improve flexibility. The major muscle groups were stretched and three sets of 30 seconds intervals were performed. Finally, a 10-minute period of muscle endurance against body weight completed the exercise session. These activities were performed in sets of three with 20 repetitions of each exercise per set. Exercises like push-ups and squats were performed to strengthen the large muscle groups. The intervention programme took place three times a week under the supervision of Biokineticists-in-training. Participant compliance was recorded in a register. Accelerometers were randomly placed on subjects to determine compliance with the required intensity levels (Puyau *et al.*, 2004) of the physical activity intervention programme.

Blood analyses

Blood samples were collected at baseline measurements and after the 10-week intervention programme. Subjects fasted for 10 hours before the blood samples were taken. Twenty milliliter venous blood was taken from the *vena cephalica* for EDTA blood. The EDTA blood samples were immediately placed on ice and centrifuged for 15 minutes within one hour of sampling to yield plasma for the analyses of total plasma homocysteine. Aliquots were stored at –80°C until the analyses were done. Homocysteine concentrations were determined by means of the ELISA-method (Abott AXSYM).

Statistical analyses

The data were analyzed using SPSS (Vers 15.0). Descriptive statistics were used to describe the baseline characteristics of the subjects in the control and experimental groups for the boys and the girls respectively. Significant differences between the control and experimental groups at baseline were determined by an independent t-test. Spearman Rank and Pearson correlations were used to examine the relationships between the homocysteine concentrations, body composition and physical activity at baseline. One - way t-test was used to determine the plasma homocysteine changes (Thomas & Nelson, 2001). The level of significance was set at $p < 0.05$ for all analyses. The influence of different levels of compliance to the physical activity intervention programme on homocysteine concentrations was determined with an ANOVA.

RESULTS

The baseline characteristics (Table 3.1) indicated that the age for the experimental group and control group ranged between 14 and 16 years. The average age of the boys in the experimental group was 16.08 (± 0.52) years and that of the boys in the control group was 14.67 (± 0.84) years. The average age of the girls in the experimental group was 15.73 (± 0.38) years and that of the girls in the control group 14.83 (± 1.01) years.

Boys in the experimental group had a mean BMI of 18.87 (± 2.57) kg/m^2 and the boys in the control group had a BMI of 18.00 (± 1.51) kg/m^2. The girls' BMI value was higher than that of the boys but no statistical significant difference was found (BMI: girls, experimental = 20.47 (± 3.26) kg/m^2, control = 20.72 (± 3.17) kg/m^2; boys, experimental = 18.87 (± 2.57) kg/m^2; control = 18.00 (± 1.51) kg/m^2). The WHR between the experimental and control group was statistically significant. WHR of the girls was higher than that of the boys [WHR: girls, experimental = 0.77 (± 0.03), control = 0.71 (± 0.04); boys, experimental = 0.84 (± 0.05); control = 0.8 (± 0.57)].

Percentage body fat of the boys in the experimental group was lower 17.97 % (± 6.19) than the percentage body fat of the boys in the control group, 21.44 % (± 9.3). The girls in the experimental group and the control group had percentages of body fat of 29.16 % (± 6.59) and 29.4 % (± 7.8) respectively. The percentage body fat of all the groups did not differ statistically significant.

Plasma homocysteine concentration for the boys in the experimental group was 7.03 (± 1.67) µmol/L and for the control group 5.93 (± 0.92) µmol/L. Plasma homocysteine concentrations for the girls in the experimental group was 6.10 (± 1.46) µmol/L and for the control group 6.22 (± 0.93) µmol/L. The average plasma homocysteine concentrations of all the subjects were within the recommended range for homocysteine of 5 – 15 µmol/L (Reddy, 1997; De-Laet *et al.*, 1999; Dinavahi *et al.*, 2003 & Ganji & Kafai. 2005).

The results of the cardio - respiratory fitness - test indicated that the VO_2max value for the boys was higher than the VO_2max for the girls. Indirect VO_2max of the boys in the experimental group was 34.13 (± 6.77) ml/kg/min and of the boys in the control group was 32.84 (± 8.59) ml/kg/min. The girls' values for the experimental group were 24.59 (± 4.03) ml/kg/min and for the control group 23.89 (± 5.46) ml/kg/min respectively. The VO_2max values of the girls were statistically significantly different from the VO_2max values of the boys.

Table 3.1: Descriptive statistics of the subjects at baseline for the experimental and control schools (mean and ± standard deviation)

VARIABLES	EXPERIMENTAL				CONTROL			
	N	BOYS	N	GIRLS	N	BOYS	N	GIRLS
Age (yr)	106	16.08 (±1.52)	106	15.73 (±1.38)	18	14.67 ±0.84	42	14.83 (±1.01)
Mass (kg)	104	50.27 (±9.58)	103	49.19 (±8.29)	17	45.51 (±6.09)	41	50.56 (±9.53)
Height (cm)	**104**	162.70 (±8.65)	103	155.01 (±6.09)	17	158.81 (±8.99)	41	155.81 (±7.37)
BMI (kg/m²)	**104**	18.87 (±2.57)	103	20.47 (±3.26)	17	18.00 (±1.51)	41	20.72 (±3.17)
Waist (cm)	**99**	66.40 (±5.38)	103	64.64 (±5.96)	17	63.98 (±3.31)	41	64.29 (±6.04)
Hip (cm)	**98**	78.90 (±6.25)	103	84.02 (±6.67)	17	80.05 (±5.39)	41	90.23 (±8.21)
WHR	**98**	**0.84[a] (±0.05)**	**102**	**0.77[a] (±0.038)**	**18**	**0.8[b] (±0.57)**	**41**	**0.71[b] (±0.04)**
Fat (%)	74	17.97 (±6.19)	96	29.16 (±6.59)	9	21.44 (±9.2)	38	29.45 (±7.48)
Muscle mass (kg)	**74**	40.70 (±8.12)	96	34.64 (±4.52)	9	34.01 (±5.69)	38	33.76 (±6.79)
VO_2max (ml/kg/min)	**102**	**33.73[c] (±7.48)**	**101**	**24.59[c] (±4.03)**	**19**	**32.84[d] (±8.59)**	**40**	**23.89[d] (±5.46)**
Hcy (µmol/L)	**65**	7.03 (±1.67)	83	6.10 (±1.46)	9	5.93 (±0.92)	30	6.22 (±1.52)

BMI = Body mass index; WHR = Waist hip ratio; Fat % = Fat percentage; ml = milliliter; VO_2 max = Maximum oxygen uptake ml/kg/min = milliliter/kilogram/minute; N = subjects; Hcy = Homocysteine, a.b.c.d = similar symbols indicate statiscal significant difference ($p < 0.05$)

The results of the Spearman Rank correlations between plasma homocysteine, anthropometric measurements and cardio - respiratory fitness, for the total group indicated a statistically significant correlation between plasma homocysteine and muscle mass ($r = 0.25$; $p = 0.00$).

The influence of the physical activity intervention progamme expressed as the percentage change from baseline to end for the measured variables (Figure 3.1). BMI and WHR did not change significantly although the experimental boys and girls indicated an increase in BMI [BMI: boys, experimental = 1.44 % (± 2.93), control = 0.47 % (± 2.06); girls, experimental = 1.58 % (± 3.31); control = 0.33 % (± 4.22)]. WHR in the control group for both boys and girls increased while the WHR of the experimental group decreased. Although the percentage body fat for all the groups decreased, it was not a significant decrease. Muscle mass increased during intervention but the percentage change was not significantly different between the groups. The percentage change in plasma homocysteine concentration indicated an increase for all the groups. The increase in the control group was more than 15 %, while the plasma homocysteine concentrations of the experimental group only increased with about 1 %. The percentage change of cardio respiratory fitness for the boys was 9.11 % and for the girls was 5.18 % for the experimental group while the fitness of the control group decreased with 19.24 % for the boys and slightly increased 0.31 % for the girls. Experimental group fitness increased while the control group fitness decreased. The changes in fitness for the boys and girls of the control group differed statistical significantly.

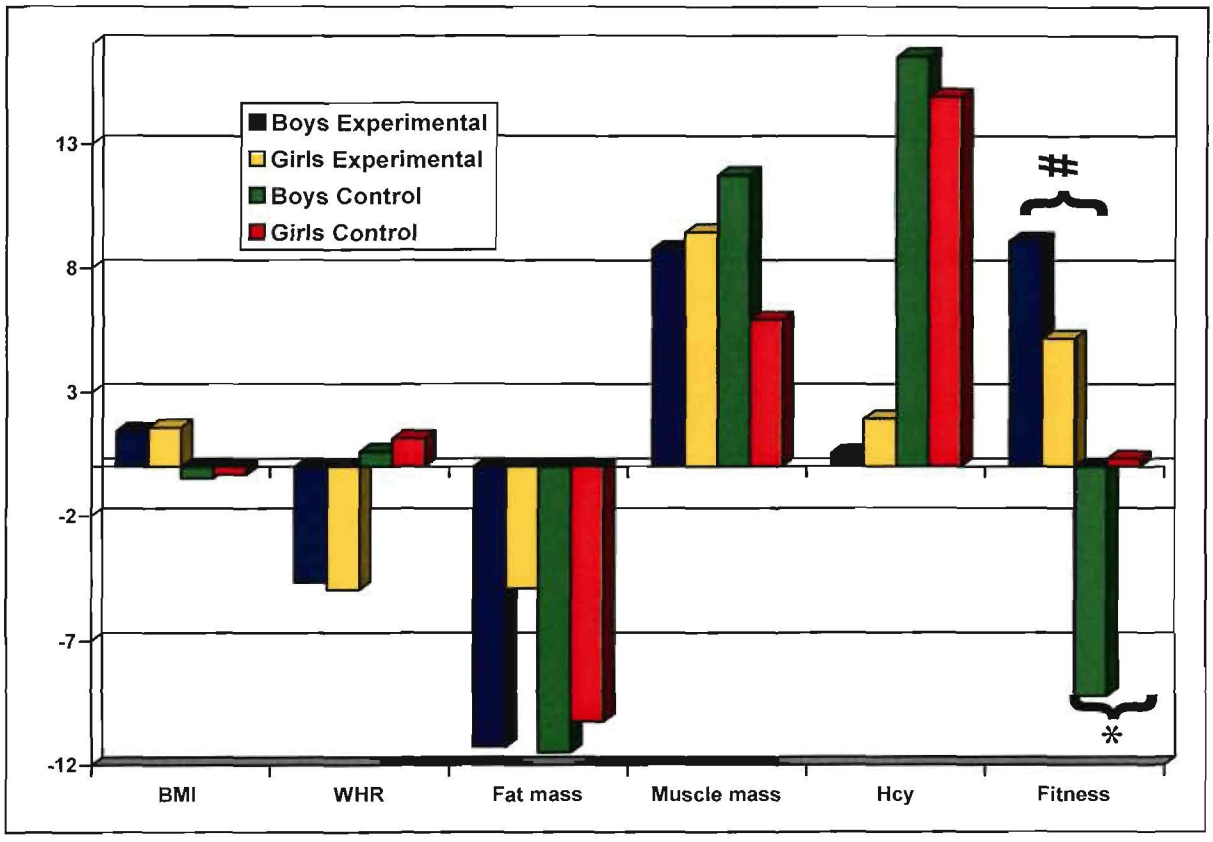

Figure 3.1: Percentage changes in the variables between boys and girls of the experimental and control group.

The mean percentage attendance of the experimental group boys and girls were 24.55 ± 24.72 % and 24.91 ± 27.13 % respectively. In order to determine whether the percentage attendance to the physical activity intervention program influenced the changes in plasma homocysteine concentrations, the attendance of the subjects were divided into tertiles. The results (Table 3.2) indicated that the subjects that participated in less than 33.3 % and more than 66 % of the activity intervention programme had a significant increase in plasma homocysteine concentrations, while the subjects participating in between 33.3 % to 66.6 % of the intervention programme a decrease in plasma homocysteine concentration was indicated.

Table 3.2: **Percentage change in homocysteine concentrations according to compliance of the physical activity intervention for the boys and the girls**

% Attendance	N	% Change Hcy
1st tertile < 33.3 %	40	7.39 ± 15.46*
2nd tertile 33.3 % - 65 %	18	- 4.35 ± 18.09
3rd tertile > 66.6 %	13	7.39 ± 18.82*

DISCUSSION

Elevated plasma homocysteine concentrations can be a risk for cardiovascular diseases and stroke, especially in black women of 15 – 44 years (Giles *et al.*, 1999). The purpose of the study was to determine the baseline value for plasma homocysteine concentration in black adolescents, as well as the changes in plasma homocysteine concentrations following a 10 week physical activity intervention programme.

Reference values published for plasma homocysteine for children younger than 12 years are 4 – 8 μmol/L. For adolescents as well as for adults of different ethnic backgrounds (Hispanic whites, Hispanic blacks and Mexican Americans) the published reference values are 5 μmol/L to 15 μmol/L (Reddy 1997; De-Laet *et al.*, 1999; Dinavahi *et al.*, 2003 & Ganji *et al.*, 2005). At baseline testing it was noted that the plasma homocysteine concentrations differed between the ages for the experimental group and the control group. The plasma homocysteine values were between 5.93 (± 0.92) μmol/L and 7.03 (± 1.67) μmol/L for the adolescents. Both study groups were within the recommended range for plasma homocysteine concentrations. The reason for the difference between the plasma homocysteine concentrations could be the relationship between muscle mass and plasma homocysteine concentrations.

A relationship was found between anthropometric measurements and plasma homocysteine concentrations. Muscle mass correlated significantly with plasma homocysteine ($r = 0.25$; $p = 0.00$). Gallist *et al.* (2001), noted that when the lean muscle mass in children and adolescents increases and the fat mass decreases in a weight reduction programme, the total plasma homocysteine concentrations will be reduced.

The VO_2max values of the boys and girls indicated that the boys had a significantly higher VO_2max compared to the girls. During the intervention the experimental group boys showed a 9.11 % increase in their fitness level and the girls 5.18 % increase. The girls in the control group also had an increase in their fitness level, which can be due to other lifestyle factors outside the scope of the study. A study on sedentary women with polycystic ovary syndrome found that plasma homocysteine concentrations lowered from 10.06 (± 3.22) μmol/L to 7.36 (± 1.96)

μmol/L over a 25-week period of moderate (30 min of low intensity) exercise (Randeva *et al.*, 2006). In a separate study by Ali *et al.* (1998), plasma homocysteine concentrations decreased with 12 % with physical activity in patients with cardiovascular risk factors. A meta analysis done by Joubert *et al.* (2006:350) found equivocal results for the effect of physical activity on plasma homocysteine.

Plasma homocysteine concentration increased in the experimental group as well as in the control group. However, the change of plasma homocysteine from baseline was not statistically significant in the experimental group. In the control group, plasma homocysteine increased by 15 %, while the experimental group only increased by 1 % with the intervention programme. This is however, consistent with studies previously mentioned (Ali *et al.*, 2000; Joubert *et al.*, 2006 & Randeva *et al.*, 2006).

The low compliance of 25 % to the physical activity intervention programme may be the reason for the changes measured in plasma homocysteine concentrations. A large number of subjects dropped out from baseline to end with regard to blood sampling for the plasma homocysteine concentration analysis. This might have influenced the power of the statistical analysis.

Controversial findings still exist especially on the influence of physical activity on plasma homocysteine concentrations in people, although evidence suggests that moderate endurance training over a period of time may induce plasma homocysteine concentrations in active people (Hermann *et al.*, 2003a; Konig *et al.*, 2003; Joubert *et al.*, 2006).

CONCLUSION

In conclusion, the study found that black adolescents of the North – West Province plasma homocysteine concentrations was within the normal range 5 – 15 μmol/L. A significant correlation was found between muscle mass and plasma homocysteine concentrations in these adolescents. The 10-week physical activity intervention pragramme had no significant change in plasma homocysteine concentrations, although subjects reporting a moderate attendance to the physical activity intervention indicated a significant 4 % decrease in the plasma homocysteine concentrations compared to very low and very high levels of physical activity compliance. Research with larger sample sizes should, however, be investigated to verify the results.

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CHAPTER 4

SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 SUMMARY

The purpose of the study was firstly to determine the average plasma homocysteine concentrations in black adolescent, boys and girls from a low - socio economic area in the North – West province. Secondly, to determine whether a physical activity intervention programme could change the plasma homocysteine concentrations in adolescents. Chapter 1 provided a brief introduction and outline of the problem statement that underlies the research questions, objectives and hypotheses that form the basis of the study.

Chapter 2 discussed the literature of the published data on plasma homocysteine concentrations, as well as the factors that influenced or determined the plasma homocysteine concentrations in people. According to literature, the reference range for plasma homocysteine concentrations is 5 – 15 μmol/L for adolescents and adults. If plasma homocysteine reaches higher ranges, the risk for cardiovascular disease and strokes increases as well. Plasma homocysteine concentrations can be determined by age, gender and ethnicity of people' these factors can influence the concentrations as well. Lifestyle factors such as physical activity, diet, smoking and alcohol

consumption influence plasma homocysteine. Positive correlations were found between diet and smoking. This, however, can increase the plasma homocysteine concentration if a healthy lifestyle is not followed.

Physical activity can change the plasma homocysteine concentrations. Plasma homocysteine concentrations are dependent on the mode, intensity and duration of the physical activity programme. Plasma homocysteine seems to decrease with moderate physical activity, where as with acute physical activity plasma homocysteine increases.

The research article "Changes in homocysteine following a physical activity intervention" by Snyman, L., Moss, S.J. & Boonstra, A. will be presented for publication to the International *Journal of Sport Nutrition and Exercise Metabolism*. This article is included in Chapter 3. The objective of this study was to investigate the plasma homocysteine concentrations in Tswana adolescents and if a 10-week physical activity intervention porgramme influence on the plasma homocysteine concentrations. Tswana boys and girls of 14 – 16 years from a low socio – economic background were recruited for the study.

All of the above mentioned articles have been written according to the guidelines of the specific journal and consist of an introduction, problem statement and the resulting research questions and purposes of the study. The research methods (subjects, measurement procedures and data analysis) were described, after which the results were presented and discussed. Each article concluded with research conclusions and implications.

4.2 CONCLUSION

The conclusion of this study will be discussed in accordance with the hypothesis of the study (Chapter 1).

4.1.1 HYPOTHESIS 1

The average plasma homocysteine concentrations for Tswana adolescents from a low socio - economic area are within the normal range as indicated by published data.

When the plasma homocysteine concentration was investigated, it was reported that the homocysteine concentration was within the normal range in both the males and females of the control and experimental group at baseline. The plasma homocysteine concentrations ranged between 5.93 (±0.92) μmol/L and 7.03 (±1.67).μmol/L.

The hypothesis is therefore accepted.

4.1.2 HYPOTHESIS 2

Physical activity intervention programme will reduce plasma homocysteine levels of Tswana adolescents from a low socio - economic area

The hypothesis is rejected based on the research findings that the homocysteine concentrations increased in the experimental group as a result of the 10-week physical activity intervention. Although the control group indicated a 15% increase compared to the 1% increase in the experimental group. The results alluded to the percentage compliance to the intervention program having an influence on the changes in the homocysteine concentrations.

Hypothesis 2 will not be accepted, as plasma homocysteine did not change significantly during the intervention period.

4.3 RECOMMENDATIONS

The results of this study emphasise the importance of further research regarding plasma homocysteine and physical activity on Tswana adolescents, as there is clearly a shortage of literature that focuses on this research theme within this ethnic group. With the race differences in mortality for strokes being greater among black than white people. It would be of interest to determine whether these differences can be ascribed to a genetic predisposition or modifiable lifestyle factors. Further research is needed, however to support these findings and determine a possible mechanism through which the association between plasma homocysteine concentrations and stroke can be explained.

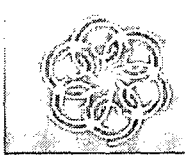
During the study the following limitations were encountered:

Certain shortcomings regarding this study as well as recommendations:

- Subjects attended intervention after school without a rest break.
- A large number of subjects for blood sampling were lost to the study at the follow up.
- The compliance to the intervention program was very low
- It was difficult to ensure that the same level of activity intensity with all the subjects.

APPENDICES

APPENDIX A



INTERNATIONAL JOURNAL OF
SPORT NUTRITION and
EXERCISE METABOLISM

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Manuscripts: Every manuscript must be in English, double-spaced with wide margins, and include an abstract of no more than 250 words. Include 3 to 6 key words that are not in the title. The abstract should contain a purpose/hypothesis statement, and a brief description of methods, results, and conclusions. Label clearly any tables and graphs, and include them on separate pages. Number all pages in the upper-right corner in this order: title page, abstract, text, references, acknowledgments if any, figure captions, tables, and figures. Include line numbers in the text. Disclose all funding sources.

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Copyright Assignment Form

Figures and Tables: Figures should be professional in appearance and have clean, crisp lines. Hand drawings and hand lettering are not acceptable. In graphs, use black and white only, no shading or color. Keep labels proportionate with the size of the figures on the journal page, which is 4.5 in. wide. Digital images should be 300 dpi at full size for photos and 600 dpi for line art. Format tables in the table function of your word-processing program rather than aligning columns in text with tabs and spaces or using text boxes. Figures can be submitted electronically in .TIF or .PDF file formats. Submit a copy of each figure with each copy of the manuscript. On each figure indicate figure number, author's name, and top side. Authors are encouraged to submit illustrations rather than tables. When tabular material is necessary, it should not duplicate the text. Tables should be double-spaced on separate sheets and include brief titles.

Use of Human and Animal Subjects: *IJSNEM* requires that all submitted studies using human or animal subjects conform to the policies established by the U.S. Department of Health, Education, and Welfare and the American Physiological Society.

UPDATE: With the first issue of 2008, Volume 18, *IJSNEM* is changing its editorial style. We will now follow the style laid out in the *Publication Manual of the American Psychological Association* (APA), 5th ed. The reference list will still be alphabetized but not numbered, and citations in text will include the authors' last names and the date in parentheses. Examples of the three most common forms of references are as follows. For other variations, please consult the APA manual.

Chisolm, D.J., Young, J.D., & Lazarus, L. (1969). The gastrointestinal stimulus to insulin release. *Journal of Clinical Investigation, 48,* 1453-1460.
Wadler, G.I., & Hainline, B. (1989). *Drugs and the athlete*. Philadelphia: F.A. Davis.
Haymes, E. Proteins, vitamins, and iron. (1983). In M.H. Williams (Ed.), *Ergogenic aids in sport* (pp. 27-55). Champaign, IL: Human Kinetics.

APPENDIX B

I CONFIRM THAT:

It has been explained to me, that:

1. The purpose of the research study is to collect information on growth and activity among Grade 9 schoolchildren in Boitshoko Secondary School, North West Province.
2. I have been told that the researchers will measure me. The participant will be weighed and his/her height as well as circumferences and skinfolds of his/her arm will be measured without causing any pain to the child. For those measurements boys and girls in separate groups will be asked to undress in the privacy of a class-room, because some measurements must be taken with the children dressed in underwear only, or a light shirt and pants/skirt. The researchers will also ask me to indicate my own level of physical maturation from pictures. The different age groups will be measured separately. The researchers and fieldworkers will work in a professional way, not to embarrass the children.
3. I will also be measured in an instrument, called the BODPOD to measure amount of muscle, bone and fat. These measurements will be done at the North West University and children will be transported to the laboratory and back.
4. Fitness testing will be done and blood pressure will be tested.
5. Blood samples will be taken during basal and final measurements. Blood will be collected by qualified personnel by using a thin needle (20ml blood per each sample) to minimize pain and discomfort. Blood samples will not be tested for HIV.
6. The measurements will be done twice, March and September, to assess growth and health.
7. The researchers will ask me about my home environment, the food that I usually eat and activities that I do. None of these questions will be to see if I am clever, or know correct answers. I can just tell them what I usually do.
8. Guidelines for appropriate, culture sensitive, practical and sustainable intervention programmes for children will be developed.
9. The information I will give shall be kept confidential, only to be used anonymously for making known the findings to other scientists.

10. It was also clearly explained to me that I can refuse to participate in this research study or I can stop answering the questions at any time during the interviews, or refuse to give a blood sample if it hurts.

The information in this consent form was explained to me by Mrs Susan Legoete (interviewer) in_____(language) and I confirm that I have a good command in this language and understood the explanations, OR it was translated to me by_____(Name of translator) in my language_____. I was also given the opportunity to ask questions on things I did not understand clearly.

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

Signed/confirmed _____

at_____on_____2005

Witness_____

*Representative of participant (parent/guardian)*_____

APPENDIX C

PLAY-PROJEK

ANTROPOMETRIE DATAKAART

Naam: _____ Van: _____

DOB: ____/___/____ Ouderdom: _____ Geslag: _____

Proefpersoonnr.: _____ RHT: _____ Toetsdatum: ___/___/2004

			Meting 1	Meting 2	Meting 3
1	Massa	kg			
2	Lengte	cm			
3	Armspan	cm			
Omtrekke					
3	Bo-arm ontspanne	cm			
4	Bo-arm gespanne	cm			
5	Maagomtrek	cm			
6	Heupomtrek	cm			
7	Kuitomtrek	cm			
Velvoue:					
8	Triseps	mm			
9	Subskapulêr	mm			
10	Kuit	mm			
11	Supraspinaal	mm			
12	Abdominaal	mm			
Deursneemates					
11	Humerus	cm			
12	Femur	cm			
BodPod:					
13	Massa	kg			
14	Vet %	%			
15	Vetmassa	kg			
16	Skraalliggaamsmassa	kg			
17	Longvolume	L			

APPENDIX D

PLAY-PROJEK

KONTROLE KAART

PLAY-STUDIE KONTROLE KAART

PLAY 2005

Subject name: _____ No _____ Gender _____

		Check control
STATION 1	RECRUITMENT	
STATION 2	DEMOGRAPHIC QUESTIONNAIRE age_____	
STATION 3	ANTROPOMETRY: weight_____height_____ armspan _____	
STATION 4	BODPOD	
STATION 5	TANNER STAGE	
STATION 6	DIETARY QUESTIONAIRE:24 HOUR	
STATION 7	PHYSICAL ACTIVITY: PDPAR (week and weekend)	
STATION 8	PHYSICAL ACTIVITY: QUESTIONAIRE	
STATION 9	POSTURE	
STATION 10	BLOOD PRESURE	
STATION 11	FITNESS TEST (strength, flexibility...)	
STATION 12	FITNESS TEST (bleep test)	
STATION 13	BLOOD SAMPLES	
STATION 14	BACK TO STATION 1	
		SIGNATURE