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The effect of folic acid supplementation on total plasma homocysteine (tHcy) concentrations in sedentary adult men

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Abstract

Elevated plasma homocysteine concentrations are associated with an increased risk of cardiovascular disease. Nutritional supplementation of folic acid may be used to reduce homocysteine levels and thus minimise the risk of cardiovascular disease. This study examines 90 days of oral supplementation of a liquid supplement, containing folic acid and vitamin B_{12}, on the plasma homocysteine levels in 20 sedentary adult men, aged 20-60 years. Supplier recommended dosage was administered daily. Blood was drawn pre- and post-test for measuring homocysteine, vitamin B_{12}, and folate levels. Over the course of the study, the mean homocysteine levels of the active group decreased and folate levels increased significantly ($p \leq 0.05$). The placebo group did not display these characteristics. The active supplement thus provided beneficial effects by decreasing disease risk. This was accomplished by a 15% reduction in homocysteine levels and increased plasma folate levels in the adult men.

Keywords: Supplement, homocysteine, folic acid, folate, vitamin B_{12}, nutrition.

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Introduction

Elevated total concentrations of homocysteine (tHcy) in human plasma are associated with an increased risk of cardiovascular disease (ACSM, 2006). Some oral nutritional supplements, like folic acid, have been shown to reduce tHcy concentrations and potential risk of cardiovascular disease (Baran, 2004; Jordan, Jurca, Abraham, Salikhova, Mann & Morss, 2004; Araki, Maruyama, Igarashi, Yoshida, Maruyama & Satoh, 2006). It is postulated that factors associated with the modern way of life, like following a sedentary lifestyle and poor nutrition, contribute to mortality and morbidity. These factors include distress, disease, little exercise, environmental toxins, smoking and radiation. Dietary supplementation with essential nutrients may provide protection against these factors and thus leading to a decreased risk of disease (Tawakol, Omland, Gerhard, Wu & Creager, 1997; Woo, Chook,

Lolin, Cheung, Chan & Sun, 1997; Jordan *et al.*, 2004; Riza Erbay, Turhan, Yasar, Ayaz, Sahin & Senen, 2005).

Homocysteine is one of the many amino acids produced by the body. Production occurs through the chemical conversion of methionine, which is a compound regularly consumed in the diet (Tucker, Olson, Bakun, Dallal, Selhub & Rosenberg, 2004; Riza Erbay *et al.*, 2005). Elevated tHcy concentrations (even in moderation, being 10-15 µmol/L) increase the risk of: cancer, neurodegenerative diseases. neural tube defects, atherosclerotic vascular disease in the coronary, cerebral and peripheral vessels, as well as arterial and venous thrombo embolisms. Very high levels of tHcy (>15 µmol/L) are linked to an increased risk of type two diabetes, rheumatoid arthritis, osteoporosis, hypothyroidism, and inflammatory bowel disease (Shimakawa, Nieto, Malinow, Chambless, Schreiner, Szklo, 1997; Tawakol *et al.*, 1997; Woo *et al*., 1997; Riza Erbay *et al*., 2005; Doncheva, Penkov, Velcheva, Boev, Popov & Niagolov, 2007; Block, 2011).

If Folic acid supplementation is capable of modifying tHcy levels favourably, it may be postulated that the risk of developing the aforementioned diseases may be reduced by taking the supplement. tHcy accumulation may have a common cause, such as a deficiency or low availability of folate or vitamin $B_{12.}$ By elevating plasma folate and vitamin B_{12} levels, tHcy concentrations can be decreased. If oral supplementation is a viable option for achieving this, it may lead to revolutionary changes in the management of patients with moderate hyper homocysteienemia (10-15µmol/L) (Shimakawa et al., 1997; Riza Erbay, 2005).

Vitamin B_{12} has been shown to reduce tHcy, whilst folic acid supplementation does not affect concentrations of tHcy (Woodside, Yarnell, McMaster, Young, Harmon & McCrum, 1998; Deshmukh, Joglekar, Lubree, Ramdas, Bhat & Naik, 2010). Other studies demonstrate that supplementation with folic acid is the most effective means of reducing tHcy concentrations (Riddell, Chisholm, Williams & Mann, 2000). It has been demonstrated that increasing plasma folate does not affect concentrations of vitamin B_{12} or tHcy (Wang, Zhang, Moslehi, Ma, Pan & Zhou, 2009). The effect of oral supplementation containing either folic acid or vitamin B_{12} on tHcy concentrations in South African men has been previously investigated; however, little is known concerning the effect of supplementation with both (Ubbink, Vermaak, van der Merwe, Becker, Delport & Potgieter, 1994).

The oral supplement investigated in this study contains folic acid and vitamin B_{12} and is available throughout South Africa. The suppliers claim that their product decreases disease risk by lowering plasma tHcy concentrations. No publications detailing this supplement and its effects could be found. This study examines oral supplementation containing nucleic acid bases, methyl groups and ATP in middle-aged men over a

period of 90 days, and its effect on the individual's concentrations of plasma tHcy, vitamin B_{12} and folate.

Methods and Material

Participants

An experimental, randomized, double-blind, placebo-controlled, pre- and post-test group comparison research design was used (Thomas & Nelson, 2001). A convenient sample of 20 sedentary men, aged 30-60 years, were selected from a group of volunteers (friends and family of the investigators), using a non-probability method. This technique is described as selection of the sample based in some part on the judgment of the researcher (Kinnear & Taylor, 1995). The sample was divided randomly into two groups of 10 participants per group. One group was exposed to the active nutritional supplement; the control group (placebo) was not.

For the purpose of this study, being sedentary was described as not regularly participating in structured physical activity. Exclusion criteria included participants who indicated that they participated in regular physical activity; had a history of impaired cardiovascular, hepatic, respiratory, or renal nature; were using medication or other nutritional supplements within six weeks prior to the study; took other supplements during the study; were ill within seven days prior to study commencement; did not fast for 8 to 12 hours before blood samples were taken, necessary for determining plasma tHcy, vitamin B_{12} and folate levels; and/or had plasma levels of tHcy below 10µmol/L.

An initial pre-study orientation and selection session was held during which volunteers were screened to ensure compliance with the participation criteria. All participants were briefed regarding the potential risks and benefits of the study, before informed consent was obtained from each participant. Participant involvement was discontinued if they: failed to comply with testing procedures; consumed any supplement not approved by the researchers; and/or experienced any side effects. The study was approved by the Research Proposal and Ethics Committee of the University of Pretoria, South Africa.

Procedures

The recommended dosage was six sprays of the liquid supplement, containing folic acid and vitamin B_{12}, sublingually. Each spray was held for 30 seconds to achieve optimal sub-lingual absorption. The supplier-recommended dosage was administered daily as per their instructions and repeated for the full 90-day duration of the study. To ensure compliance with the study, a weekly short messaging service (SMS) was sent to each participant to remind him to take the required dosage.

The control group received a placebo oral liquid spray for use in the same way. Participants were only supplied with more oral spray (active or placebo) every two to four weeks - in sufficient quantity to last only until the next date of resupply. Logbooks were provided in which participants had to record the following daily: amount of supplement ingested; time of supplement ingestion; side-effects experienced, like nausea, headaches, muscle cramps or flu-like symptoms.

All participants underwent pre- and post-test measurements for the 90 day intervention period. On the first day of the intervention period, participants underwent anthropometric assessment for estimation of body composition. On the second day, participants were rested for 5 minutes in a seated position. Blood was then drawn from the median cubital vein in the right or left cubital fossa. Samples were kept iced and transported to an independent pathology laboratory (Lancet) for analysis within an hour of collection. Lancet Laboratories is SANAS (South African National Accreditation System) accredited, adhering to international criteria set out according to ISO Standard 17025. Blood samples were stored at room temperature before delivery to the pathology laboratories for testing. Blood samples were analysed for tHcy, vitamin B_{12}, and folate levels. After completion of the intervention participants underwent the same procedures as in the beginning.

Measurement Techniques

For anthropometric assessment, the body mass was measured to the nearest 0.1kg using a Detecto Scale (Webb City, MO, USA). Stature was measured to the nearest 0.1cm using a Seca 214 Stadiometer (Seca Corporation, Hanover, USA). Body mass index (BMI) was calculated as mass (kg) divided by participant's height in meters squared and body fat percentage was estimated using the Durnin and Womersley equation (Durnin & Womersley, 1974). From each participant's blood samples, the following were measured: tHcy (µmol/L); vitamin B_{12} (pmol/L); and folate (nmol/L).

Data Analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) software. The aim of the data analysis was to determine whether statistically significant differences existed in the pre- and post-test values between the experimental and control groups. Non-parametric analysis included the Wilcoxon Signed Ranks Test. This test is used in situations where there are two sets of scores to compare, but the scores come from the same participants. It is the distribution-free analogue of the t-test for related samples. It tests the null hypothesis that two related samples were drawn either from identical populations or from symmetric populations with the same mean. In this case, it was used to determine whether statistically significant differences existed between pre- and post-tests scores obtained for all variables measured within the same group. Statistically significant

differences are marked ($p \leq 0.05$) in Table 1 with an asterix*, and presented in Figure 1.

Results

The study commenced with 10 participants in each group. However, before initial pre-tests were completed, one participant from the active supplement group withdrew from the study. Over the 90-day intervention period, a further 3 participants withdrew from the study. At the post-test session, there were 7 participants in the active supplement group and 9 in the placebo group. Table 1 summarizes the results of the comparison between pre- and post-test results for the Active supplement group and the Placebo group.

Table 1: Summary of anthropometric measurements and plasma concentrations

Group	Test Description	Pre-test		Post-test		p-value
		Mean	SD	Mean	SD	
Active Supplement	Age (years)	39.8	5.0	41.7	3.5	
	Mass (kg)	86.8	14.2	85.2	14.3	0.612
	Stature (cm)	175.6	8.4	173.3	11.7	0.317
	BMI ($kg.m^2$)	28.0	2.1	27.7	2.2	0.917
	Body fat (%)	28.6	3.0	28.6	3.9	0.498
	Homocysteine (µmol/L)	10.0	0.5	8.5	0.8	0.018*
	VitaminB12 (pmol/L)	468.7	214.1	408.8	396.1	0.398
	Folate (nmol/L)	24.9	5.9	33.9	6.8	0.018*
Placebo	Age (years)	40.1	8.7	41.1	8.7	
	Mass (kg)	84.2	11.7	86.5	11.2	0.441
	Stature (cm)	178.7	7.8	180.4	6.2	1.000
	BMI ($kg.m^2$)	26.4	3.7	26.6	4.0	0.513
	Body fat (%)	25.2	6.4	25.3	6.3	0.906
	Homocysteine (µmol/L)	13.9	3.4	13.6	3.3	0.109
	Vitamin B12 (pmol/L)	364.9	154.1	325.0	97.2	0.953
	Folate (nmol/L)	18.1	5.1	18.7	7.9	0.260

Anthropometric measurements showed no difference between groups at the start of the study or within either group after the 90-day intervention. This is an expected result as substantial body composition changes after three months can only be mediated by starvation, disease, over nutrition and physical activity.

However, the active supplement group showed a significant decrease and increase in homocysteine (µmol/L) and folate (nmol/L) levels, respectively (Figure 1). Homocysteine levels decreased significantly ($p=0.0180$) from 10 µmol/L in the active supplement group (pre-intervention) to 8.5 µmol/L (post-intervention) and the

Folate levels increased from 24.9 nmol/L to 33.9 nmol/L (p=0.018). No significant changes were measured in the vitamin B12 concentration (p=0.398) Figure 1 graphically represents the significant changes observed in homocysteine and folate levels. In the placebo group, no statistically significant differences were found.

Discussion

No statistically significant differences were found between the active supplement group and the placebo group's anthropometrical pre- or post-test measurements. This may be attributed to the participants in both groups being sedentary. However, the ingredients ingested by the active supplement group did not have a significant effect on their body composition as measured by mass or body fat %.

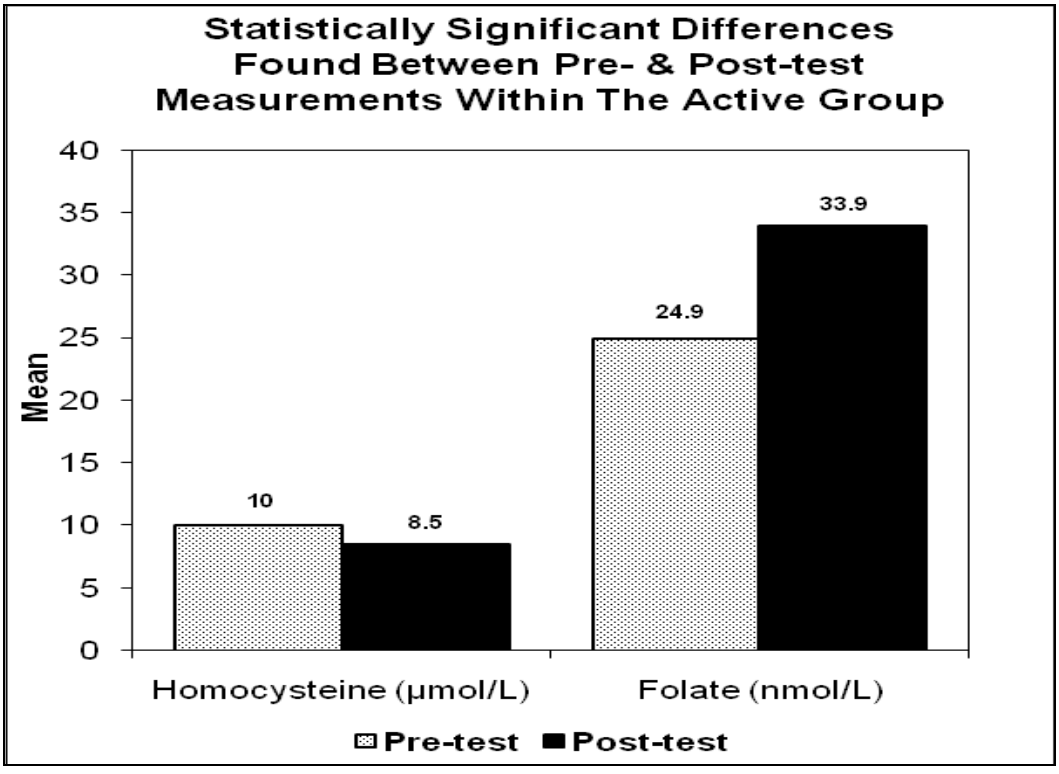

Figure 1: Summary of statistically significant changes in the active supplement group

Significant differences were found in the active supplement group: homocysteine mean (pre-test: 10.0 µmol/L > post-test: 8.5 µmol/L, p-value = 0.018); folate mean (pre-test: 24.9 nmol/L < post-test: 33.9 nmol/L, p-value = 0.018). These differences were not observed in the placebo group. The effect of folate on tHcy concentrations is not surprising since folate has a dominant tHcy lowering effect in the blood; it is

greater among participants whose blood has higher levels of tHcy; or lower folate (Clarke, Frost, Leroy & Collins R, 1998; Araki *et al.*, 2006).

The objective of this study was to determine whether or not treatment with the active supplement (folic acid and vitamin $B_{12)}$ will improve measures of disease risk. From the results, it appears that the active supplement treatment did provide beneficial effects in improving disease risk as it resulted in a 15% reduction in tHcy concentrations and an increase in plasma folate levels. This finding is consistent with that reported in another study on South African males which showed that supplementation with folic acid can reduce plasma tHcy concentrations by up to 41.7% and supplementation with vitamin B_{12} does reduce plasma tHcy concentrations by up to 14.8% (Ubbink *et al.*, 1994).

In conclusion, this study provides insight into the effects of oral nutritional supplementation on middle-aged men by examining the effect of supplements on plasma tHcy, vitamin B_{12} and folate concentrations. The study is unique as it shows that folic acid supplementation, not vitamin B_{12}, decreases tHcy (Deshmukh *et al.*, 2010). As no significant difference was found in the concentration of vitamin B_{12}, the decrease in tHcy must be attributed to the supplementation of folic acid. This differs from results of other studies which attributed the decrease in tHcy to vitamin B_{12} (Woodside *et al.*, 1998). A statistically significant reduction in plasma tHcy concentrations did occur in the active supplement group, as claimed by the supplier. There was also a significant increase in folate levels within this group. Optimally, daily diets should contain adequate amounts of folate to keep the risk of chronic disease to a minimum (ACSM, 2006). When compared to other vitamins, folate has a dominant effect on lowering plasma tHcy (Clarke *et al.*, 1998). Future studies should focus on different intervention periods, hereditary differences between study participants and sampling participants with plasma tHcy concentrations lower than10 µmol/L.

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