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ARTICLE

The effect of vitamins B12, B6 and folate supplementation on homocysteine metabolism in a low-income, urbanised, black elderly community in South Africa

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Objectives: The aim of this study was to assess the effect of vitamins B12, B6 and folate supplementation at >100% Recommended Dietary Allowances (RDA) for six months on serum homocysteine (Hcy) levels of an elderly urbanised black South African community.
Design: An experimental, non-equivalent control group intervention study design was used in a 104 purposively selected sample. Two groups were compared: hyperhomocysteinaemic (hyperHcy) ($n = 61$) and normo-homocysteinaemic (normoHcy) ($n = 43$).
Setting: Elders attending a day-care centre in Sharpeville, Gauteng, South Africa.
Subjects: All subjects were equivalent in age (> 60 years), race (black) and unemployed/pensioner.
Outcome measures: The following parameters were determined at baseline and after the six-month supplementation: serum Hcy, vitamins B6, B12 and folate levels, red cell count, mean cell volume, haemoglobin, haematocrit and the nutritional intake of vitamin B6, B12 and folate.
Results: A very high incidence (66.36%) of hyperhomocysteinaemia was present in the sample. The mean ± standard deviation (SD) serum Hcy level in hyperhomocysteinaemic individuals decreased statistically significantly from 25.00 ± 8.00 umol/l to 18.80 ± 12.00 umol/l after the intervention. The number of respondents with an increased Hcy level decreased from 100% (baseline) to 67% after the intervention.
Conclusions: It is concluded that supplementation of vitamins B6, B12 and folate is an effective Hcy-lowering approach to reduce hyperhomocysteinaemia in an elderly population, and thereby reduce their risk of cardiovascular disease (CVD).
Summary The supplementation had a beneficial effect on the respondents' serum vitamin B6 as well as their haemopoiesis (decreased macrocytosis).

Keywords: Cardiovascular risk, folate, Hcy, supplementation, vitamin B6, vitamin B12

Introduction

The wave of cardiovascular disease (CVD) that was considered endemic to the developed world is expanding to developing countries.[1] The emphasis in South African health policy is largely placed on children, youth and maternal care, whereas the health needs of the elderly tend to be marginalised. At primary health care level, no dedicated geriatric services are offered in the public health sector and older people thus need to be accommodated in facilities where overcrowding and severe staff shortages limit the attention given. This limits the detection and treatment of nutritional deficiencies of the low-income aged in South Africa (SA).[2,3] A previous study conducted in the current population (Sharpeville elderly community) confirmed that these respondents are at an increased risk of CVD.[4–6]

McCully proposed an association between elevated plasma Hcy and the development of atherosclerosis.[7,8] Multiple studies have also reported on this hypothesis and confirmed it.[9,10] In the elderly, elevated Hcy levels could result from glomerular filtration rate together with decreased vitamin B12 and folate absorption.[11] Supplementation studies have been recommended, but inconsistent results were reported in previous supplementation studies with an insufficient dosage of supplements as a possible explanation.[12–15] Lowering of Hcy was found in studies using between 100% and 200% of the Recommended Daily Allowance (RDA) of vitamin B6, B12 and folate.[12–16]

The aim of this study was to evaluate the effect of vitamins B12, B6 and folate supplementation at > 100% RDA for six months on serum Hcy levels as a cardiovascular risk marker in a random sample of black elderly men and women of low socioeconomic status ($n = 104$). To the researcher's knowledge, no study of this nature has been conducted in an elderly community in the African or international context and the novelty of this study is that it is the first of its kind in SA.

Methods

Study population and sampling

This study was conducted in 2012 at a day care centre in Sharpeville (Vaal Region, SA) that offers skills training and religious activities aimed at low-income elderly persons, and provides breakfast and lunch on the days the elderly voluntarily attend the centre. Initial visits were made to the day-care centre, explaining the objectives of the project to the

management of the day-care centre and the attendees in order to obtain approval from both parties. All the participants who completed and signed the informed consent forms were included in the study ($N = 104$). Individuals who could not give consent due to conditions such as Alzheimer's and senile dementia were excluded. Due to the low prevalence (2–3%) of human immunodeficiency virus (HIV) infection amongst the elderly in SA[17] it was not considered as a possible confounding variable.

A baseline study was conducted determining the serum levels of Hcy, vitamins B6, B12 and folate, the red cell count (RCC), haematocrit (HCT), haemoglobin (Hb) and mean cell (corpuscular) volume (MCV), as well as the dietary intake of vitamins B6, B12 and folate. The baseline results were used to purposively select subjects with elevated Hcy levels for the hyperHcy (group A, $n = 61$) and normoHcy group (group B, $n = 43$).

Intervention

A six-month supplementation programme, with a commercially available product, was designed and implemented. The supplement contained > 125% RDA of vitamins B12 (25 µg), B6 (50 mg) and folate (400 µg). Previous supplementation studies varied in duration from 3 to 24 months. However, a significant effect was observed after only a six-month period in most studies.[9–11] Based on these reports, six months was chosen for the intervention period.

All the subjects (experimental and control group) received a 30-day supply of vitamins B12, B6 and folate per month for six months. A weekly follow-up visit was made to the day-care centre to remind the participants and improve compliance. Fieldworkers also made random house visits to count the remaining tablets to further ensure compliance. The following measurements were taken at baseline and repeated after the six months' supplementation.

Data collection

Dietary measurements

Total vitamin B6, B12 and folate intakes were measured to monitor the possible confounding effect of the dietary intakes on the serum levels of the parameters. Due to the short attention span of the participants, and because the questionnaire is fast and easy to administer, it was decided to use a 24-hour recall questionnaire, using a validated data collection method[15] to measure actual dietary intakes. Food models were used to correctly estimate the food items and portion sizes consumed. Two (weekend and weekday) 24-hour recalls were completed for each subject and an average for the two days was calculated for vitamin B6, B12 and folate intake before and after the intervention. The recommended standard for estimating the prevalence of inadequate intakes within a group was determined by the estimated average requirements (EAR) for these vitamins for the age group > 70 years.[18]

Blood collection procedure and biochemical measurements

A fasting blood sample was collected by a qualified phlebotomist using a standardised venepuncture method. Standard laboratory operational procedures were followed to ensure accurate and precise results. Intra-assay variation coefficients were lower than 6% for all the measurements. Serum Hcy (enzymatic method) was determined on an automated Konelab™ analyser (Thermo Fisher Scientific, Waltham, MA, USA). Quantitative serum folate and vitamin B12 were analysed by means of a competitive immunoluminometric assay on an automated Maglumi 1000 system (Snibe Diagnostics, Shenzhen, China). Serum vitamin B6 levels were determined by the preferred high performance liquid chromatography (HPLC) method on an Agilent 1 260 Infinity HPLC system (Agilent, Santa Clara, CA, USA) using a complete commercialised analytical kit from Recipe® (RECIPE Chemicals + Reagents, Munich, Germany). A fully automated Beckman Coulter™ (Beckman Coulter, Brea, CA, USA) AC.T™ 5 diff haematology analyser was used to measure the red cell parameters, which included RCC, Hb, Hct and MCV.

Ethics approval

This study was ethically approved both by the ethics committee of the University of the Witwatersrand, Johannesburg (M070126) and by the ethics committee of Durban University of Technology (DUT). Participation in this study was voluntary and participants gave consent.

Data analysis

All the data were captured in Microsoft Office Excel (Microsoft Corp, Redmon, WA, USA) and exported to IBM SPSS, version 22 (IBM Corp, Armonk, NY, USA). Most data were normally distributed; therefore, means and standard deviations were computed for each biochemical and dietary intake variable. The nutrient intakes (vitamin B6, B12 and folate) were compared with the Dietary Reference Intakes (DRI);[18] specifically, the EAR and blood parameters were compared with recommended reference values. Paired t-tests were done to determine statistically significant differences before and after intervention within the hyperHcy (A) and normoHcy group (B), respectively. Independent t-tests were used to determine the statistically significant changes between the groups. Correlation coefficients were used to examine the association between the study variables ($p \leq 0.05$). Only significant correlations are reported in the results.

Results

The dropout rate was less than 10% in both group A (1.65% dropout) and group B (4.65%) from baseline to follow-up measurements. It was therefore assumed that the dropouts would not affect the outcome of this study.

The mean dietary intakes of the two groups were homogeneous, as no statistically significant differences were observed between the baseline nutrient intakes of the two groups. As presented in **Table 1**, at baseline the mean vitamin B12 in both groups was within the recommendations, but the mean vitamin B6 and folate intake in both groups was below the recommendations.

The percentage of respondents not meeting the recommended intake in group A at baseline was 85% for vitamin B6, 92% for folate and 59% for vitamin B12. The percentage of respondents for group B was 95%, 98% and 58% in group B, respectively. In both groups, the supplement ensures that the intakes of these vitamins were 100% of EAR for all the respondents in both groups after the intervention at follow-up. Significant ($p \leq 0.05$) increases in vitamin B12, B6 and folate intakes were observed after the intervention. Due to the fact that most of the variables measured in this study showed homogeneity between the two groups, except for Hcy, the dependent variable, it can be assumed that the significant changes observed after the intervention study can thus be attributed to the vitamin B6, B12 and folate supplementation.

Table 1: Effect of the intervention on the dietary intakes of vitamin B6, B12 and folate of the sample

Nutrient		Baseline				Follow-up			
Variable/ nutrient	EAR > 70 years	Actual intake (mean ± SD)		% of respondents with abnormal intakes		Actual intake (mean ± SD)		% of respondents with abnormal intakes	
		Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
Vitamin B6 (mg)	1.4*	0.81 ± 0.47[a]	0.63 ± 0.44[b]	85[#]	95[#]	50.65 ± 0.35[a]	50.65 ± 0.35[b]	0[#]	0[#]
Folate (µg)	*	113 ± 79[a]	105 ± 124[b]	92[#]	98[#]	497 ± 72[b]	491 ± 48[b]	0[#]	0[#]
Vitamin B12 (µg)	2*	2.81 ± 3.89[a]	3.27 ± 4.90[b]	59	58	26.00 ± 1.43[a]	26.30 ± 1.30[b]	0	0

[#]Deficient dietary intake.
*Dietary reference intakes according to the Institute of Medicine.[16]
[a,b]Statistically significant ($p \leq 0.05$) differences in the same row.

The results in **Table 2** demonstrated that the mean ± SD serum vitamin B12 results of group A at baseline was 677 ± 348 picograms per millilitre (pg/ml) with only 7% of the respondents diagnosed as having a deficiency (< 200 pg/ml). The mean ± SD serum vitamin B12 of group B was 679 ± 364 pg/ml with only 2% of the respondents having been diagnosed with a deficiency. The follow-up results indicated that in group A the mean ± SD serum vitamin B12 levels decreased statistically significant ($p = 0.000$) to 677 ± 304 pg/ml. In group B there was a significant ($p > 0.022$) increase in the mean ± SD vitamin B12 levels to 705 ± 290 pg/ml. The prevalence of vitamin B12 deficiency (< 200 pg/ml) increased from 7% (baseline) to 12% (follow-up) in group A compared with group B, where it was unchanged at 2%. The difference between group A and B at follow-up was not significant ($p > 0.05$).

The mean ± SD serum folate levels of group A at baseline (12.94 ± 8.00 nanograms per millilitre [ng/ml]) was statistically significantly ($p = 0.041$) different from group B (14.89 ± 11.22 ng/ml). The mean values for both groups were within the normal range (5.21–20.00 ng/ml); however, the prevalence of folate deficiency (< 5.21 ng/ml) was 8% and 12% for groups A and B, respectively. The follow-up folate measurements indicated that the mean ± SD of group A decreased ($p < 0.05$) to 9.75 ± 5.20 ng/ml; however, this was not significant ($p = 0.391$). The same trend was observed in group B, where the mean ± SD folate decreased to 8.66 ± 5.28 ng/ml ($p = 0.114$). The prevalence of folate deficiency increased during the intervention period to 15% for group A and 27% for group B ($p < 0.05$).

Extremely low serum vitamin B6 values were observed in both groups at baseline with a 100% prevalence of vitamin B6 deficiency (of 8.60 micrograms per millilitre [µg/ml]) in both groups A and B. Group A had a mean ± SD serum vitamin B6 of 1.35 ± 0.69 µg/ml compared with 1.40 ± 0.80 µg/ml in group B at baseline. The difference between group A and B was not statistically significant ($p = 0.736$). After the intervention the mean ± SD vitamin B6 levels in group A increased significantly ($p = 0.002$) to 5.03 ± 2.49 µg/ml and the prevalence of folate deficiency decreased to 93%. In group B, the mean ± SD serum vitamin B6 increased significantly ($p < 0.001$) to 5.72 ± 6.15 µg/ml after the intervention and the folate deficiency improved by only 2%. The difference between groups A and B after the intervention was not statistically significant ($p > 0.05$).

At baseline, 100% of subjects in group A had an increased (> 15.00 micromole per litre [umol/l]) serum Hcy level and 0% in group B as this was the selection criterion. The mean ± SD serum Hcy level at baseline for group A was 25.23 ± 8.47 umol/l and in group B 11.94 ± 3.40 umol/l. This difference was statistically significant ($p = 0.001$). After the intervention the mean ± SD serum Hcy levels of the respondents in group A had shown a statistically significant decrease ($p < 0.001$) to 19.07 ± 11.85 umol/l. The number of respondents with an increased Hcy level decreased from 100% (baseline) to 67% (follow-up). In group B none of the respondents had an increased serum Hcy level at baseline, but this increased to 34% after the intervention. The mean ± SD Hcy level in group B increased to 14.30 ± 5.21 umol/l after the intervention, but this was not statistically significant ($p = 0.281$). The difference in follow-up measurements between group A and group B was also not statistically significant ($p > 0.05$).

The red cell parameters of group A at baseline (as indicated in Table 2) showed the mean ± SD RCC was $4.60 \pm 0.50 \times 10^{12}$/l, which did not differ significantly ($p > 0.05$) from group B ($4.45 \pm 0.60 \times 10^{12}$/l). Only 7% of the respondents in group A and 9% in group B had a decreased RCC at baseline. A slight decrease in the mean RCC was observed in both groups: group A decreased to $4.50 \pm 0.80 \times 10^{12}$/l and group B to $4.30 \pm 1.10 \times 10^{12}$/l ($p < 0.05$ at baseline and $p < 0.05$ at follow-up). The number of respondents with a decreased RCC ($< 3.90 \times 10^{12}$/l) decreased to 3% in group A and to 7% in group B after the intervention. The mean ± SD Hb of both groups A (13.80 ± 1.40 gram per decilitre [g/dl]) and B (13.50 ± 1.60 g/dl) was within the normal reference range at baseline. The number of respondents with decreased Hb (< 11.50 g/dl) increased in both groups from baseline to follow-up measurements: 3% to 11% (group A) and 7% to 15% (group B) respectively. The mean ± SD Hb levels in both groups also decreased significantly ($p < 0.05$) to 12.70 ± 2.30 g/dl (group A) and to 12.20 ± 3.10 g/dl (group B) after the intervention with no statistically significant difference ($p < 0.05$) between groups A and B. The Hct levels were within the normal range at baseline with mean ± SD levels of 40 ± 7% for group A and 44 ± 1% for group B with significant differences ($p < 0.05$) between the groups. The number of respondents with a decreased Hct (< 36%) was 3% and 12% in group A and B respectively. After the intervention the mean ± SD Hct level for group A was significantly ($p < 0.05$) higher than baseline at 43 ± 0.4%. In group B the mean ± SD Hct increased to 47 ± 6% ($p < 0.05$). The difference between groups A and B after the intervention was statistically significant ($p < 0.05$). The number of respondents not meeting the minimum reference value for Hct in group A increased to 10% and in group B to 15%. The mean ± SD MCV as indicator for macrocytosis (> 95 pg) at baseline was 91.14 ± 9.83 pg (group A) and 93.11 ± 6.26 pg (group B) respectively ($p < 0.05$).

Table 2: Effect of the intervention on Hcy metabolic markers of the sample

Variable	Reference	Baseline				Follow-Up			
		Group A		Group B		Group A		Group B	
		(mean ± SD)	Number of respondents abnormal value (%)	(mean ± SD)	Number of respondents abnormal value (%)	(mean ± SD)	Number of respondents abnormal value (%)	(mean ± SD)	Number of respondents abnormal value (%)
Vitamin B12	200–1100 pg/ml	677 ± 345[a]	7	681 ± 355	2	567 ± 311[a]	12	671 ± 321	2
Folate	5.21–20 ng/ml	12.94 ± 8.00[a,c]	8	14.66 ± 11.00[b,c]	12	9.58 ± 5.31[a]	15	8.24 ± 5.47[b]	27
Vitamin B6	8.6–27.2 µg/ml	1.35 ± 0.69[a]	100	1.40 ± 0.80[b]	100	5.03 ± 2.49[a]	93	5.72 ± 6.15[b]	98
Hcy	>15 µmol/l	25.30 ± 8.00[a,b]	100	12.04 ± 3.40[b]	0	18.76 ± 12.00[a]	67	13.64 ± 6.00	34
RCC	$3.9–5.6 \times 10^{12}$/l	4.60 ± 0.50	7	4.45 ± 0.60	9	4.50 ± 0.80	3	4.30 ± 1.10	7
Hb	11.5–15.5 g/dl	13.80 ± 1.40[a]	3	13.50 ± 1.60[b]	7	12.70 ± 2.30[a]	11	12.20 ± 3.10[b]	15
Hct	36–48%	40 ± 7[a]	3	44 ± 1[b]	12	43 ± 0.4[a,c]	10	47 ± 6[b,c]	15
MCV	80–95 pg	91 ± 10[a]	31	93 ± 6[b]	23	85 ± 13[a]	7	84 ± 19[b]	5

[a,b]Statistical significant ($p \leq 0.05$) differences in the same row.

The prevalence of macrocytosis was 31% and 23% in groups A and B, respectively. Follow-up measurements indicated that the MCV levels decreased statistically significantly ($p < 0.001$) in both groups. After the intervention the mean ± SD MCV for group A decreased to 86.63 ± 5.97 pg and in group B to 88.34 ± 4.92 pg ($p < 0.001$). The prevalence of macrocytosis decreased to 7% and 5% in groups A and B respectively.

Discussion

As early as 1969 McCully[7] proposed an association between elevated plasma Hcy and the development of atherosclerosis. Animal studies have revealed that increased oxidative stress, impaired endothelial function and increased thrombogenicity is a result of elevated Hcy and promoted atherosclerosis.[19–22] Retrospective and prospective epidemiological studies have complemented these findings. Furthermore, Hcy has been reported as a strong independent risk factor for atherothrombotic vascular disease[22–24] and it was concluded that this is related to multiple pathophysiological mechanisms. In this study a supplementation intervention approach instead of a food-based approach was followed as an Hcy-lowering strategy. The supplementation resulted in significant changes in dietary intake from baseline to follow-up for vitamins B6, B12 and folate in the hyperHcy and normoHcy groups respectively.

Hcy as a cardiovascular risk marker and strategies to lower plasma Hcy levels have been a topic of investigation for many years.[25,26] An association between cardiovascular risk and elevated Hcy has been confirmed by epidemiological studies, but intervention studies with an Hcy-lowering approach have not been consistent in their findings.[26–28] Vitamins B12, B6 and folate are directly involved in the Hcy metabolism.[11] However, in this study no direct relationship was observed, but a possible indirect relationship with haemopoiesis was indicated. This study used serum Hcy, vitamins B6, B12, folate as well as MCV (macrocytosis) as Hcy metabolic markers. A very high prevalence (66.36%) of hyperhomocysteinaemia is present in the sample of elderly people, resulting in a much larger group A than group B. The mean serum Hcy level in group A decreased significantly, whereas the mean Hcy level in group B increased during the intervention, despite very high supplemented dietary intake. Mean serum vitamin B12 and folate levels were within the normal reference range at baseline. Although a slight but statistically significant decrease of these two parameters occurred after the intervention in both groups, the mean levels were still within the respective normal reference ranges. This phenomenon can be due to normal physiological action. Both vitamin B12 and folate are absorbed mainly by highly specific binding glycoprotein. The availability of the binding glycoproteins is indirectly proportional to the vitamin B12 and folate status.[29] It may thus be that because vitamin B12 or folate deficiency was not present in a large percentage of the sample at baseline or follow-up, no additional absorption took place in spite of excess availability. Very low serum vitamin B6 levels were observed in both groups and improved significantly after the intervention, although in both groups the serum levels did not reach the minimum reference value. The prevalence of macrocytosis (MCV > 95 pg) decreased significantly in both groups after the intervention.

No statistically significant relationships were found between Hcy and dietary vitamin B6, vitamin B12 and folate as suspected due to the intervention; however, Hcy levels were significantly reduced in the hyperHcy group, indicating metabolic change. Hcy, dietary vitamin B6, vitamin B12 and folate correlated similarly with the red cell parameters, however. It can therefore be concluded that an indirect relationship exists between Hcy and dietary vitamin B6, vitamin B12 and folate. This can be explained by the biochemical interaction between vitamin B6, vitamin B12 and folate with Hcy during haemopoeiesis.[30]

The results of this study should be interpreted with caution due to the following limitations: first, this was not a case-control design where a control group received placebo tablets and an experimental group received the vitamin B6, B12 and folate supplementation. Due to ethical considerations, it was decided not to deprive any elderly included in this study from the possible health benefit of the supplement. Elderly attending the day care centre, but who had not given consent to participate in the study, also received the supplement, but no measurements were taken. This was so as not to exclude anyone from the study. Second, this was a purposively selected sample and not necessarily a representative sample of the elderly in South Africa and the results thus cannot be generalised.

Conclusion and recommendations

It can be concluded that vitamin B12, B6 and folate supplementation at > 125% RDA for a period of six months had a Hcy-lowering effect in hyperhomocysteinaemic individuals, but not for normo-homocysteinaemic individuals. The supplementation was thus beneficial to all the individuals (independently of their Hcy status) on their vitamin B6 serum levels and on their haemopoiesis (decreased macrocytosis).

This study recommends that an acute and drastic intervention is needed to reduce the high risk of CVD prevalent amongst the poor black elderly, and to provide better preventative health care to the aged in SA. Determining homocysteine levels as a screening test at primary health care level would be beneficial in preventative treatment.

Furthermore, it is recommended that future studies (case controlled) should evaluate the effect of the homocysteine supplements (vitamin B6, vitamin B12 and folate) individually and not in combination. The effect of a genetic polymorphism of the homocysteine metabolites, present in the black African population as a possible contributory factor to the high prevalence of hyperHcy, should be studied.

The nutritional supplementation programme as part of the national Department of Health Integrated Nutrition Program (INP) for South Africans is not targeted towards the elderly. This study proposes that such programmes should be adopted by the Department of Health INP and vitamin B6, B12 and folate supplementation should be considered as a homocysteine-lowering strategy.

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