African Journal for Physical, Health Education, Recreation and Dance (AJPHERD),
Vol. 15, No. 4 (December) 2009, pp. 650-659.

The effect of oral nutritional supplementation on selected pathology and anthropometric markers of health status in middle aged men

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(Received: 27 July 09 Revision Accepted: 9 October 2009)

Abstract

This study was aimed at determining whether treatment with nutritional supplementation would improve pathology measures of disease risk. A total of twenty (20) healthy males, 30-60 years of age, served as participants. Participants were randomly divided into an Active Group (AG) (n=10) and a Placebo Group (PG) (n=10). Participants took the treatment (with active or placebo ingredients) for 90 days. On day 1 and 90th day blood samples were collected and analysed for cardiovascular disease risk markers (HDL, LDL, Triglycerides, total cholesterol and C reactive protein). To evaluate renal function urea, electrolytes creatinine and g GFR were analysed. White cell count and differential count were used as indicators of immune function. Vit B_{12} and s-folate were also analysed. No significant differences were found between the pre-test and post-test readings in the PG, whilst the AG experienced significant differences between the pre- and post-test in sodium and s-folate readings. On sodium readings the AG presented more cases with significantly lower levels during the post-test whilst s-folate was statistically higher during the post-test. No significant changes were found in any of the other pathology disease markers. It can be concluded despite the significant improvement in folic acid and sodium level observed in the AG participants that the nutritional supplement rich in folic acid, Vit B_6, Vit B_1, Vit $B_{12,}$ zinc, calcium, amino acid, Vit E and Vit C used for a period of 90 days has no effect on selected pathology markers of health status in middle-aged sedentary men.

Key words: *Supplementation, groups, health status, sedentary, sodium, s-folate.*

Introduction

It is postulated that factors associated with the modern way of life such as stress, disease, poor nutrition, lack of exercise, environmental toxins, smoking and radiation all contribute to morbidity and mortality, and that diet supplements may confer protection against these factors (Thomas, Baker & Davie, 2003; Reddy & Katan, 2004). Among the claims which some suppliers make, is that immune function and cardiovascular disease risk – notably the lowering of cholesterol levels – are modified by these products (Vivekananthan, Penn, Sapp, Hsu & Topol, 2003, Cromarty, 2006).

Elevated blood levels of triglycerides and low-density lipoprotein cholesterol (LDL-C) have been associated with increased risk of cardiovascular disease

(ACSM, 2006). Some nutritional supplementations, for example folic acid, Vitamin B and Niacin have been shown to reduce low-density lipoprotein cholesterol (LDL-C), triglycerides, and potentially cardiovascular disease risk (Araki, Maruyama & Igarashi, 2006).

Levels of high-density lipoprotein cholesterol (HDL-C) appear to be protective against the development of atherosclerosis (Craig, 1998). There is some evidence that Niacin has high-density lipoprotein cholesterol (HDL-C) raising effects (Sanyal, Karas & Kuvin, 2007).

Amino acid supplementation has been associated with enhanced immune function in athletes (Bassit, Sawada & Bacurau, 2002). It has also been proposed that nucleotide supplementation may improve immunological function (Yu, 2002). There is some evidence to suggest that oral supplementation with a variety of micronutrients is useful in reducing the risk of diseases (Baran, 2004).

Vitamin E, (alpha-tocopherol) reduces the rate of nonfatal myocardial infarction with beneficial effects, apparently after one year (Stephens, Parsons, Schofield, Kelly, Cheeseman & Mitchinson, 1996). Adequate Vitamin C intake can play a role in lipid composition, and could be potentially important in the genesis and prevention of artherogenic disease (Okamoto, 2002). Zinc is known to be essential for all highly proliferating cells in the human body, especially the immune system (Ibs & Rink, 2003).

The objective of this study was to determine whether or not treatment with an oral nutritional supplementation, containing folic acid, Vit B_6, Vit B_1, Vit B_{12}, zinc, calcium, amino acid, Vit E and Vit C would improve pathological markers of disease risk.

Materials and Methods

A random double-blind, placebo-controlled pre-test/post-test group comparison was conducted. Sedentary, healthy males (n=20) aged 30-60 years participated in this investigation. All subjects completed and signed a document of informed consent, approved by the faculty of the Health Science Research Committee, University of Pretoria – approved protocol. For the purpose of participant recruitment *sedentary* was described as “no regular participation in structured physical activity”. All subjects were free from chronic disease or contra-indications to exercise, as assessed by written medical history. Participants using medication or other nutritional supplementation were excluded. The study

protocol was approved by the medical ethics committee of a leading South African University.

Anthropometric measurements were taken before and after 90 days of the study. The body mass was measured to the nearest 0.1 kg using a Detecto scale (Webb City, MO, USA). Stature was measured to the nearest 0.1 cm using a Seca 214 stadiometer (Seca Corporation, Hanover, USA). Body mass index (BMI) was calculated as mass in kg divided by height in meters squared. The skinfold thickness of seven sites (biceps, triceps, subscapula, suprailiac, abdominal, thigh, calf) were measured with a Harpenden skinfold caliper (British Indicators, West Sussex, England). These were summed to obtain the sum-of-7-skinfolds in mm. The equation of Durnin and Womersley (1974) was used to estimate percentage body fat (%BF DW).

Blood samples were drawn from the antecubital vein in the morning after an overnight fast, and having refrained from strenuous physical activity for 24 hours, at 0 and 90 days by an independent pathology lab. Each participant thus served as his own control. Samples were analysed for lipids (HDL, LDL triglycerides, total cholesterol), urea, creatinine, uric acid, c-reactive protein, white blood cell count, differential count, electrolytes, Vit B_{12} and s-folate.

Concentrations of selected blood markers of cardiovascular disease risk (lipogram-triglycerine, LDL-C, HDL-C, total cholesterol) and c-reactive protein were measured. Atherosclerosis is not solely a disease of lipid deposits, as systemic inflammation also plays a role. Studies demonstrate that plasma c-reactive protein levels predict the likelihood of coronary artery events in healthy people (Hansson, 2005; Boudik, Reissigova & Hrach, 2006).

To evaluate renal function, urea, creatinine, Glomerulo Filtration Rate (GFR) and electrolytes were tested. White cell count and differential count were used as indicators of immune function. Uric acid was measured because the purine nucleic acids, adenine and guanine are metabolised to uric acid in the body. Persons with elevated uric acid or a history of gout may have a slightly increased risk of gout while taking nucleic acid supplements.

Participants were randomly divided into an Active Group (AG) (n=10) and Placebo Group (PG) (n=10). The participants (n=20) received their treatment (with active or placebo ingredients) every 2 to 4 weeks with sufficient products supplied to last only until the next date of product supply.

The treatment administered included a supplement rich in folic acid, Vit B_6, Vit B_1, Vit B_{12}, zinc, calcium, amino acid, Vit E and Vit C. The dosing schedule specified that the AG took six sprays of the supplement daily in the morning under the tongue, held there for 30 seconds for optimal sublingual absorption. The PG received a placebo oral liquid spray for use in the same way for a 90 day period. Studies have found a 90-day intervention period of nutritional supplementation of adequate length to assess changes to baseline pathology measurement (Zhang, Bateman, Metzger & Lanigan, 2006; de Oliveira, Donangelo, de Oliveira, da Silveira & Koury, 2009).

On completion of the study one participant from the AG and three participants from the PG failed to comply with the supplementation requirements and their data were thus excluded from the data analysis.

The statistical analysis was performed by an independent statistician. Non-parametric tests, also known as distribution-free tests, were used. This is a class of test that does not rely on a parameter estimation or distribution assumptions. The major advantage attributed to these tests is that they do not rely on any seriously restrictive assumptions concerning the shape of the sampled population and thus accommodates small samples, as in the case of this study.

Friedman's rank test for K correlated samples and the Kruskal-Wallis one-way analysis of variance were used for data analysis. All differences are reported at the 5% level of confidence ($p<0.05$).

Results

The physical characteristics of both the AG and the CG are presented in Table 1. There were no statistically significant differences in anthropometrical and physical measurement found between groups at pre and post-testing and within groups after the 90 day treatment period.

Analysis of between group differences the AG indicated a statistically significant higher s-Folate (nmol/L) measure both at pre-testing and at post-testing. Additionally the AG showed statistically significant differences on the post-test readings of potassium, CO_2, and urea.

Within group analysis to test if any statistically significant differences existed between the pre-test and post-test values within each group showed that whilst the PG had no significant differences, the AG experienced significant differences between the pre- and post-tests of sodium and s-folate values (Table 2). Sodium

values in the AG presented more cases with significantly lower levels during the post-test.

Table 1. Comparison of the active and placebo groups' pre-test/post-test anthropometrical results.

Parameter	**Active group**					**Control group**				
	Pre-test (n=10)		**Post-test (n=9)**			**Pre-test (n=10)**		**Post-test (n=9)**		
	Mean	**Std Dev**	**Mean**	**Std Dev**	**P Value**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**P value**
Age (yrs)	39.8	5.0	41.7	3.5	0.81	40.1	8.7	41.1	8.7	0.92
Mass (kg)	86.8	14.2	85.2	14.3	0.61	84.2	11.7	86.5	11.2	0.44
Stature (cm)	175.6	8.4	173.3	11.7	0.32	178.7	7.8	180.4	6.2	1.00
BMI (m/h^2)	28.0	2.1	27.7	2.2	0.92	26.4	3.7	26.6	4.0	0.51
Sum of 7 skinfolds (mm)	149.6	24.6	141.5	30.6	0.24	130.8	44.2	132.9	50.8	0.31
Body fat (%)	28.6	3.0	28.6	3.9	0.50	25.2	6.4	25.3	6.3	0.90

The s-folate was statistically higher during the post-test than during the pre-test. The s-folate levels of the AG measured higher when compared to the PG levels, indicating that s-folate levels increased over the 90 day intervention period.

Discussion

A supplement rich in folic acid, Vit B_6, Vit B_1, Vit $B_{12,}$ zinc, calcium, amino acid, Vit E and Vit C. used by this study's sedentary participants did not have a significant effect on the body composition as measured by skinfolds and mass (Table 1). The PG experienced no significant changes in pathology measured tested over the 90 day intervention period with the placebo product (Table 2).

In the AG significant differences were present between the sodium and s-folate levels of the pre- and post-test. The sodium levels decreased whilst the s-folate increased with the 90 day supplementation period with the pre-test mean value at 24.89 nmol/L and the post-test mean value at 33.19 nmol/L; p=0.018.

As folic acid is a water-soluble vitamin which serves as part of a coenzyme that plays a role in the metabolism of methionin, an essential amino acid, it is critical to the formation of DNA, the generic material that regulates cell division. It is also essential for the production of red blood cells and has been shown to

improve endothelial function in coronary artery disease (Doshi, McDowell, Moat, Payne, Durrant, Lewis & Goodfellow, 2002).

Table 2. Comparison of the active and placebo groups' pre-test / post-test pathology results.

Parameter	**Active group**					**Control group**				
	Pre-test (n=10)		**Post-test (n=9)**			**Pre-test (n=10)**		**Post-test (n=9)**		
	Mean	**Std Dev**	**Mean**	**Std Dev**	**P Value**	**Mean**	**Std Dev**	**Mean**	**Std Dev**	**P Value**
T.Chol (mmol/L)	5.50	0.71	5.21	0.80	0.14	5.10	0.7	5.2	0.8	0.16
Tge (mmol/L)	1.76	0.70	1.86	0.61	0.74	1.41	0.7	1.9	0.6	0.44
HDL.Chol (mmol/L)	1.14	0.20	1.13	0.18	0.32	1.42	0.6	1.1	0.2	0.89
LDL.Chol (mmol/L)	3.83	0.64	3.67	0.52	0.41	3.20	0.7	3.7	0.5	0.08
Sodium (mmol/L)	140.66	1.41	139.14	1.35	0.02*	139.42	3.6	139.1	1.3	0.35
Potassium (mmol/L)	4.37	0.40	4.21	0.30	0.75	4.52	0.4	4.2	0.3	0.57
Chloride (mmol/L)	103.00	1.12	102.71	0.49	0.48	101.91	2.9	102.7	0.5	0.31
CO_2 (mmol/L)	23.89	2.15	23.57	1.99	0.58	25.57	1.4	23.6	1.0	0.40
Anion Gap (mmpl/L)	18.22	2.28	17.00	1.53	0.10	16.60	1.5	17.0	1.5	0.52
Urea (mmol/L)	5.66	1.20	4.99	1.04	0.61	5.12	1.2	5.0	1.0	0.09
Urate (mmol/L)	0.45	0.05	0.44	0.29	0.67	0.4	0.1	0.4	0.0	0.46
Creatinine (mmol/L)	96.89	11.08	89.29	13.03	0.15	95.8	10.0	89.3	13.0	0.86
GFR (ml/min)	80.33	9.57	88.29	15.76	0.15	81.4	9.9	88.3	15.7	0.86
WCC (10^a/L)	5.33	0.53	5.21	0.63	0.50	5.9	0.8	5.1	0.6	0.94
Vit B_{12} (qmol/L)	468.73	214.06	391.51	228.41	0.40	364.9	154.1	408.8	396.1	0.953
S-folate (nmol/L)	24.89	5.85	33.19	18.87	0.02*	18.1	5.1	33.9	6.8	0.26

* Significant change from the pre-test to the post-test at the end of 90 days intervention ($p<0.05$).

Endothelial dysfunction is a key process in atherosclerosis (Ross, 1993) and independently predicts cardiovascular events (Schachinger, Britten & Zeiher, 2000). High-dose folic acid (5 mg daily), alone or in combination with other B-group vitamins has been shown to improve endothelial function (Doshi et al. 2002).Additionally folate intake has also been positively associated with performance on a variety of measures of cognition (speed, memory, and fluency) in a healthy female populations across the lifespan (Bryan, Calvaresi & Hughes, 2002). An increase in plasma folic acid is therefore an important finding.

Although a large prospective cohort study on 1980 Finnish healthy middle aged men observed a significant inverse association between quantitatively assessed moderate-to-high folate intakes and incidence of acute coronary events several clinical trails have shown that folic acid therapy does not improve Chronic Vascular Disease (CVD) outcomes in older adults with CVD (Title, Cummings, Giddens & Bassar, 2000; Verhaar, Stroes & Rabelink, 2002; Carlsson, 2006).

Megadoses of niacin have been used in attempts to reduce high serum lipid levels (McKenny, 2004). McKenny (2004) noted that niacin favourably affects the lipid profile, as it reduces levels of total cholesterol, LDL-C triglycerides and lipoprotein, and increases HDL-C. Other researchers have documented that niacin can result in regression of atherosclerosis (Hunninghake, 1999; Zhao, Morse, Dowdy, Heise, DeAngelis, Frohlich, Chait, Albers & Brown, 2004). However, no significant reduction in LDL-C or an increase of HDL-C was noted in this study.

Although the sodium readings in the AG presented more cases with significantly lower values during the post-test (mean value in pre-test 140.66 mmol/L and mean value of the post-test 139.14 mmol/L, p= 0.02), however as the change noted was only 1.52mmol/L it was too small to hold any clinical significance (Cutler, Follmann & Allender, 1997).

Conclusion

The purpose of this investigation was to determine whether or not nutritional supplementation would improve pathology readings of disease risk in healthy middle-aged men. Although there was no changes in anthropometric measures and the majority of pathology markers tested and the increase in s-folate observed may be of importance, the main finding of the current investigation is that there was no effect on the pathological readings of disease risk with a nutrition supplementation rich in folic acid, Vit B_6, Vit B_1, Vit B_{12},, zinc,

calcium, amino acid and Vit C after a treatment period of 90 days in middle-aged healthy men.

Recommendations

Randomised controlled studies are suggested to investigate the use of this supplement in participants with already existing pathological conditions, such as atherosclerosis and CVD. Additionally the role of this supplement on plasma homocysteine levels should also be investigated due to the conflicting results which exist between plasma homocysteine levels and CVD across the spectrum of ages and risk subgroups (Araki et al. 2006, Carlsson, 2006). With adequate funding and resources more randomised clinical trials over a longer period with repeated measurements may be needed to clarify the role of this nutrition supplementation on disease risks.

Acknowledgement

The author would like to thank Christine Smit, the independent statistician who assisted in the data analysis.

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