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Supplementation with fruit and vegetable concentrate decreases plasma homocysteine levels in a dietary controlled trial

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Abstract

An elevated level of total plasma homocysteine (tHcy) is considered to be a predictor of the mortality risk for all diseases. A high consumption of vegetables and citrus fruit, both good sources of folate, decreases the concentration of tHcy. We investigated if supplementation of concentrated fruit and vegetables is able to decrease tHcy concentrations. For this purpose, we used a product that is presently on the market, made up of fruit and vegetable powders. 26 subjects participated in a cross-over design intervention trial. At the end of the study, each participant received 2 capsules of fruit and 2 capsules of vegetables a day for 4 weeks and then acted his/her own control for another 4 weeks. Daily extract intake decreased plasma tHcy from an expected mean \pm SD level of 12.71 ± 3.23 $\mu\text{mol/L}$ observed after the 28-day control period to 7.98 ± 1.70 $\mu\text{mol/L}$, a difference of 4.73 ± 1.153 ($p < 0.001$).

Elevated tHcy is a risk factor causally linked to chronic disease. The plasma tHcy concentration was decreased as a result of taking a powdered fruit and vegetable extract on a daily basis. © 2003 Elsevier Inc. All rights reserved.

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1. Introduction

An increase in the consumption of fruit and vegetables has a high impact on public health. Many studies have shown that 6 to 8 portions of fruit and vegetables a day reduce the risk associated with increased oxidative cellular stress [1–3]. Homocysteine is directly involved in oxidative damage and is considered to be an independent risk factor for cardiovascular and kidney diseases, for vascular dementia and for Alzheimer disease [4–9]. Recently, it has been considered to be predictive for risk of all-cause adult mortality [10–11].

Hyperhomocysteinemia can be congenital or acquired. The causes of the congenital form relate to deficiencies of the enzymic systems (N^5, N^{10} methylene-tetrahydrofolate reductase, cystathionine- β -synthase, cobalamin reductase, methionine synthase); while acquired hyperhomocysteinemia can be nutritional (deficiencies of vitamin B6, folate and vitamin B12), pharmacological (intake of methotrexate, carbamazepin, phenytoin, isoniazid, L-dopa), endocrine (hypothyroidism) or due to other pathologies (such as kidney failure and psoriasis) [12–13]. Approximately 80% of total plasma homocysteine is bound to albumin by a disulfide bridge. Unbound homocysteine species exist mainly as homocysteine-cysteine or homocysteine-homocysteine (homocystine) disulfides. Only 1% of all circulating homocysteine exists as truly free homocysteine. Total homocysteine (tHcy) describes the sum of all these free and protein-bound biochemical homocysteine species [14–17]. In fruit and vegetables there is a good concentration of vitamins in the B group, such as B-6 and folic acid, although vitamin B-12 is not present. The efficacy of these vitamins, in non-vegetarian subjects, suggest that a nutritional intervention with fruit and vegetables is practicable and can be successful. We have studied the hypothesis that supplementation with concentrated fruit and vegetables decreases tHcy levels.

2. Subjects and methods

2.1. Subjects

The primary end point was plasma tHcy reduction at 4 weeks. Assuming a two-sided type I error of 5 percent, we estimated that we would need 19 subjects for the study to have 95 percent power to demonstrate a mean (\pm SD) plasma tHcy reduction that was 1.5 (\pm 1.7) μ mol/L [18], greater in the treated group than in the control group [19]. Given an anticipated dropout rate of 35 percent, we set the enrolment at 30 subjects.

Thirty healthy men and women (15 men and 15 women), aged 20–56 yrs were recruited. Exclusion criteria were: pregnancy, breast-feeding, gastrointestinal disorders, use of vitamins and/or minerals, vegetarianism restricted caloric diets, regular intake of medicine, yeast or seaweed, malaria prophylactics or anti-convulsants in the 4 months prior to the study. On the basis of these criteria, 26 subjects (12 men and 14 women) were considered eligible for participation in the trial. Written consent was requested from all participants for enrolment in the study. The subjects under treatment took the fruit and vegetables concentrate twice a day for 4 weeks. No alimentary restrictions or changes in life-style were requested.

2.2. Product

For the test, a product (Juice Plus^{TM+}, manufactured by NSA International Inc., Fleet, UK GU13 8UY) that is presently on the market and made up of fruit and vegetable powder was used. The supplementation consisted in 2 capsules containing 1.7 grams of concentrated, powdered fruit extract (from apples, oranges, pineapples, blueberries, peaches and papaya) in the morning and 2 capsules containing 1.7 grams of concentrated, powdered vegetable extract (from carrots, parsley, beet-root, broccoli, black cauliflower, cabbage, spinach and tomato) in the evening, taken during meals.

The micronutrient concentrations declared on the label for 2 capsules of fruit are: vitamin-C 90 mg, vitamin-E 15mg, vitamin B-1 0.4 mg, vitamin B-2 0.3 mg, vitamin B-6 2.0 mg, niacin 7 mg and folic acid 100 mcg; for the vegetable ones: vitamin-C 50 mg, vitamin-E 15 mg, vitamin B-1 0.6 mg, vitamin B-2 1.0 mg, vitamin B-6 1.5 mg, niacin 13 mg and folic acid 100 mcg, magnesium 45 mg and zinc 2.5 mg. The same product was examined in relation to its constituents and its antioxidant power in a pilot study [20].

2.3. Design

The study was conducted according to a case-cross-over study design, with subjects assigned at random to the treatment group and to the control group (Fig. 1). The cross-over, using subjects as their own controls enabled control of the possible confounding factors such as sex, age, dietetic habits and smoking. The trial was divided into three time periods, each one consisting of 28 days: the first treatment period, wash-out and the second treatment period. During the 1st 27-day treatment period (days 0 to 27) the 13 subjects assigned to Group 1 were supplemented daily with 2 capsules each of the powdered fruit and vegetable extract in the morning and evening, respectively, while the 13 subjects of Group 2 received nothing. Following treatment period 1, both groups were subjected to a 28-day wash-out period (days 28 to 55). During the 2nd 27-day treatment period (days 56 to 82), Group 2 received capsules of fruit and vegetable extract daily while Group 1 received nothing.

One plasma sample was collected of each participant on days 0, 14, 27, 56, 69 and 83, in order to detect tHcy concentrations; another sample collected for determine serum folic acid levels: on day 0 and on day 27 for group 1; on day 56 and on day 83 for group 2.

Dietary intake was estimated by means of food diary in which participants kept a record of their daily food intake throughout the study period.

2.4. Analytic methodology

Levels of folic acid in serum were determined using the immunoenzymatic method [21]. Colorimeter Enzymatic method was used to measure total cholesterol in serum [22]. For homocysteine, the analyses were carried out by an immunoenzymic methodology “Axis homocysteine EIA” in order to determine the presence of tHcy in plasma. The range of measurement is 2-50 umol/L, the intra-assay coefficient of variation was < 5%, the inter-assay vc < 4%. In addition, an excellent correlation was found with the H.P.L.C. method [23].

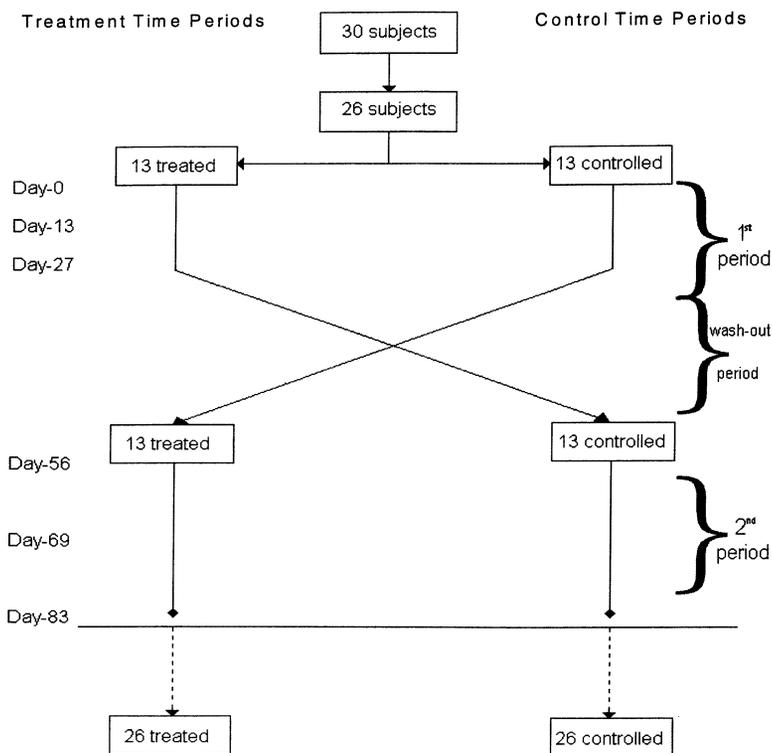


Fig. 1. Design of study.

2.5. Statistics

In order to compare the changes which took place in tHcy and serum folic acid levels from the starting point of both groups paired t-tests were used, for the analysis of the data the programme SPSS for Windows version 6.0.1 (SPSS Inc.,1989-1993) was utilized. P values <0.05 were considered moderately significant, <0,001 highly significant. Values in the text are means \pm SD.

3. Results

The characteristics of the participating subjects are shown in Table 1. 12 men and 14 women with an average age of 35.6 years and a body mass of 24.2 participated. 10 subjects regularly performed physical activities, 3 were smokers. The average level of cholesterolemia was at 155.7 mg/dL. Most of the subjects normally consumed 1-2 portions of fruit and vegetables a day. The tHcy levels in plasma decreased throughout each period of treatment but not during the control periods of the study. In Table 2 the results relative to supplementation and its effect on tHcy in the subjects during the period of the trial are shown. tHcy

Table 1
Characteristics of subjects that participated in the study

	Men (n = 12)	Women (n = 14)	Total (n = 26)
Age (years)*	34.5 ± 10.1	36.5 ± 11.2	35.6 ± 10.7
Body Mass index (Kg/m ²)	24.3 ± 2.18	24.1 ± 2.34	24.2 ± 2.22
Smokers	1	2	3
Regular physical activity (2-5 dy/5)	3	7	10
Total serum cholesterol (mg/dL)*	153.3 ± 15.44	157.7 ± 23.31	155.7 ± 19.83
Diet			
Fruit and vegetables (portions a day)			
<1	1	2	3
1-2	6	5	11
3-4	3	5	8
>4	2	2	4

* mean ± SD.

levels decreased significantly in both groups. In group 1 the decreased was 4.1 (± 2.06) umol/L from day 0 (starting day of consumption of product) to day 27 (end of treatment); for group 2, tHcy decreased by 5.03 (± 1.39) from day 56 (beginning of consumption) to day 83 (end of treatment), whereas plasma tHcy changed little during control periods for both groups. In Table 3 the results of plasma tHcy levels are shown for subjects during both of the case cross-over periods of the study. During treatment periods when subjects took the powdered extract plasma tHcy steadily decreased, by 2.34 (± 2.82) umol/L half-way through and by 4.73 (± 1.53) umol/L by the 27th day of supplementation compared to controls.

Folic acid serum levels were for group 1: 4.1 (± 0.6) nmol/L on day 0 and 10.7 (± 1.1) nmol/L on day 27 (P value < 0.001); for group 2: 4.2 (± 0.4) nmol/L on day 56 and 11.3 (± 2.4) nmol/L on day 83 (P value < 0.001).

Table 2
Effects of supplementation with concentrated fruit and vegetables on tHcy in subjects, during the period of the trial

	Group-1 (n = 13)	Group-2 (n = 13)
Plasma homocysteine, umol/L		
Day-0	12.64 ± 3.70	<i>12.23 ± 3.62</i>
Day-13	9.51 ± 1.63	<i>12.33 ± 4.57</i>
Day-27	8.54 ± 1.64	<i>12.86 ± 3.41</i>
Day-56	<i>12.79 ± 4.06</i>	12.44 ± 3.01
Day-69	<i>12.15 ± 4.02</i>	10.28 ± 1.05
Day-83	<i>12.56 ± 3.17</i>	7.41 ± 1.62
Change from baseline	-4.1 ± 2.06**	-5.03 ± 1.39**

Values are mean ± SD. Values in cursive (italics) refer to the period of control, those in normal print refer to the period of treatment.

Baseline: for subjects of group 1 = day 0; for subjects of group 2 = day 56.

Student's t-test: *P < 0.05; **P < 0.001.

Table 3
Concentrations of plasma tHcy in the cross-over treatment periods

	Subjects not treated (n = 26)	Subjects treated (n = 26)	Change from baseline
Starting period ¹	12.51 ± 3.78	12.54 ± 3.31	−0.03 ± 0.47 ^{NS}
Half period ²	12.24 ± 4.22	9.90 ± 1.40	−2.34 ± 2.82*
Endpoint ³	12.71 ± 3.23	7.98 ± 1.70	−4.73 ± 1.53**

Values are mean ± SD.

¹ = subjects treated: day 0 for group 1 and day 56 for group 2; subjects not treated: day 0 for group 2 and day 56 for group 1;

² = subjects treated: day 13 for group 1 and day 69 for group 2; subjects not treated: day 13 for group 2 and day 69 for group 1;

³ = subjects treated: day 27 for group 1 and day 83 for group 2; subjects not treated: day 27 for group 2 and day 83 for group 1.

Significance level of P: ^{NS} = not significant; * P < 0.005; **P < 0.001.

4. Discussion

This controlled dietary intervention study demonstrated that the daily consumption of 2 capsules of concentrated fruit and 2 capsules of concentrated vegetables has significantly decreased plasma tHcy levels in 26 subjects whose normal fruit and vegetable consumption is low. THcy levels decreased from an average of 12.71 (±3.23) during the control period to 7.98 (±1.70) during the treatment period. Subjects, were asked not to change their lifestyle, and each participant acted as his/her own control.

Many studies have demonstrated that providing folic acid or food rich in folate improved folate status and at the same time decreased tHcy. Brouwer et al. (1999) discovered an accentuated reduction in tHcy concentration after being given vegetables and citrus fruit [18]. However, the quantity of vegetables and citrus fruit provided every day (350 g. of vegetables, 1 citrus fruit and 200ml. of citrus juice, reaching a level of dietary folate equal to 560 µg/day) was higher than levels that may expected to be consumed in a general population. In our study the level of folate supplied by the capsules was 200 µg/day which resulted in a steady, significant decrease of tHcy, reflected by a moderate decrease half-way through the treatment period that continued to decline through the last day of treatment. The average values and changes in plasma tHcy in both periods observe in this study are in agreement with results of other studies, where supplemental folic acid or 5,10-MTHF was used [24–27]. The decrease in tHcy values, with a difference of means of 4.73 (±1.53) µmol/L, occurred with all 26 participants of the study.

In conclusion, we have shown that daily intake of supplements of powdered fruit and vegetables extracts in capsular form significantly decreased plasma levels of tHcy in healthy subjects, just as the interruption of intake allowed tHcy return to pre-supplementation levels. A homocysteine lowering effect may be only part of the health benefit associated with consuming fruit and vegetable extracts on a daily basis. In the other study, the same product decreased DNA damage in the peripheral lymphocytes of an elderly population [28].

Further studies are in course to demonstrate if the decrease of tHcy is associated with a decrease of the risk of pathologies in which it is implicated. However, already the results of

the first studies are encouraging. For example, in a prospective study of 4.4 years, Peterson et al. found that after Hcy-lowering treatment with vitamin supplementation, the rate of progression of carotid plaque formation before and after supplementation was significantly reduced [29]. If this were to be confirmed by other research it would mark a decisive move forwards in the nutritional prevention of disease.

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