

Changes in folate status in overweight/obese women following two different weight control programmes based on an increased consumption of vegetables or fortified breakfast cereals

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The modification of folate status was analysed in a group of sixty-seven overweight/obese women of childbearing age (20–35 years). Subjects were randomly assigned to one of two slightly hypocaloric diets: diet V (increased consumption of vegetables) or diet C (increased consumption of breakfast cereals). Dietetic, anthropometric and biochemical data were collected at the start of the study and again at 2 and 6 weeks. At 6 weeks a weight loss of 2.0 (SD 1.3) kg was achieved in V subjects and of 2.8 (SD 1.4) kg in C subjects ($P < 0.05$). At the start of the study, 64.2% of all subjects had a folate intake of < 67% of the recommended intake; this fell to just 3% (7.14% of V subjects and 0% of C subjects) by week 6. Significant increases were only seen in C subjects in serum folate concentrations (both at 2 and 6 weeks), accompanied by a significant reduction in serum homocysteine (at week 6). Some 62.1% of all subjects had serum folate concentrations of ≥ 13.6 nmol/l (associated with a very low risk of neural tube defects) at the start of the study, while 87.0% (85.2% of V subjects and 88.9% of C subjects) had concentrations of ≥ 13.6 nmol/l at 6 weeks ($P < 0.01$). Increasing the relative consumption of vegetables/cereals in the context of a slightly hypocaloric diet may therefore be a good way to lose body weight. Breakfast cereals may be of special help with respect to folate status and serum homocysteine levels in overweight/obese young women following energy restriction diets.

Folic acid: Young women: Overweight: Obesity: Homocysteine

In September 1992, the US Public Health Service recommended that women of childbearing age who were capable of becoming pregnant should consume 400 $\mu\text{g}/\text{d}$ folic acid to reduce the number of cases of neural tube defects (Public Health Service, Centers for Disease Control and Prevention, 1992). In 1998, the Food and Drug Administration (1998) required the fortification of enriched cereal grain products with folic acid, and manufacturers have voluntarily added more folic acid to many ready-to-eat breakfast cereals (Centers for Disease Control and Prevention, 2002). The result has been a considerable improvement in folate status in this population group, accompanied by an impressive reduction in the prevalence of neural tube defects (Centers for Disease Control and Prevention, 2002; Hertrampf *et al.* 2003; Dietrich *et al.* 2005; Pfeiffer *et al.* 2005). A secondary possible benefit of fortification might be a reduction in the risk of developing CVD (Boushey *et al.* 1995; Lee *et al.* 2003) and certain cancers (Dietrich *et al.* 2005).

A number of studies (Ortega *et al.* 1996a, 1999b, 2000; Navia *et al.* 2003) have shown that, for the Spanish population, the greatest distances between the observed and

recommended intakes of foods correspond to vegetables (natural sources of folate) and cereals (important sources of folate only when fortified). The consumption of foods fortified with folic acid is rare; extra folate is added to very few foods and the fortification of cereals with this vitamin is not obligatory (Ortega *et al.* 2003a). This explains why the folate status of Spanish women leaves clear room for improvement (Ortega *et al.* 2003a, 2004b). Women who are concerned about their weight have an additional problem; dieting per se conceptually in an effort to lose weight can lead to restrictions in folate intake (Neumark-Sztainer *et al.* 2004). Similarly, obese women tend to consume fewer folate-containing fruits and vegetables (Serdula *et al.* 1996), and some authors (Tungtrongchitr *et al.* 2003; Mojtabai, 2004; Hirsch *et al.* 2005) have reported an inverse association between serum folate and BMI.

The results of previous studies (Ortega & Andrés, 1998; Ortega *et al.* 1999a, 2005; Ortega & López-Sobaler, 2005) suggest that approximating the diet to the theoretical ideal by increasing the relative consumption of vegetables and cereals is nutritionally beneficial, and helps to achieve a hypocaloric diet that aids

Abbreviations: DFE, dietary folate equivalents; diet C, increased consumption of breakfast cereals; diet V, increased consumption of vegetables; RI, recommended intake; tHcy, homocysteine.

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weight control. An increased consumption of vegetables or fortified breakfast cereals might be of particular benefit to those who need to improve their folate status (Schorah & Wild, 1993; Hertrampf *et al.* 2003; Department of Nutrition, 2004a; Dietrich *et al.* 2005; Pfeiffer *et al.* 2005).

The aim of the present work was to determine the folate status of a group of overweight/obese young women and to analyse the changes produced by following two slightly hypocaloric diets, one rich in vegetables, the other rich in cereals, especially fortified breakfast cereals.

Materials and methods

Study subjects

The study subjects were sixty-seven women aged 20–35 years (mean 27.8 (SD 4.6) years). Most were university students.

The subjects were enrolled through a public offer to take part in a study on 'The assessment of nutritional status and improvement of weight control'. The study was publicised using posters, radio announcements and via publications directed towards young, female university students.

Initially, all interested parties were interviewed by telephone to ensure that they met the inclusion criteria, which were: female sex, age 20–35 years, BMI 24–35 kg/m², not having quitted smoking in the previous two months, to be free of all disease that might interfere with the results (such as diabetes, hyperthyroidism, metabolic disease, hypertriglyceridaemia, lactose or gluten intolerance (coeliac disease) and food allergies etc.), to not be currently involved in a weight-loss programme, to have lost no more than 4.5 kg in the 2 months prior to the study, to have not lost or gained more than 3 kg between the first interview and the start of the study, to have a regular menstrual cycle, to take no more than two alcoholic drinks per day, and to be neither pregnant nor lactating.

Those interested in taking part declared themselves to meet all inclusion criteria and were invited to the Department of Nutrition at the Universidad Complutense de Madrid. Here, their weights and heights were recorded, and questionnaires were completed to collect personal, health and dietary information etc. All persons who were confirmed as meeting the inclusion requirements were informed of the aim of the study, of the clinical tests they would undergo, and of the number and type of interviews and testing to which they would be subject. Following the requirements of the Ethics Committee of the Faculty of Pharmacy, all subjects signed a witnessed form of consent to be included.

The final number of aspirants was 193, but only sixty-seven met all the inclusion criteria. Fifty-seven women concluded the 6-week dietary intervention period.

Interventions

The experimental diets to which the subjects were randomly assigned were designed to provide a mean of approximately 20% less than their theoretical energy requirements. Theoretical energy expenditure was established by taking into account the body weight, age and physical activity of all subjects, using equations proposed by the World Health Organization (1985). Both diets were structured with the idea of

approximating them to the theoretical ideal by increasing the relative consumption of either vegetables or cereals; earlier studies have shown that these foods are those with the greatest differences between their observed and recommended intakes (Ortega *et al.* 1996a, 1999b, 2000; Navia *et al.* 2003).

Diet C. With this diet, the weight control measures were based on restricting the consumption of energy-rich foods and increasing the consumption of cereals. Breakfast cereals and cereal bars were particularly recommended (a minimum of three times per day) since, apart from carbohydrate, they also provide fibre, minerals and vitamins (particularly folic acid: 250–300 µg/100 g). However, the subjects were also advised to eat other cereals, e.g. bread, rice and pasta etc.

Diet V. With this diet, the weight control measures were based on restricting the consumption of energy-rich foods and increasing the intake of vegetables (minimum three times per day).

Increasing the consumption of these foods may also be useful for improving folate status since vegetables are a natural source of folate and the breakfast cereal was enriched in this vitamin (Department of Nutrition, 2004a).

The full characteristics of the diets followed and other methodological details are described elsewhere (Ortega *et al.* 2005).

Compliance with dietary rules

Over the intervention period (a total of 6 weeks), the subjects attended a weekly appointment to record anthropometric data and to discuss (and solve) any difficulties in following the diet assigned.

Methods

The following data were collected from all subjects during the pre-intervention stage, and again at 2 and 6 weeks.

Physical activity. The subjects completed a questionnaire on their normal physical activity; this information was used to calculate their energy expenditure (Ortega *et al.* 2003b). Subjects indicated the length of time spent sleeping, eating, playing sport etc. during both working days and weekend days. An activity coefficient was established for each subject (World Health Organization, 1985; Ortega *et al.* 1996b). No overt attempt to increase the activity of the subjects was made, but all were encouraged to maintain moderate activity without introducing any great changes into their normal activity routines (the aim of the study was to analyse the effects of changes in food consumption, not exercise).

Anthropometric information. Weight and height were determined using a Seca Alpha digital electronic balance (range 0.1–150 kg) and a Harpenden digital stadiometer (range 70–205 cm), respectively. For both measurements, subjects were barefoot and wore only underwear. All data were collected at the Department of Nutrition by trained personnel following norms set out by the World Health Organization (1995). The BMI was calculated as weight (kg)/height² (m²).

Health variables. Information was collected on any disease problems, the consumption of medications, supplements and the consumption of manufactured diet foods. Tobacco use and alcohol consumption were also recorded (both are

known to affect folate status; Ortega *et al.* 2004b; Hirsch *et al.* 2005; Stark *et al.* 2005).

Dietetic study. A 3 d food diary was used to register all intakes (both at home and away) for 3 d, including a Sunday (Ortega *et al.* 2003c). Subjects were instructed to record the weights consumed if possible, and household measurements (spoonfuls, cups etc.) if not. The aim was to have as true a record as possible; subjects were asked to record all intakes, even though they broke the 'rules' of their diet.

The energy and nutrient contents of these food were then calculated using food composition tables (Department of Nutrition, 2004a). The values obtained were compared to those of the recommended intake (RI) (Department of Nutrition, 2004b) to determine the adequacy of the diets. Special attention was paid to the intake of energy and folates. DIAL software (Alce Ingeniería, Madrid, Spain) was used to process all data (Ortega *et al.* 2004a).

Folate intake was recorded in the form of dietary folate equivalents (DFE), which takes into account the higher bio-availability of straight folic acid compared to food folate (DFE; 1 DFE = 1 µg food folate = 0.6 µg folic acid from fortified food) (Food and Nutrition Board and Institute of Medicine, 2000). Thus, the dietary data presented in the present study are in micrograms of total folate, the term 'total folate' referring to the combination of food folate and folic acid provided by fortified foods. Thus dietary folate (µg DFE) = µg food folate + (1.7 × µg folic acid added to or provided by fortified foods).

Blood biochemical analysis. Blood samples were taken from the cubital vein first thing in the morning (after a 12 h overnight fast) at the Department of Nutrition. Part of this blood was collected in tubes with no anticoagulant to allow the separation of serum. Samples were kept at -40°C until analysis.

Serum folate was determined by RIA (Lindenbaum, 1983) (CV 4.5%), and plasma homocysteine (tHcy) levels by

polarized fluorescence immunoanalysis using an Imx Analyzer (Shipchandler & Moore, 1995) (CV 6.3%).

Statistical analysis

Means and standard deviations were calculated for all variables. ANOVA was used to analyse the changes in the different variables over time in each diet group. Weight losses at 2 and 6 weeks and the results for group C and V subjects were compared using the Student's *t* test or the Mann-Whitney *U* test (if the distribution was not homogeneous). Linear correlation coefficients were calculated using the Pearson test. Analysis of covariance and multiple regression analysis were used where indicated. Comparisons between proportions were made using an approximation of the binomial distribution to the normal distribution, employing continuity correction. All calculations were made using RSIGMA BABEL Software (Horus Hardward, Madrid, Spain). Significance was set at $P < 0.05$.

Results

No initial significant differences were seen between V and C subjects with respect to tobacco use (10.4 (SD 6.4) cigarettes/d for V subjects and 11.4 (SD 6.7) for C subjects), or alcohol consumption (Table 1), nor were any modifications detected over the study period.

Some 11.1% of V subjects and 9.7% of C subjects (NS) declared taking supplements containing folic acid on a sporadic basis. Similarly, 41.7% of V subjects and 41.9% of C subjects (NS) declared taking breakfast cereals or other foods (dairy, juice) fortified with folic acid. However, at the start of the study, no significant differences were seen in the serum folate concentrations of those who had taken and those who had not taken supplements or fortified foods.

Table 1. Changes in anthropometric data, activity coefficient and food intake over the dietary intervention period§ (Mean values and standard deviations)

	Pre-intervention data				Results at 2 weeks				Results at 6 weeks			
	Diet V (n 36)		Diet C (n 31)		Diet V (n 32)		Diet C (n 30)		Diet V (n 28)		Diet C (n 29)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Anthropometric data												
Weight (kg)	73.3	7.7	77.8	10.9	72.3	8.1	75.6	10.6	70.1	7.3	74.0	10.9
Height (cm)	161.8	5.0	164.8†	5.9	161.8	5.0	164.9†	6.0	161.7	5.3	164.6	5.8
BMI (kg/m ²)	28.0	2.8	28.6	3.6	27.6	2.8	27.4	3.5	26.8	2.6	27.3	3.6
Weight loss since start (kg)					0.9	0.6	1.5††	0.9	2.0†††	1.3	2.8††††	1.4
Physical activity level	1.53	0.05	1.51	0.07	1.51	0.05	1.50	0.09	1.51	0.06	1.52	0.07
Alcohol intake (% energy)	0.62	1.27	0.88	1.48	0.41	0.92	0.34	0.99	0.23	0.83	0.25	0.53
Consumption of food groups (servings/d)												
Cereals and pulses	3.9	1.3	4.1	1.5	3.2	1.2	4.9*†††	0.9	3.8	1.3	4.9*†††	1.1
Greens and vegetables	2.8	1.2	3.0	1.2	4.7**	1.7	3.9	1.8	4.8**	1.5	3.5†††	1.2
Fruits	0.9	0.7	1.3	1.0	3.4**	1.5	3.6**	1.3	3.7**	1.7	3.5**	1.1
Milk products	1.7	1.0	2.1†	0.7	1.9	0.6	1.8	0.6	1.9	0.8	1.9	0.7
Meat, fish and eggs	3.7	1.6	4.1	1.6	3.1	1.4	2.4**†	1.1	2.3**†	1.1	2.5**	1.0

Diet C, increased consumption of breakfast cereals; Diet V, increased consumption of vegetables.

Mean values were significantly different from those of the pre-intervention group: * $P < 0.05$; ** $P < 0.01$.

Mean values were significantly different from those of the 2-week group: † $P < 0.05$; †† $P < 0.001$.

Mean values were significantly different from those of the diet V group: ‡ $P < 0.05$; ‡‡ $P < 0.01$; ‡‡‡ $P < 0.001$.

§ For details of procedures, see pp. 713–714.

During the intervention period, the consumption of cereals increased among C subjects and the consumption of vegetables increased among V subjects (both at 2 and 6 weeks). The intake of fruit increased in both groups at 2 and 6 weeks, while the consumption of meat, fish and eggs fell at 2 weeks in C subjects, and was lower at 6 weeks in both V and C subjects (Table 1).

Energy intake was reduced in both groups (Table 2), however, the weight loss of C subjects was significantly greater than that of V subjects at both 2 and 6 weeks (Table 1). This remained the case after adjusting for initial weight (covariance analysis). No changes in the activity coefficient were seen that might have contributed to this weight loss (Table 1).

Despite this reduction in energy intake, folate intake, folate density, the contribution of dietary folate to the coverage of the RI and the index of nutritional quality for folate increased in both V and C subjects, although more so in the latter. In addition, the percentage of women with folate intakes below the RI fell with both diets, but again, more so in C subjects (Table 2).

Significant increases in serum folate were only seen in C subjects (both at 2 and 6 weeks). The percentage of subjects with serum folate concentrations <14.9 nmol/l fell significantly in V subjects at 6 weeks and in C subjects at 2 and 6 weeks. Serum tHcy levels were significantly reduced in group C subjects by week 6 (Table 3).

Discussion

The initial consumption of meat/fish/eggs was somewhat higher than that recommended, while that of cereals/pulses, vegetables/greens and fruit was lower (Ortega *et al.* 1999b; Federación de Industrias de Alimentos y Bebidas *et al.* 2004) (Table 1). The same situation has been reported in other studies (Ortega *et al.* 1996a, 2000, 2005; Navia *et al.* 2003). This justifies the following of diets C and V during the intervention period: these interventions approximate the diet to the theoretical ideal while providing a slightly hypocaloric energy intake and a greater intake of folate (Tables 1 and 2). The results obtained over the study period show that the goals set out during the planning period were attained (Tables 1 and 2), including a slight weight reduction (more obvious in C subjects) (Table 1). This agrees with the results of other studies (Ortega & López-Sobaler, 2005; Ortega *et al.* 2005).

The initial dietetic and anthropometric data (Tables 1 and 2) were similar to those obtained for other groups of overweight women (Ortega *et al.* 1996a, 2000; Navia *et al.* 2003). The initial blood analysis results were also similar to those recorded in other studies (Lee *et al.* 2003; Ortega *et al.* 2003a, 2004a; Tucker *et al.* 2004).

At the start of the intervention, the mean folate intake was far below the recommended 400 $\mu\text{g}/\text{d}$ (Department of Nutrition, 2004b); 64.2% of all subjects had a folate intake of $<67\%$ of this RI (Table 2). This clearly inadequate intake (with respect to protection against congenital malformations, cancer and CVD) coincides with that reported in other studies (Ortega *et al.* 2004a; Stark *et al.* 2005), in which the difficulty of reaching an optimum intake without taking supplements or consuming fortified foods was discussed (Stark *et al.* 2005).

At the start of the study an association was seen between folate intake and serum folate concentrations (r 0.346), as reported by other authors (Stark *et al.* 2005). This is to be expected since serum folate is a very sensitive indicator of dietary folate intake (Food and Nutrition Board and Institute of Medicine, 2000). A correlation was also seen between folate intake and the number of rations of cereals (r 0.252) and vegetables (r 0.542) consumed.

During the intervention period, the increased consumption of breakfast cereals clearly increased the intake of folate (Table 2). This is in good agreement with the results of Schorah & Wild (1993). The latter authors monitored the effects of introducing folic acid-fortified foods to the diets of young women by measuring their total folate intakes between 1986 and 1988, a period during which breakfast cereal manufacturers in the UK began to fortify their products with folic acid. These authors estimated the total folate intake to be 43% higher in consumers of fortified breakfast cereals than in non-consumers. Folate intake also increased in V subjects because of the folate content of the vegetables (Department of Nutrition, 2004a), although this increase was smaller than in the C subjects (Table 2).

Mandatory folic acid fortification of all enriched cereal-grain products in the USA (with the aim of increasing folate intake in women of childbearing age to reduce the incidence of neural tube defects) has been associated with significant increases in blood folate concentrations (Dietrich *et al.* 2005; Pfeiffer *et al.* 2005). For example, Dietrich *et al.* (2005) report the median serum folate concentration of women aged 15–44 years to have increased from 10.9 to 29.5 nmol/l from the time of the NHANES III study (reflecting the time prior to folate fortification) to that of the NHANES 1999–2000 study (reflecting the time period after mandatory fortification). The mean dietary total folate intake of the study population increased from 275 to 351 $\mu\text{g}/\text{d}$ (Dietrich *et al.* 2005). In Chile, after the mandatory fortification of wheat flour with folic acid, which aimed to increase daily folate consumption by women of childbearing age by 400 $\mu\text{g}/\text{d}$, serum folate concentrations in a representative sample of the population increased from 9.7 (SD 4.3) to 37.2 (SD 9.5) nmol/l (Hertrampf *et al.* 2003).

The initial folate status of the subjects in the present study (Table 3) was better than that reported by Hertrampf *et al.* (2003) and Dietrich *et al.* (2005), but in agreement with these authors, a substantial improvement in folate status was seen in C subjects (Table 3).

With regard to the prevention of neural tube defects, Dietrich *et al.* (2005) also report that, as a consequence of the mandatory folic acid fortification of cereals, a larger percentage of women of childbearing age have attained the lower limit of the range of serum folate concentrations associated with very low risk for neural tube defects (≥ 13.6 nmol/l; Sauberlich *et al.* 1974). In the present work, 62.1% of women had serum folate concentrations of ≥ 13.6 nmol/l at the start of the study, rising to 87.0% (85.2% of V subjects and 88.9% of C subjects) at 6 weeks ($P < 0.01$).

The influence of folate intake on serum folate concentrations was further reflected in that women who increased their folate intake by <200 $\mu\text{g}/\text{d}$ (P_{50}) at 6 weeks showed significantly smaller elevations of serum folate (3.04 (SD 15.7) nmol/l) compared to those who increased their intake by

Table 2. Changes in dietary data over the dietary intervention period§
(Mean values and standard deviations)

Intake	Pre-intervention data						Results at 2 weeks						Results at 6 weeks					
	Diet V (n 36)		Diet C (n 31)		Diet V (n 32)		Diet C (n 29)		Diet V (n 28)		Diet C (n 29)		Diet V (n 28)		Diet C (n 29)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Energy (kJ/d)	8788	2226	9913†	2112	6544**	959	6602**	1185	6638**	1144	6726**	1173	6638**	1144	6726**	1173		
Proteins (g/d)	80.5	21.3	94.2‡	23.9	68.3*	12.8	69.7**	13.8	66.3**	14.9	69.9**	11.4	66.3**	14.9	69.9**	11.4		
Lipids (g/d)	104.2	33.3	119.9	37.5	65.1***	14.1	48.0***‡‡‡	16.8	60.0***	22.1	51.0***	16.2	60.0***	22.1	51.0***	16.2		
Carbohydrates (g/d)	195.7	51.7	212.0	50.4	159.9**	31.1	201.6‡‡‡	36.4	188.5††	44.7	202.8	38.3	188.5††	44.7	202.8	38.3		
Vitamin B12 (µg/d)	6.4	4.5	6.3	3.2	4.9	4.7	4.8‡‡	1.4	3.7	1.8	4.8†	1.3	3.7	1.8	4.8†	1.3		
Vitamin B6 (mg/d)	2.02	0.52	2.02‡	0.52	3.26**	1.15	3.26**†††	1.15	2.74**††	0.83	4.13**††††	0.73	2.74**††	0.83	4.13**††††	0.73		
Folate (µg/d)‡	224.3	69.9	269.6‡	77.1	337.1**	114.2	544.9**‡‡‡	120.8	418.4**†††	115.9	533.6**‡‡‡	103.1	418.4**†††	115.9	533.6**‡‡‡	103.1		
Folate density (µg/MJ)	25.9	6.8	28.4	10.1	51.8**	16.7	83.2**‡‡‡	15.9	64.5**††	19.6	80.9**‡‡	17.6	64.5**††	19.6	80.9**‡‡	17.6		
Coverage of RI (%)	56.1	17.5	67.4‡	19.3	84.3*	28.5	136.2**‡‡‡	30.2	120.2**††	89.9	133.4**‡‡	25.8	120.2**††	89.9	133.4**‡‡	25.8		
Folate intakes < RI (%)	97.2		93.5		75**		6.89***‡‡‡		46.4***†		13.8***‡‡		46.4***†		13.8***‡‡			
Folate intakes < 67% RI (%)	72.2		54.8		21.9		0†		7.14		0		7.14		0			
INQ	0.65	0.18	0.73	0.28	1.27**	0.41	2.10**‡‡‡	0.51	1.55**††	0.45	2.04**‡‡‡	0.47	1.55**††	0.45	2.04**‡‡‡	0.47		
INQ < 1 (%)	94.4		83.9		21.9***		0***‡‡		17.6***		0***†		17.6***		0***†			

Diet C, increased consumption of breakfast cereals; Diet V, increased consumption of vegetables; INQ, index of nutritional quality (folate density (µg/MJ)/density recommended (µg/MJ)); RI, recommended intake. Mean values were significantly different from those of the pre-intervention group: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Mean values were significantly different from those of the diet V group: † $P < 0.05$; ‡ $P < 0.01$; ‡‡ $P < 0.001$. Mean values were significantly different from those of the diet V group: †† $P < 0.01$; ††† $P < 0.001$.

§ For details of procedures, see pp. 713–714.

|| Folate intake is presented in dietary folate equivalents: DFE = µg food folate + (1.7 × µg folic acid added to or provided by fortified foods).

Table 3. Changes in folate concentrations and homocysteine levels over the dietary intervention period§
(Mean values and standard deviations)

Intake	Pre-intervention data						Results at 2 weeks						Results at 6 weeks					
	Diet V (n 35)		Diet C (n 31)		Diet V (n 29)		Diet C (n 30)		Diet V (n 27)		Diet C (n 27)		Diet V (n 27)		Diet C (n 27)			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Folate (nmol/l)	16.9	8.5	18.4	15.1	20.8	10.5	30.5**‡‡	15.5	21.0	7.9	32.9**‡‡‡	15.1	21.0	7.9	32.9**‡‡‡	15.1		
Subjects with folate < 6.8 nmol/l (%)	0		3.23		0		0		0		0		0		0			
Subjects with folate < 14.9 nmol/l (%)	47.2		41.9		31.2		13.8*		14.3**		10.3**		14.3**		10.3**			
Homocysteine (µmol/l)	7.57	2.31	7.18	1.71	7.13	1.60	6.54	1.44	6.67	1.47	6.12*	1.19	6.67	1.47	6.12*	1.19		
Subjects with homocysteine > 10.4 µmol/l (%)	5.6		3.2		0		0		0		0		0		0			

Diet C, increased consumption of breakfast cereals; Diet V, increased consumption of vegetables.

Mean values were significantly different from those of the pre-intervention group: * $P < 0.05$; ** $P < 0.01$.

Mean values were significantly different from those of the diet V group: †† $P < 0.01$; ††† $P < 0.001$.

§ For details of procedures, see pp. 713–714.

>200 µg/d (14.8 (SD 14.3) nmol/l). At the end of the study, and for all subjects considered together, a relationship was found between serum folate and folate intake (r 0.369) and the number of rations of cereals consumed (r 0.401).

Folic acid is a strong predictor of total plasma tHcy levels (de Bree *et al.* 2001; Tungtrongchitr *et al.* 2003; Nurk *et al.* 2004; Tucker *et al.* 2004). de Bree *et al.* (2001) report that mean plasma tHcy concentrations in women with low (first quintile: 160 µg/d) and high (fifth quintile: 262 µg/d) folate intakes were 13.7 and 12.4 µmol/l, respectively. The same influence was seen in the present study, although the tHcy levels recorded were lower than those reported by de Bree *et al.* (2001) (the mean plasma tHcy concentrations in women with low (first quintile: 174 µg/d) and high (fifth quintile: 305 µg/d) folate intakes were 8.78 (SD 2.60) and 6.33 (SD 1.55) µmol/l, respectively; $P < 0.01$).

In agreement with Tungtrongchitr *et al.* (2003), a negative correlation was found between serum folate and tHcy for all subjects at the beginning of the study (r -0.429). In addition, an inverse relationship was seen between tHcy and folate intake (r -0.345) at this time-point. These correlations were maintained at 2 weeks (r -0.523 for that between tHcy and serum folate; r -0.346 for that between tHcy and folate intake). Also at the beginning of the study, the subjects with tHcy concentrations $< P_{50}$ (7 µmol/l) had higher folate intakes (276.0 (SD 78.4) µg/d) than did subjects whose tHcy levels were $\geq P_{50}$ (214.8 (SD 62.3) µg/d).

The C diet reduced tHcy concentrations (Table 3). This could be partly due to the greater increase in folate intake and serum folate in subjects on this diet (Tables 2 and 3). Although vegetables are very rich in folate, the intake of V women was lower than that of C women and cooking may cause its thermal breakdown; it may also be eliminated with cooking water during straining (Melse-Boonstra *et al.* 2002). Thus, although the folate intake of V subjects increased, the serum concentrations of these subjects did not increase significantly; this might explain the difference seen in the tHcy levels of the V and C subjects. However, multiple regression analysis revealed no significant influence of the change in folate, vitamin B₁₂, vitamin B₆ or protein intake on tHcy levels.

The reduction in tHcy concentrations seen in group C (Table 3) coincides with that reported by Tucker *et al.* (2004). In a relatively healthy group of volunteers these authors observed that the consumption of one cup of fortified breakfast cereal per day significantly increased B vitamin concentrations and reduced tHcy levels.

The present results agree with those obtained in many studies which have shown dietary folate improves folate status and decreases tHcy levels (Lee *et al.* 2003; Skibinska *et al.* 2004). This is important since every 1 µmol/l decrease in tHcy may be associated with a 10% reduction in the risk of developing CVD (Boushey *et al.* 1995).

Ubbink (2001) suggested that a tHcy concentration of ≤ 9 µmol/l would be desirable should the outcome of controlled clinical trials show that a lowering of plasma tHcy concentrations reduces the incidence of CVD. In the NHANES 1999–2000 study, 78% of the US population (72% of males and 85% of females) had plasma tHcy concentrations of ≤ 9 µmol/l (Pfeiffer *et al.* 2005). In the present study, 81.8% of subjects (83.9% of the C subjects) had serum

tHcy concentrations of ≤ 9 µmol/l at the start of the study, rising to 100% for C subjects and 96.3% for V subjects at 6 weeks.

The results of the present study suggest that the folate status of the studied women could be improved. Bearing in mind the results of similar studies (Ortega *et al.* 2004a), the present results show the need for better education on the importance of an adequate supply of folate during a woman's fertile period (Stark *et al.* 2005). Increasing the consumption of vegetables and fortified breakfast cereals would seem to approximate folate intake to the theoretical ideal. With respect to weight control, increasing the relative intake of vegetables or cereals would seem to be helpful; both hypocaloric diets brought food intake closer to the theoretical ideal, and are reasonable from a nutritional and health point of view (Ortega & López-Sobaler, 2005; Ortega *et al.* 2005). The weight lost by the subjects, the improvement in folate status and the fall in tHcy levels seen with the increase in the consumption of fortified breakfast cereals is of particular interest.

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