Folate Status in Young Overweight and Obese Women: Changes Associated with Weight Reduction and Increased Folate Intake

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Summary Objective: To analyze folate status changes in a group of overweight/obese young women following two different weight control programs. Methods: Fifty-seven women (BMI=24–35 kg/m²) were randomly assigned to one of two slightly hypocaloric diets: diet V, in which the consumption of vegetables was increased, or diet C, in which the consumption of cereals (especially breakfast cereals) was increased. Dietetic, anthropometric and biochemical data were collected at the start of the study and again at 6 wk. Results: At the beginning of the study, the obese women (BMI≥30 kg/m²) were at greater risk of showing serum folic acid concentrations of <14.9 nmol/L, even though there were no differences in folate intake between them and the women with a lower BMI. Energy intake was reduced and folate intake increased with both the V and C diets. Weight was lost as a consequence of this lower energy intake. Serum folic acid concentration increased and the plasma homocysteine concentration diminished only in those who lost ≥2.5 kg; this was the case of the subjects as a whole and of those who followed the C diet. Among those who lost the most weight (>2.5 kg), the chances of having an increased serum folate concentration were higher, although no significant differences were seen in folate intake with respect to women who lost less weight. Conclusions: Following a hypocaloric diet could lead to a better folate status through increased intake, but especially among those who lose the most body weight.

Key Words folate status, homocysteine, overweight, obesity, young women

The importance of adequate folate nutrition for the maintenance of health and the prevention of diseases such as neural tube defects (NTDs), cancer and occlusive vascular disease, is well recognized (1–3). Women of fertile age who are overweight/obese form a group at greater risk of having children with certain birth defects: higher pre-pregnancy body mass index (BMI) is associated with an increased risk of NTDs and perhaps other negative birth outcomes (4, 5). The reason for this association remains unknown, but may involve folate deficiency (4). Certainly, obese women tend to consume fewer folate-containing fruits and vegetables (6) and some researchers report an inverse association between serum folate and BMI and increased plasma homocysteine levels in overweight subjects (4, 7, 8). In addition, a number of weight loss interventions have been described to have a positive effect on folate status—a consequence of weight loss independent of diet composition (4, 9).

The aim of the present work was to determine the folate status of a group of overweight/obese young women and to observe the changes in this produced by two hypocaloric weight loss diets also designed to increase folic acid intake (relative increase in the consumption of vegetables or fortified breakfast cereals).

Materials and Methods

Study subjects. The study subjects were 67 women aged 20–35 y (mean 27.8±4.6 y). Most were university students; the others were women who worked in the university environment or who had come to know of the study through recruitment advertisements.

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 publicized 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 y, BMI 24–35 kg/m², not having quit smoking in the previous 2 mo, free of all disease that might interfere with the results, such as diabetes, hyperthyroidism, metabolic disease, hypertriglyceridemia, lactose or gluten intolerance (celiac disease) and food allergies etc., not currently involved in a weight loss program, not having lost more than 4.5 kg in the 2 mo prior to the study, not having lost or gained more than 3 kg between the first interview and the start of the study, a regular menstrual cycle, taking no more than two alcoholic drinks per day, and neither pregnant nor breastfeeding.

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Those interested in taking part declared themselves to meet all inclusion criteria, and were invited to the Department of Nutrition at the University Complutense of 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. To meet the requirements of the Ethics Committee of the Faculty of Pharmacy (University Complutense), all subjects signed a witnessed form of consent to be included.

The final number of aspirants was 193, but only 67 met all the inclusion criteria. Ten of the women originally included in the study failed to attend appointments for some of the initial tests or withdrew within the first few weeks (usually because of time problems). Fifty-seven women concluded the 6-wk dietary intervention period; these women made up the final study population.

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 age, body weight and physical activity of all subjects, using equations proposed by the World Health Organization (10). 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 (11–13).

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 3 times/d) since, apart from carbohydrate, they also provide fiber, 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.

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 3 times/d).

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 (14).

The full characteristics of the diets followed and other methodological details are described elsewhere (15).

Compliance with dietary rules: Over the intervention period (a total of 6 wk, 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 6 wk:

Physical activity: The subjects completed a questionnaire on their normal physical activity, and this information was used to calculate their energy expenditure (16). Subjects indicated the length of time spent sleeping, eating, playing sport, etc., during both working days and weekends. An activity coefficient was established for each subject (10, 17).

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 Dept. of Nutrition by trained personnel following norms set out by WHO (18). 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 (8, 19, 20).

Dietetic study: A “food and drink record” was used to register all intakes (both at home and away) for 3 d, including a Sunday (21). Subjects were instructed to record the weights of foods consumed if possible, and to use 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 if they broke the ‘rules’ of their diet.

The energy and nutrient contents of these foods were then calculated using food composition tables (14). The values obtained were compared to those recommended (RI) (22) to determine the adequacy of the diets. Special attention was paid to the intake of energy and folates. DIAL software (Alce Ingenieria, 2004) was used to process all data (23).

Folate intake was recorded in the form of dietary folate equivalents (DFE), which takes into account the higher bioavailability of straight folic acid compared to food folate (DFE: 1 DFE = 1 μg food folate = 0.6 μg folic acid from fortified food) (24). Thus, the dietary data presented in this 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 of food folate + (1.7× μg of 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 Dept. of Nutrition. Part of this blood was collected in tubes with no anticoagulant to allow the separation of serum. Serum samples were kept at −40°C until analysis.

Serum folate was determined by radioimmunoassay (25) (CV=4.5%), and plasma homocysteine levels by polarized fluorescence immunoanalysis using an Imx Analyzer (26) (CV=6.3%). Bearing in mind the proposals of Wartanowicz et al. (27), serum folate concentra-
tions of <6.8 nmol/L were understood to reflect a high risk of folate deficiency, while values of ≥14.9 nmol/L were understood to represent optimum status. The upper limit for homocysteine was taken as 10.4 μmol/L, in agreement with the proposal of Tucker et al. (28) for the female population.

Statistical analysis: The women were grouped by diet (C or V) and by the weight loss achieved (those who lost more and those who lost ≤2.5 kg [50th percentile; P50]) (Tables 1–4). Means and standard deviation (SD) were calculated for all variables. ANOVA was used to determine differences between means. Linear correlation coefficients were calculated using the Pearson test. The Student t test or the Mann-Whitney U test (if the distribution was not homogeneous) was used to compare differences where indicated. Comparisons between proportions were made using an approximation of the binomial distribution to the normal distribution, employing continuity correction. Logistic regression analysis was used to identify risk or protection factors that might modify any variables. All calculations were made using RSIGMA BABEL Software (Horus Hard- ward, Madrid). Significance was set at p<0.05.

RESULTS

At the beginning of the study, no significant personal, dietetic, anthropometric or biochemical differences were seen between the women of the different groups established (Tables 1–4). However, the obese women (BMI ≥ 30 kg/m² (29.9%)) were at greater risk of having serum folate levels of <14.9 nmol/L (odds ratio: OR = 6.2; 95% CI: 1.895–20.280; p<0.01) even though there were no differences in folate intake between these and the lighter women (229.8 ± 74.3 μg/d in obese women compared to 251.8 ± 76.9 μg/d in those with a smaller BMI) (non significant: NS).

After 6 wk following the diets, the consumption of fruit increased in all the established groups (i.e., independent of the weight lost or dietary intervention). The consumption of vegetables also increased while that of meat diminished among all women who lost ≤2.5 kg and among all those who lost more, but also among V women who lost >2.5 kg. Among the women who lost the most weight (>2.5 kg), those who followed diet C had a higher cereal consumption but lower vegetable consumption than those who followed diet V (Table 2).

The two diets led to a similar reduction in energy intake in all the established groups, and a significant linear correlation was seen between the reduction of BMI at 6 wk and the reduction of energy intake (r=0.272). An increase in folate intake was also seen, through greater in C women (260.1 ± 121.7 μg/d) than in V women (185.6 ± 125.2 μg/d) (p<0.05) (Table 3).

However, the blood data showed an increased serum folate concentration and lower plasma homocysteine levels only in those who lost more weight (>2.5 kg), both for the entire population studied (n=26) and for the C subjects (n=16) (Table 4).

Linear regression analysis, taking into account the influence of the changes in serum folate levels, changes in body weight, and changes in folate intake (at 6 wk), showed significant correlations between the change in serum folate and the reduction in body weight (r = –0.359), and between the change in serum folate

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**Table 1.** Personal and anthropometric data. Changes as a result of the dietary interventions and differences with respect to the weight loss achieved at 6 wk (mean±SD).

| | Women that lost ≤2.5 kg (P50) in the 6 wk of the intervention | Women that lost >2.5 kg (P50) in the 6 wk of the intervention |
|---|---|---|
| **Diet V** | **Diet C** | **Total** | **Diet V** | **Diet C** | **Total** |
| **Baseline data (n)** | 18 | 13 | 31 | 10 | 16 | 26 |
| **Personal data** | | | | | | |
| Age (y) | 28.3±5.5 | 27.3±4.5 | 27.9±5.0 | 29.3±4.1 | 26.8±4.2 | 27.7±4.3 |
| Smokers (%) | 27.8 | 7.7 | 19.4 | 40 | 31.3 | 34.6 |
| Smoking (cigarettes/d) | 5.2±3.4 | 20.0±0.0 | 7.7±6.8 | 13.3±5.4 | 9.0±6.2 | 10.9±5.9 |
| Sporadic taking of folate supplements (%) | 11.1 | 15.4 | 12.9 | 20.0 | 6.3 | 11.5 |
| **Anthropometrics data** | | | | | | |
| Weight (kg) | 71.6±7.9 | 80.0±10.0 | 75.1±9.6 | 73.2±6.2 | 74.2±10.6 | 73.8±9.0 |
| Height (cm) | 161.2±5.7 | 164.7±6.3 | 162.7±6.1 | 162.7±4.6 | 164.4±5.6 | 163.8±5.2 |
| BMI (kg/m²) | 27.5±2.7 | 29.5±3.5 | 28.4±3.1 | 27.7±2.3 | 27.4±3.1 | 27.5±2.8 |
| **Final data (6 wk) (n)** | 18 | 13 | 31 | 10 | 16 | 26 |
| **Anthropometrics data** | | | | | | |
| Weight (kg) | 70.3±8.0 | 78.4±10.2 c* | 73.7±9.7 | 69.9±6.1 | 70.4±10.3 a* | 70.2±8.8 |
| BMI (kg/m²) | 27.0±2.8 | 28.9±3.6 | 27.8±3.3 | 26.4±2.3 | 26.0±3.0 a* | 26.2±2.7 a* |
| Weight loss since start (kg) | 1.28±0.9 | 1.56±0.7 | 1.40±0.85 | 3.31±0.42 a** | 3.78±0.82 a** | 3.60±0.72 a** |

ANOVA, analysis of variance; BMI, body mass index; P50, 50th percentile.

*p<0.05; **p<0.01; a: difference between women who lost ≤2.5 kg and those who lost more, c: difference between diets C and V (t-test or Mann-Whitney test).
and the increase in folate intake \((r=0.266)\). The correlation between serum folate and weight loss was also significant \((p<0.01)\), but that between serum folate and folate intake was not. The influence of weight loss on serum folate is therefore greater.

Multiple regression analysis showed a positive association between the increase in serum folic acid and the increase in folic acid intake and weight loss at the end of the study. Thus, by increasing the relative intake of folic acid by 1 mg/d, the expected increase in serum folic acid would be 0.032 nmol/L. Taking also into account the increase in folic acid intake and weight loss at the end of the study \((2.5, 0.198)\), the expected increase in serum folic acid by 1 mg/d, the expected increase in serum folate is therefore greater.

The initial dietetic, anthropometric and biochemical data (Tables 1–4) were similar to those obtained for other groups of overweight women \((11–14, 28, 29)\); they were also similar across the different groups established in the present study.

In agreement with that reported by other authors \((2, 19, 20)\), folate status was inadequate (with respect to protection against congenital malformations, cancer and cardiovascular disease) at the start of the study: 64.2% of all subjects had a folate intake of <67% of the RNI and 45.5% had serum folate concentrations of <14.9 nmol/L (Tables 3 and 4).

The present study also showed that obese women \((BMI \approx 30 \text{ kg/m}^2)\) were more likely to have serum folate levels of 14.9 nmol/L—even though there were no significant differences in their folate intake compared to that of the lighter women. These results appear to support the findings of other authors \((4, 7, 8)\), who indicate that folic acid status is affected negatively by the condition of being overweight/obese, and that women with a higher BMI need a higher folic acid intake to achieve the same serum folate level as women of normal weight \((4)\).

At the beginning of the study, the consumption of meat/fish/eggs was somewhat higher than that recommended \((30, 31)\), while that of cereals/pulses, vegetables/greens and fruit was lower (Table 2). This justifies the following of diets C and V during the intervention period, i.e., approximating the diet to the theoretical ideal while providing a slightly hypocaloric energy intake and a greater intake of folate (Table 3). The

### Table 2. Food intake changes as a result of the dietary interventions: differences with respect to the weight loss achieved at 6 wk (mean±SD).

|                      | Women that lost ≤2.5 kg (P50) in the 6 wk of the intervention | Women that lost >2.5 kg (P50) in the 6 wk of the intervention |
|----------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|                      | Diet V | Diet C | Total | Diet V | Diet C | Total |
| Baseline data (n)    | 18     | 13     | 31    | 10     | 16     | 26    |
| Food intake (servings/d) |
| Cereals and pulses   | 4.4±1.3 | 4.7±1.6 | 4.5±1.4 | 4.8±1.8 | 5.4±1.5 | 5.2±1.6 |
| Greens and vegetables| 3.0±1.3 | 3.0±1.6 | 3.0±1.4 | 2.5±0.8 | 3.1±1.0 | 2.9±1.0 |
| Fruits               | 1.2±0.9 | 1.2±1.1 | 1.2±1.0 | 0.9±0.7 | 1.4±1.0 | 1.2±0.9 |
| Milk products        | 1.7±0.8 | 2.2±0.7 | 1.9±0.8 | 1.9±1.4 | 2.2±0.8 | 2.1±1.1 |
| Meat, fish and eggs  | 3.4±1.9 | 4.2±2.0 | 3.7±1.9 | 4.1±1.4 | 4.1±1.5 | 4.1±1.4 |
| Final data (wk) (n)  | 18     | 13     | 31    | 10     | 16     | 26    |
| Food intake (servings/d) |
| Cereals and pulses   | 4.2±1.4 | 4.8±1.0 | 4.5±1.3 | 4.1±1.4 | 5.4±1.0 | 4.9±1.3 |
| Greens and vegetables| 5.9±6.2 | 3.8±1.0 | 5.1±4.8 | 5.0±1.5 | 3.3±1.4 | 4.0±1.6 |
| Fruits               | 3.6±1.7 | 3.6±1.4 | 3.6±1.5 | 4.1±1.7 | 3.4±1.0 | 3.7±1.3 |
| Milk products        | 1.8±0.9 | 1.8±0.6 | 1.8±0.7 | 2.1±0.7 | 2.3±0.8 | 2.2±0.7 |
| Meat, fish and eggs  | 2.6±1.2 | 2.9±1.3 | 2.7±1.2 | 2.1±1.0 | 2.3±0.9 | 2.2±0.9 |

ANOVA, analysis of variance; BMI, body mass index; P50, 50th percentile.

\*\(p<0.05\); **\(p<0.01\); b: difference between pre-intervention and 6 wk data (ANOVA and Newman-Keuls post-test), c: difference between diets C and V (\(t\)-test or Mann-Whitney test).
Table 3. Changes in dietary data: differences with respect to the weight loss achieved at 6 wk (mean±SD).

|                          | Women that lost ≤2.5 kg (P50) in the 6 wk of the intervention | Women that lost >2.5 kg (P50) in the 6 wk of the intervention |
|--------------------------|---------------------------------------------------------------|-------------------------------------------------------------|
|                          | Diet V | Diet C | Total | Diet V | Diet C | Total |
| Baseline data (n)        |        |        |       |        |        |       |
| Energy intake (kJ/d)     | 18     | 13     | 31    | 10     | 16     | 26    |
| Alcohol intake (% energy)| 8,361 ±2,348 | 9,777 ±2,374 | 8,955 ±2,426 | 9,460 ±2,499 | 10,265 ±1,941 | 9,955 ±2,161 |
| Folate intake (µg/d)     | 0.29 ±0.76 | 1.07 ±2.03 | 0.62 ±1.50 | 1.27 ±1.94 | 0.84 ±0.97 | 1.01 ±1.41 |
| Folate density (µg/MJ)   | 226.4 ±69.2 | 273.5 ±100.4 | 246.2 ±85.5 | 244.3 ±86.1 | 273.4 ±57.9 | 262.2 ±69.9 |
| Coverage of RI (%)       | 27.6 ±7.2 | 29.9 ±13.8 | 28.6 ±10.3 | 26.0 ±7.3 | 27.3 ±6.7 | 26.8 ±6.8 |
| INQ (%)                  | 56.6 ±17.3 | 68.4 ±25.1 | 61.5 ±21.4 | 61.1 ±21.5 | 68.3 ±14.5 | 65.5 ±17.5 |
| Folate intakes<RI (%)    | 0.69 ±0.18 | 0.79 ±0.37 | 0.73 ±0.28 | 0.66 ±0.21 | 0.69 ±0.20 | 0.68 ±0.20 |

|                          | 100 | 84.6 | 93.5 | 90 | 100 | 96.2 |

|                          | 44.4 | 7.7 c* | 29 | 15.9 ±0.39 | 1.83 ±0.42 | 1.74 ±0.42 |
|                          | 50 | 18.8 | 30.8 |

*ANOVA, analysis of variance; BMI, body mass index; RI, recommended intake; INQ, index of nutritional quality; folate density, (µg/MJ)/density recommended (µg/MJ); P50, 50th percentile.
*p<0.05; **p<0.01; b: difference between pre-intervention and 6 wk data (ANOVA and Newmal-Keuls post-test), c: difference between diets C and V (t-test or Mann-Whitney test).

Results obtained over the study period show that the goals set out during the planning period were attained, including a slight weight reduction (2.0±1.3 kg in V women and 2.8±1.4 kg in C women; p<0.05) (Table 1). This agrees with the results of other studies (II–I3, I5, 32).

The particularly strong increase in folate intake in the C group (Table 3) is due to the fact that fortified breakfast cereals are an important source of folate (33, 34), even more so than natural sources of the vitamin such as vegetables (2, 35).

The initial folate status of the subjects in the present study (Table 3) was better than that reported by Dietrich et al. (2), Hertrampf et al. (1) and Mojtabai (4), but after the intervention the final figures were similar to those reported by these authors when analyzing the effects of the mandatory folic acid fortification of all enriched cereal-grain products in the US.

Folic acid intake and weight loss determined serum folate acid levels at the end of the study. Different studies have shown the effect of the former or the latter factors on serum folate acid levels (4, 9). Nevertheless, according to our knowledge, there is not any investigation about how each of these factors condition serum folic acid. In agreement with the results obtained in the present study, weight loss seems to have more effect on serum folic acid levels than the intake of this vitamin.

It was also found that the greatest increase in serum folate was recorded in the women who showed the greatest weight loss, irrespective of folate intake. This also agrees with that reported by other authors (4, 9).

Mojtabai (4) indicated that after controlling for intake of folate in food and nutritional supplements, increased BMI in childbearing age women was associated with a lower serum folate level in NHANES III and NHANES 1999–2000. In addition, using data from NHANES 1999–2000, it was estimated that women in the ≥30.0 kg/m² BMI category would need to take an additional 350 µg/d of folate to achieve the same serum folate level as women in the <20.0 kg/m² category.
Future studies need to examine the mechanisms mediating the association between higher BMI and reduced serum folate levels. It may be that excess weight increases the need for some vitamins if the protection against certain diseases they afford in lighter people is to be obtained. It has already been shown that heavier women may need to take a larger dose of folate to achieve the same serum folate level as their lighter counterparts. If this is the case, current recommendations for pre-conception folate supplement use may have to change to take the greater needs of heavier women into account (4). The most important finding of the present study is the confirmation that weight loss can help to improve the folate status of overweight/obese women.

This study shows there to be room for improvement in the folate status of the studied population—especially in the women with the highest BMI. The results also show that approximating the diet to the theoretical ideal by increasing the relative consumption of vegetables or breakfast cereals in the context of a slightly hypocaloric diet is a good way to lose body weight (15, 32) and improve folate status in overweight/obese persons. The consumption of fortified breakfast cereals is thus of particular interest in both weight loss and the improvement of folate status.

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