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# *Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years*

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## SUMMARY

*Background*: Patients with coeliac disease are advised to keep to a lifelong gluten-free diet to remain well. Uncertainty still exists as to whether this gives a nutritionally balanced diet.

*Aim*: To assess the vitamin nutrition status of a series of coeliac patients living on a gluten-free diet for 10 years.

*Methods*: Thirty adults with coeliac disease (mean age, 55 years; range, 45–64 years; 60% women), in biopsy-proven remission following 8–12 years of dietary treatment, were studied. We measured the total plasma homocysteine level, a metabolic marker of folate, vitamin B-6 and vitamin B-12 deficiency, and related plasma vitamin levels. The daily vitamin intake level was assessed using a 4-day food record. Normative data were obtained from the general population of the same age.

*Results*: Coeliac patients showed a higher total plasma homocysteine level than the general population, indicative of a poor vitamin status. In accordance, the plasma levels of folate and pyridoxal 5′-phosphate (active form of vitamin B-6) were low in 37% and 20%, respectively, and accounted for 33% of the variation of the total plasma homocysteine level ($P < 0.008$). The mean daily intakes of folate and vitamin B-12, but not of vitamin B-6, were significantly lower in coeliac patients than in controls.

*Conclusions*: Half of the adult coeliac patients carefully treated with a gluten-free diet for several years showed signs of a poor vitamin status. This may have clinical implications considering the linkage between vitamin deficiency, elevated total plasma homocysteine levels and cardiovascular disease. The results may suggest that, when following up adults with coeliac disease, the vitamin status should be reviewed.

## INTRODUCTION

Removing gluten from the diet permanently is essential for patients with coeliac disease in order to maintain a normal intestinal mucosa and to reduce the risk of complications, such as malignant lymphomas and osteoporosis.[1, 2]

In practice, treatment of patients with a gluten-free diet allows them to select appropriate foods by omitting and substituting for gluten-containing products. Considering the increasing number of adults diagnosed with coeliac disease,[3, 4] remarkably few studies have questioned whether the composition of a strict gluten-free diet is nutritionally balanced. Studies that have so far addressed the dietary history of adult coeliac patients agree that it is nutritionally adequate within the first few years of treatment.[5–7] However, as recently pointed out by Bardella *et al.*, this may not necessarily hold true later in the course of treatment.[8] Indeed, Thompson, assessing the vitamin content of gluten-free cereal products, concluded that coeliac patients under

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dietary treatment may be vulnerable to the inadequate intake of folate, which may have important clinical implications.[9]

This study was therefore designed to evaluate the vitamin nutrition status of adults with coeliac disease adhering to a gluten-free diet for 8–12 years. To do this, we determined the total plasma homocysteine level, a metabolic marker of folate, vitamin B-6 and vitamin B-12 deficiency, and an independent risk factor for cardiovascular disease.[10] We also assessed the plasma and intake levels of the related vitamins in these patients.

## METHODS AND MATERIALS

### *Patients*

The study comprised a series of 30 adults (12 males and 18 females), aged 45–64 years [mean age, 55 years; 95% confidence interval (CI), 53.2–56.8], characterized by healed intestinal mucosa at repeat biopsy after 8–12 years of treatment with a gluten-free diet. They represented 64% of a coeliac cohort ($n = 47$) diagnosed at six gastroenterology units in south-east Sweden in 1984–88 and proven to be in histological remission 8–12 years later. The remaining patients of this cohort were either unwilling to participate ($n = 11$) or took folate supplementation ($n = 6$).

The most common principal reasons for referral for diagnosis were abnormal blood tests (33%), diarrhoea (33%), dyspepsia (17%) and tiredness (10%). The diagnosis was confirmed by an intestinal biopsy showing a flat mucosa corresponding to a lesion of Marsh type 3.[11] All patients were started on a gluten-free diet and the early clinical and histological responses were unequivocal as stated in the medical records. At the time of the study, major concomitant disorders included autoimmune diseases, mainly thyroid disorders, seen in four patients, depression in three and seizures in two.

### *Methods*

Blood samples were drawn for routine laboratory investigations. Plasma folate and vitamin B-12 levels were estimated by radioimmunoassay, and plasma pyridoxal 5′-phosphate levels by enzymatic photometry with high-performance liquid chromatography separation (Mimelab AB, Söråker, Sweden). The total plasma homocysteine level was determined by high-performance liquid chromatography in samples immediately centrifuged and stored in the dark at − 20 °C pending analysis (Mimelab AB, Söråker, Sweden). Normative total plasma homocysteine values, as determined by high-performance liquid chromatography, were obtained from a general Nordic population sample aged 43–64 years ($n = 592$; 51% females).[12]

Compliance with a gluten-free diet was assessed by histological evidence of remission and was classified as follows: normal; borderline (i.e. $>25$ intraepithelial lymphocytes per 100 enterocytes, but without the other stigmata of gluten enteropathy); or diagnostic for gluten sensitivity (i.e. an increased intraepithelial lymphocyte count, signs of enterocyte damage or distortion of mucosal architecture). Of the 30 patients included in the study, 25 showed normal histology and five borderline histology, i.e. findings fully compatible with healed coeliac mucosa.[13] Anti-endomysium antibodies, determined according to Grodzinsky *et al.*,[14] were not detected in any patient.

The dietary history of coeliac patients was obtained from a 4-day food record covering one holiday using household measures. During a visit to the clinic, the height and weight were measured and the patients were requested to fill out a form covering smoking habits, current medication and physical activity. The patients were reviewed by a dietitian prior to the initiation of the food diary and emphasis was placed on eating as usual. At revisit, the food records were reviewed by the patient and dietitian together. A dietitian translated the record data into weights and portion sizes that were subsequently converted into nutrients by means of Dietist software (Kost- och näringsdata AB, Stockholm, Sweden). Validation of the food record data was performed by comparing the registered energy intake with the calculated basal metabolic rate, taking into account the lifestyle using a relevant physical activity level factor outlined by Black *et al.*[15] The basal metabolic rate was predicted from the weight, height, age and gender using the Schofield equations.[16] Control intake data were obtained from a national dietary survey carried out in 1989 comprising 504 patients (50% females) aged 45–64 years.[17]

### *Statistics*

The distribution of the total plasma homocysteine values was positively skewed allowing for the use of geometric means. Student's *t*-test for independent samples was used to compare group means. The degree

Table 1. Mean values (95% confidence interval, CI) of routine laboratory profiles for 30 adult coeliacs after 8–12 years on a strict gluten-free diet

| | Males ($n$ = 12) | Females ($n$ = 18) | Reference range |
|---|---|---|---|
| Blood haemoglobin (g/L) | 144 (139–149) | 132 (127–137) | 130–165 [120–150]* |
| Erythrocyte MCV (fL) | 89 (85–93) | 90 (89–91) | 82–101 |
| Plasma albumin (g/L) | 42 (40–44) | 42 (40–44) | 36–48 |
| Plasma creatinine ($\mu$mol/L) | 85 (76–94) | 77 (69–84) | 70–115 [55–100]* |
| Plasma ferritin ($\mu$g/L) | 133 (86–180) | 99 (58–140) | 30–230 [30–150]* |
| Serum calcium (mmol/L) | 2.4 (2.35–2.41) | 2.4 (2.34–2.44) | 2.2–2.6 |
| Serum alkaline phosphatase ($\mu$kat/L) | 3.0 (2.5–3.5) | 2.9 (2.2–3.6) | < 4.6 |
| Serum zinc ($\mu$mol/L) | 15.4 (14.2–16.6) | 13.4 (12.8–14.0) | 11–17.7 |

MCV, mean corpuscular volume.
*Females.

of relationship between dependent variables was expressed by Spearman's coefficient of correlation. Stepwise multiple regression analysis was carried out using STATISTICA '99 Edition (StatSoft, Tulsa, OK, USA). The significance level was set at a two-sided $P$ value equal to or less than 0.05.

The study was approved by the Ethics Committee at the Faculty of Sciences, University of Linköping, Sweden.

## RESULTS

The study comprised 30 coeliac patients, aged 45–64 years, who were diagnosed in 1984–88 and who showed signs of histological remission 8–12 years later, corroborated by normal routine laboratory findings (Table 1). Following the start of treatment, male coeliac patients increased in body weight from 70.4 ± 5.1 kg (mean ± s.d.) to 79.2 ± 10.8 kg ($P < 0.001$), and female patients from 62.1 ± 8.1 kg to 71.0 ± 10.6 kg ($P < 0.001$).

### *Homocysteine distribution*

The mean total plasma homocysteine concentration for the coeliac sample was 11.8 $\mu$mol/L. Values ranged from 7.2 to 24.5 $\mu$mol/L. Male coeliac patients showed a (geometric) mean total plasma homocysteine concentration of 13.6 $\mu$mol/L (95% CI, 10.99–16.87), to be compared with male controls with 11.2 $\mu$mol/L (95% CI, 10.87–11.58) ($P < 0.05$). The corresponding values for female coeliac patients and controls were 10.8 $\mu$mol/L (95% CI, 9.53–12.33) and 9.9 $\mu$mol/L (95% CI, 9.53–10.25) (N.S.), respectively.

### *Vitamin nutrition status*

In order to determine the degree of the nutritional origin of the hyperhomocysteinaemia of coeliac patients, we determined the plasma levels of the three essential dietary cofactors for enzymes of homocysteine metabolism, i.e. folate, pyridoxal 5′-phosphate (active form of vitamin B-6) and vitamin B-12. Low pyridoxal 5′-phosphate levels were found in 11 patients (37%; 95% CI, 20–54), low plasma folate in six (20%; 95% CI, 6–34) and low plasma vitamin B-12 in none (Table 2). The total plasma homocysteine level correlated negatively with pyridoxal 5′-phosphate ($r = -0.50$) ($P < 0.01$), folate ($r = -0.46$) ($P < 0.01$) and vitamin B-12 ($r = -0.01$) (N.S.) and, in multivariate analysis, 33% of the variation in the total plasma

Table 2. Mean (95% confidence interval, CI) plasma levels of pyridoxal 5′-phosphate, folate and vitamin B-12 in 30 coeliac patients treated with a gluten-free diet for 8–12 years

| | Males ($n$ = 12) | | Females ($n$ = 18) | | Total |
|---|---|---|---|---|---|
| | | Low in | | Low in | Low in |
| Plasma pyridoxal 5′-phosphate (nmol/L) | 19.9 (15.3–24.5) | 6/12 (50%) | 28.7 (21.8–35.6) | 5/18 (28%) | 11/30 (37%) |
| Plasma folate (nmol/L) | 10.5 (8.3–12.7) | 3/12 (25%) | 12.6 (9.4–15.8) | 3/18 (17%) | 6/30 (20%) |
| Plasma vitamin B-12 (pmol/L) | 329 (241–417) | Nil | 259 (211–307) | Nil | Nil |

Reference range for plasma pyridoxal 5′-phosphate, 20–60 nmol/L; plasma folate, 7–39 nmol/L; plasma vitamin B-12, 120–770 pmol/L.

Table 3. Mean (95% confidence interval, CI) daily energy and vitamin intakes in adult coeliac patients and controls

| | Males | | Females | | |
|---|---|---|---|---|---|
| | Coeliacs ($n$ = 12) | Controls ($n$ = 251) | Coeliacs ($n$ = 18) | Controls ($n$ = 253) | NNR |
| Energy intake (MJ) | 9.5 (8.6–10.4) | 9.3 (9.0–9.6) | 7.5 (6.8–8.3) | 7.4 (7.1–7.7) | — |
| Vitamin B-6 intake (mg) | 2.0 (1.8–2.2) | 2.1 (2.0–2.2) | 1.7 (1.5–1.9) | 1.7 (1.6–1.8) | 1.5 (1.2)‡ |
| Folate intake ($\mu$g) | 180 (153–207)* | 215 (207–223) | 187 (164–210) | 197 (190–204) | 300 |
| Vitamin B-12 intake ($\mu$g) | 5.1 (4.4–5.8)† | 8.9 (8.0–9.8) | 4.1 (3.5–4.7)† | 7.0 (6.4–7.6) | 2.0 |

NNR, Nordic Nutrition Recommendations.[18]
*Coeliac males vs. controls ($P < 0.05$).
†Coeliacs vs. controls ($P < 0.001$).
‡Females.

homocysteine concentration could be explained by the plasma pyridoxal 5′-phosphate and folate levels ($F$ ratio = 5.87) ($P < 0.008$).

Altogether, 14 patients (47%; 95% CI, 29–65) showed a low pyridoxal 5′-phosphate level ($n$ = 8), a low folate level ($n$ = 3) or both ($n$ = 3). The series showed a mean daily intake of folate (184 ± 54 $\mu$g) that was lower than for controls (206 ± 60 $\mu$g) ($P < 0.05$) and well below the Nordic Nutrition Recommendations[18] (Table 3). For vitamin B-6, similar intake levels were seen in the coeliac patients (1.8 ± 0.4 mg) and controls (1.9 ± 0.5 mg), all above the Nordic Nutrition Recommendations. The intake of vitamin B-12 was lower in coeliac patients than in controls and consistently higher than recommended in both groups. The correlations between vitamin intakes and plasma levels were generally poor ($r < 0.18$).

Validation of the diet records was performed by comparing the energy/basal metabolic rate ratio to the physical activity level factor. For coeliac males, the ratio was 1.3/1.8 (72%) and, for coeliac females, it was 1.3/1.7 (76%). This may indicate that the coeliac patients underestimated their energy intake by about 25%, the same as found in controls (mean, 25%).

## DISCUSSION

It is generally assumed that coeliac patients adhering to a strict gluten-free diet for years will consume a diet that is nutritionally adequate.[19] This is supported by the demonstration of a normal bone mineral density up to 10 years of dietary treatment.[20]

Our results may indicate otherwise. We found signs indicative of a poor vitamin status in 56% of treated adult coeliac patients, including six on folate supplementation. This adds evidence to suggestions[9, 21, 22] that patients adhering to a strict gluten-free diet for years are prone to the development of various vitamin deficiency states, notably folate deficiency.

Our findings can hardly be explained by persistent intestinal malabsorption. The series was in biopsy-proven remission and showed normal serum zinc recordings, a highly sensitive marker of the mucosal state of the upper intestinal tract according to Jameson *et al.*[23] The results may even argue against the notion that signs of malnutrition encountered in treated coeliac patients indicate poor dietary compliance or suggest an alternative diagnosis.[24]

The assessment of vitamin nutrition status is a matter of current contention and was thus approached using different methods. We used the total plasma homocysteine level as a metabolic indicator of folate, vitamin B-6 and vitamin B-12 deficiency, as advocated by Ubbink.[25] Admittedly, the interpretation of plasma vitamin profiles can be difficult with the advent of modern techniques which may measure only one of several biologically active vitamins. This could possibly explain the weak association between the vitamin plasma and intake levels.

We also collected dietary intake data, as described by others,[5] covering 4 days to ensure patient compliance; recordings lasting longer than this may not necessarily be superior.[26] This measurement may be associated with bias, such as changes in normal dietary patterns during the study, or underreporting, or both. This is reflected by the discrepancy between the ratio of the reported energy intake to the basal metabolic rate of 1.3 in coeliac patients, corresponding to a state of pure survival, and the self-assessed physical activity level of 1.7, indicative of sedentary living. However, this was no different from that seen in the controls. Furthermore, when interpreting vitamin

intake data, it should be recalled that vitamin losses through industrial and household food processing are unknown, as is the vitamin bioavailability in humans.[27]

A gluten-free diet does not contain a single food, and so a major determinant of the poor vitamin status of coeliac patients may well be their choice of foods. Indeed, Grehn *et al.*, studying adults with long-standing disease, found significant differences in intake levels from those of population controls in half of 28 food groups.[22] Among folate-rich foods, coeliacs consumed less fresh fruit than controls, whereas they ate more greens and root vegetables; this was particularly true for coeliac women, which is in good agreement with our results. The study also showed that, whilst they ate bread to a similar extent, there was a significantly lower contribution of folate from bread consumed by coeliac patients than from that consumed by the general population, adding further support to the crucial role of food choices in the vitamin status of treated coeliac patients. It should be noted that, in Sweden, neither gluten-free products nor their gluten-containing counterparts are folate-enriched.

The significance of the relatively lower intake level of vitamin B-12 in coeliac patients compared to controls remains to be determined, as the vitamin B-12 status is more dependent on intestinal absorption than on intake.

Another new finding in this study is the raised total plasma homocysteine level in adult coeliac patients on a strict gluten-free diet for years. Besides being indicative of a poor vitamin status, it may imply an independent increased risk for cardiovascular disease in the same range as hypercholesterolaemia and hypertension.[28] Evidence is emerging that an elevated total plasma homocysteine level may be a causal factor,[29] and a dose–response relationship has been found between total plasma homocysteine and mortality,[28] which is particularly strong for a level at or above 15 $\mu$mol/L. Hence, the observation that this may apply to half of the male coeliac patients (five of 11) in this study raises concern about the long-term safety of living on a strict gluten-free diet. It is supported by the observation that men with long-standing coeliac disease tend to consume 50% less folate-rich foods than do coeliac women.[22] That the total plasma homocysteine level is partly regulated by genetic factors may be clinically less important, with studies showing that daily supplementation with 0.5–5 mg of folic acid or 0.5 mg of vitamin B-12 can normalize the concentration of total plasma homocysteine.[30] This is supported by our finding that the six coeliac patients on folic acid supplementation showed a median total plasma homocysteine level of 6.5 $\mu$mol/L.

It is possible that our findings may have further clinical implications in the light of an observation that depression in coeliac patients can be a vitamin B-6-responsive condition.[31] Depression is reported to be a feature of coeliac disease also in the treated state,[32] and is ranked as its most common neuropsychiatric disturbance.[33] Of interest in this context, Fava *et al.* found that low blood folate levels were associated with a poorer response to antidepressant treatment.[34] In addition, preliminary data suggest that the poorer quality of life of women with long-standing coeliac disease[35] may be closely related to their vitamin intake levels.

The strengths of this study include the 10-year follow-up of a cohort of coeliac patients diagnosed in a defined area and the use of intestinal biopsy to assess adherence to diet. We were anxious to use age- and sex-related normative values obtained in the general population, knowing that the total plasma homocysteine concentration is sex dependent and increases with age. Additionally, the number of smokers (10%) was clearly lower in the coeliac series than in the controls. The list of concomitant disorders was unremarkable and similar to that reported by others,[33, 36] and is unlikely to account for the raised total plasma homocysteine levels of the coeliac series.

The study has several limitations. It may be argued that changes in the dietary habits might have occurred since the time of the surveys of the controls. However, according to preliminary data from the Swedish National Food Administration, as cited by Grehn *et al.*,[22] no dramatic changes have taken place over the past decade in this respect. Furthermore, general conclusions are necessarily limited as the series consumed a Nordic diet, although Mariani *et al.*, assessing the diet of Italian coeliac adolescents, proposed that the gluten-free diet may be a nutritional risk factor.[37] Obviously, more studies are needed to assess the full vitamin nutrition status of patients treated with a gluten-free diet for years; this population may amount to 400 000 people in Europe alone. Such studies should preferably include the iron status, although, in this study, we found no evidence of iron deficiency, as reflected by generally normal plasma ferritin levels.

In conclusion, the observation that half of the coeliac series carefully treated for years showed signs of vitamin deficiency casts doubt over their dietary habits and choice of foods. It may have clinical implications considering the linkage between vitamin deficiency, elevation of total plasma homocysteine and cardiovascular disease. Hence, we agree with Parnell & Ciclitira[19] that an annual follow-up of coeliac patients should include a measure of the vitamin status, for example folate, and, when appropriate, advice to help the patients to increase their intake of folate-rich foods, such as fresh fruits and greens.

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## REFERENCES

1 Holmes GKT, Prior P, Lane MR, Pope D, Allen RN. Malignancy in coeliac disease — effect of a gluten-free diet. Gut 1989; 30: 333–8.
2 Valdimarsson T, Löfman O, Toss G, Ström M. Reversal of osteopenia with diet in adult coeliac disease. Gut 1996; 38: 322–7.
3 Collin P, Reunala T, Rasmussen M, *et al.* High incidence and prevalence of adult coeliac disease. Augmented diagnostic approach. Scand J Gastroenterol 1997; 32: 1129–33.
4 Feighery C. Coeliac disease. Br Med J 1999; 319: 236–9.
5 Kemppainen T, Uusitupa M, Janatuinen E, Järvinen R, Julkonen R, Pikkarainen P. Intakes of nutrients and nutritional status in coeliac patients. Scand J Gastroenterol 1995; 30: 575–9.
6 Ciacci C, Maurelli L, Klain M, *et al.* Effects of dietary treatment on bone mineral density in adults with celiac disease: factors predicting response. Am J Gastroenterol 1997; 92: 992–6.
7 Sategna-Guidetti C, Grosso SB, Grosso S, *et al.* The effects of 1-year gluten withdrawal on bone mass, bone metabolism and nutritional status in newly-diagnosed adult coeliac disease patients. Aliment Pharmacol Ther 2000; 14: 35–43.
8 Bardella MT, Fredella C, Prampolini L, Molteni N, Giunta AM, Bianchi PA. Body composition and dietary intakes in adult celiac disease patients consuming a strict gluten-free diet. Am J Clin Nutr 2000; 72: 937–9.
9 Thompson T. Folate, iron, and dietary fiber contents of the gluten-free diet. J Am Diet Assoc 2000; 100: 1389–96.
10 Graham IM, Daly LE, Refsum HM, *et al.* Plasma homocysteine as a risk factor for vascular disease. The European concerted action project. J Am Med Assoc 1997; 277: 1775–81.
11 Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunologic approach to the spectrum of gluten sensitivity (celiac sprue). Gastroenterology 1992; 102: 330–54.
12 Nygård O, Vollset SE, Refsum H, *et al.* Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. J Am Med Assoc 1995; 274: 1526–33.
13 Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease. Am J Gastroenterol 1999; 94: 888–94.
14 Grodzinsky E, Hed J, Skogh T. IgA antiendomysium antibodies have a high positive predictive value for celiac disease in asymptomatic patients. Allergy 1994; 49: 593–7.
15 Black AE, Coward WA, Cole TJ, Prentice AM. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. Eur J Clin Nutr 1996; 50: 72–92.
16 Schofield WN, Schofield C, James WPT. Basal metabolic rate. Hum Nutr Clin Nutr 1985; 39C(Suppl. 1): 5–41.
17 Becker W. Food Habits and Nutrient Intake in Sweden 1989. Uppsala: Swedish National Food Administration, 1994.
18 Nordic Nutrition Recommendations 1996 [Copenhagen: Nordic Ministry Board, Nord 1996: 28]. Scand J Nutr 1996; 40: 161–5.
19 Parnell NDJ, Ciclitira PJ. Coeliac disease and its management. Aliment Pharmacol Ther 1999; 13: 1–13.
20 Valdimarsson T. Bone in coeliac disease. MD Thesis No 605. Linköping, Sweden: Linköping University, 1999.
21 Thompson T. Thiamin, riboflavin, and niacin contents of the gluten-free diet: Is there cause for concern? J Am Diet Assoc 1999; 99: 858–62.
22 Grehn S, Fridell K, Lilliecreutz M, Hallert C. Dietary habits of Swedish adult coeliac patients treated by a gluten-free diet for 10 years. Scand J Nutr 2001; 45: 178–82.
23 Jameson S, Björklund O, Lööf L, Ulfberg J. Zinc and folate malabsorption in coeliac disease. In: Howell JMC, Gawthorne JM, White CI, eds. Trace Element Metabolism in Man and Animals. Canberra, Australia: Australian Academy of Science, 1981: 495–7.
24 Collins BJ, Bell PM, Thomson JM, Fee DB, Wilson EA, Love AHG. Dietary history and nutritional state in treated coeliac patients. J Roy Soc Med 1986; 79: 206–9.
25 Ubbink JB. Metabolic markers of vitamin nutritional status. Am J Clin Nutr 1999; 70: 789–90.
26 Livingstone MBE, Prentice AM, Strain JJ, *et al.* Accuracy of weighted dietary records in studies of diet and health. Br Med J 1990; 300: 708–12.
27 Witthöft CM, Forssén K, Johannesson L, Jägerstad M. Folates — food sources, analyses, retention and bioavailability. Scand J Nutr 1999; 43: 138–46.
28 Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM. Total homocysteine and cardiovascular disease. J Intern Med 1999; 246: 425–4l.

29 Schnyder G, Roffi M, Pin R, *et al.* Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. N Engl J Med 2001; 345: 1593–600.
30 Homocysteine Lowering Trialists' Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Br Med J 1998; 316: 894–8.
31 Hallert C, Åström J, Walan A. Reversal of psychopathology in adult coeliac disease with aid of pyridoxine (vitamin $B_6$). Scand J Gastroenterol 1983; 18: 299–304.
32 Ciacci C, Iavarone A, Mazzacca G, De Rosa A. Depressive symptoms in adult coeliac disease. Scand J Gastroenterol 1998; 3: 247–50.
33 Holmes GKT. Neurological and psychiatric complications in coeliac disease. In: Gobbi G, Andermann F, Naccarato S, Banchini G, eds. Epilepsy and Other Neurological Disorders in Coeliac Disease. London: John Libbey, 1997: 251–64.
34 Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B12, and homocysteine in major depressive disorder. Am J Psychiatr 1997; 154: 426–8.
35 Hallert C, Grännö C, Grant C, *et al.* Quality of life of adult coeliac patients treated for 10 years. Scand J Gastroenterol 1998; 33: 933–8.
36 Collin P, Salmi J, Hällström O, Reunala T, Pasternack A. Autoimmune thyroid disorders and coeliac disease. Eur J Endocrinol 1994; 130: 137–40.
37 Mariani P, Grazia Viti M, Montuori M, *et al.* The gluten-free diet. A nutritional risk factor for adolescents with celiac disease. J Pediatr Gastroenterol Nutr 1998; 27: 519–23.