NUTRITION

Folate Supplementation and Neural Tube Defects: A Review of a Public Health Issue.

MICHAEL J. GONZÁLEZ, DSc, PhD. FACN*; KAREN J. SCHMITZ, MS†; MARÍA I. MATOS, LND, MHSN*; DELISABEL LÓPEZ, BS*; JOSÉ R. RODRÍGUEZ MD, Ph.D, MPH‡; JOSÉ J. GORRÍN, MD, MPH* FACOG*

ABSTRACT. Folate deficiency is one of today's most common vitamin deficiencies in women. Women who consume a low level of folate during pregnancy are at risk for poor pregnancy outcomes including neural tube defects (NTD). However, other factors such as heredity, social class, maternal age, birth order, maternal diet, length of time between pregnancies, maternal zinc deficiency, use of anticonvulsant drugs, abnormal homocysteine metabolism and the use of oral contraceptives, have also been implicated as causes of NTD. Animal studies have shown that fetuses are highly depedent on the folate status of the mother. In addition, several retrospective and prospective human studies have provided evidence that folate lowers the incidence of NTD. *Key words: Folic acid, Neural tube defects.*

Folate nutritional status in the U.S. and Puerto Rico is currently of concern for several reasons. The deficiency of this nutrient, leading to megaloblastic anemia, is one of the most common vitamin deficiencies observed, especially among lower socio-economic groups (1), women and African-Americans (2). Pregnant women consuming a low level of folate are at risk for poor pregnancy outcomes including prematurity, low birthweight (LBW), abruption of the placenta, spontaneous abortions, stillbirths (2) and neural tube defects (NTD) (3-7).

Neural tube defects are major defects in the central nervous system which leave the brain or spinal cord exposed (8). Each year in the U.S., 2,500 to 3,000 infants are born with NTD; the worldwide estimate is approaching 400,000 births per year (9). In many cases the occurence of NTD in a fetus results in death; however, in some cases the child survives with handicaps involving the nervous, musculoskeletal, gastrointestinal and/or urinary systems (8, 10). Women with a previous history of NTD in pregnancy are considered high risk for recurrence, however, almost 95% of NTD babies are born to families with no previous history of NTD (11). Heredity, social class, maternal age, birth order, maternal diet, the length of time between pregnancies (12), maternal zinc deficiency (9), anticovulsant drug use, abnormal homocysteine metabolism (13) and oral contraceptive use (14) have all been implicated as causes of NTD.

Dietary deficiency of folic acid associated with an increased rate of NTD was first noted following World War II in Germany (15), and Holland (16). In 1983 a recurrence rate of 0.7% of babies with NTD of mothers taking folate supplements as compared with a rate of 4.7% among mothers receiving no supplementation. However, this study was criticized for the lack of appropriate controls. In 1992 a properly designed Hugarian study (17) substantiated the evidence for vitamin supplementation (including 0.8 mg of folic acid) as a mean for decreasing the occurence or neural tube defects.

Folate is necessary as a coenzyme for several pathways in amino acid metabolism and nucleic acid synthesis involving the transfer of one-carbon groups (Fig. 1). Through the coenzyme functions, folate assists in the

*From the School of Public Health, University of Puerto Rico, Medical Sciences Campus.
†Michigan State University Department of Food Science and Human Nutrition, East Lansing MI, 48824.
‡Caribbean Center of Postgraduate Studies Psychological Institute of Puerto Rico, PO Box 3711, Old San Juan Station, San Juan, P.R. 00902.

Address correspondence to: Dr. Michael J. González, University of Puerto Rico, Medical Sciences Campus, School of Public Health, Nutrition Program, PO Box 365067, San Juan, P R 00936-5067.

synthesis of hemoglobin and deoxyribonucleic acid (DNA). Deficiency of folate in a tissue that is actively replicating, may result in insuffient DNA synthesis, and in cells with impaired functions, or dysplasia, a type or precancerous lesion (18). Megaloblastic bone marrow and folic acid deficiency anemia are also consequences of folate deficiency (19,20).

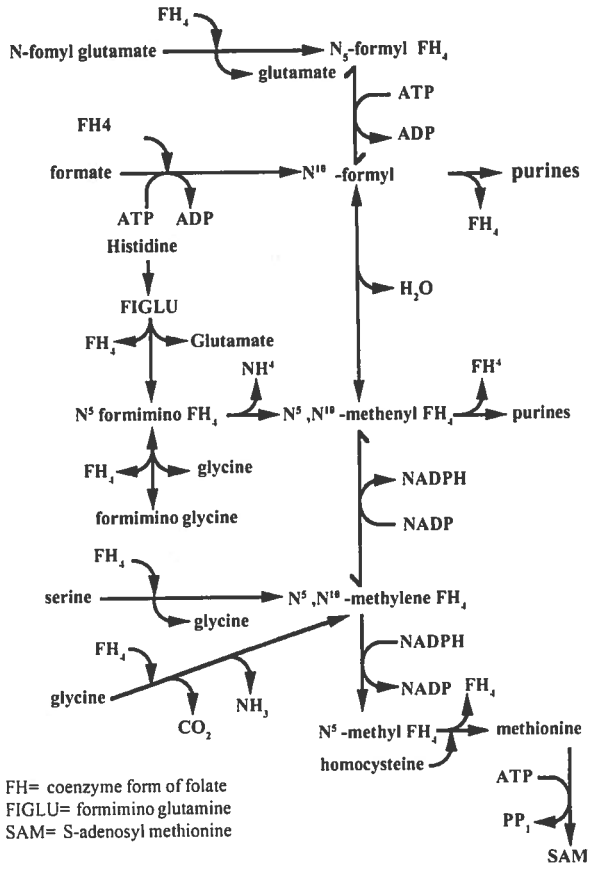

Figure 1. Folate-mediated reactions

Animal Research

Animal studies demonstrate the dependence of the fetus on the mother to maintain adequate folate stores. In studies with *Cebus albifrons* monkeys, dietary folic acid intake during pregnancy was compared with folate levels in the mother and offspring postpartum. Maternal, infant and non-pregnant hemotologic indices, blood and liver folate concentrations and urinary formimino glutamine acid excretion all varied with dietary folate intake and pregnancy status. The results of this study suggested that since fetal tissues do not have preferential access to maternal folate stores and if low, the fetus is highly dependent on the folate intake of the mother (2).

Human Research

Retrospective Studies

Bowers and Stanley (3) conducted a retrospective study on the protective effect of folate supplementation in the first six weeks of pregnancy against NTD during pregnancy. The matched 77 cases (mothers of infants with neural tube defects) with 77 controls (group I, mothers of infants with birth defects other than neural) and with a second group of 77 controls (group II, mothers of infants with no birth defects) to test whether the risk of occurrence of neural tube defects is inversely associated with maternal dietary intake of free or total folate in early pregnancy. Infants with anencephaly, spina bifida, or encephalocele were defined as having NTD. Information on maternal characteristics and eating patterns was gathered through a phone interview and a food frequency questionnaire about foods consumed over a 12 month period.

Folate supplementation in the first six weeks of pregnancy was reported by 15% of the mothers of cases, 21% of the mothers of group I subjects and 15% of the mothers of group II subjects. After adjusting for potentially confounding variables, such as social class, number of pregnancies, country of birth and birth order, odds ratios showed a protective effect against the occurence of NTD in infants with an increasing intake of free folate as assessed by dietary intake and supplementation in the first six weeks of pregnancy. The first six-to-eight weeks of gestation are important because the neural tube forms and closes during this period. Bower and Stanley reported that dietary free and total folate without addition of folate supplements showed also a protective effect with an increased folate intake, however, data to support this claim was not presented (3). This protective effect was similar to that observed when dietary folate was supplemented with additional folate.

In a similar study, the relation of folic acid intake during the first six weeks of pregnancy and the risk of neural tube defects were also investigated (4). For this study 23,491 women were recruited from various states in the U.S., undergoing maternal alpha-protein screening or amniocentesis at 16 weeks gestation. The results of these tests were recorded at the women's own clinics and forwarded to the researchers. Nurses, unaware of the results, interviewed the women by telephone. However, 7% of the patients knew the results of their amniocentesis at the time of the interview. A food frequency survey was used to determine dietary intake. The estimation of which week of pregnancy supplementation of folate began depended solely on self-reported pregnancy duration based on the date of the woman's last menstrual period. Near the delivery date, a questionnaire was mailed to each physician to ascertain information on the outcome of the

pregnancy. In cases where the physician did not respond (23.5%), the same questionnaire was mailed directly to the mother. The pregnancy-outcome questionnaire covered topics such as the results of prenatal tests, presence of any birth defects or chromosomal abnormalities, complications during pregnancy or delivery, complications in the newborn, and perinatal maternal illness. A neural tube defect was defined to be any occurence of spina bifida, anencephaly or encephalocele.

The women were divided into four groups for analysis. The prevalence of NTD in pregnancy was compared in the following groups of women: 1) women who had a family history of NTD and who did not use multivitamins; 2) women who had a family history and who began using multivitamins in the first six weeks of pregnancy; 3) women who had no family history of NTD and who did not use multivitamins; and 4) women who had no family history of NTD and who used multivitamins in the first six weeks of pregnancy. The potential effect of maternal age, family history of NTD, number of previous pregnancies, history of smoking, education level and dietary folic acid intake on the relation of multivitamin/ folic acid intake to NTD was evaluated by multiple regression analysis.

The prevalence of NTD among women with a family history of NTD and taking folic acid supplements was 3.5 per 1000, whereas, among those women who had a history of NTD and who did not use folic acid supplements the rate increased to 13.0 in 1000 births (prevalence ratio = 0.27, C.I. = 95%), Among those women with no family history of NTD, the prevalence rate for those women who used folic acid supplements in the sixth week of pregnancy was 0.9 in 1000 births, whereas, among those who did not use folic acid supplements the rate increased to 3.0 in 100 births (prevalence ratio = 0.29, C.I. = 95%). A woman was considered to be a multivitamin user if she reported taking at least one multivitamin per week, which really is a low level intake. Moreover, a varied intake of folic acid was reported. The folic acid intake for these women varied from 100 to 1000 ug/day. It was concluded that the data from the study provided good evidence that multivitamins containing folic acid taken during the first six weeks of pregnancy will prevent the occurence of NTD by more than 50%. Two others studies had similar results showing a reduction on NTD's (21,22).

While these two previous studies (3 and 4) provide indication that folate may lower the incidence of NTD, both studies encountered problems that often arise when doing human studies on the relationship of diet and disease. In both studies, the researchers measured dietary intake of folic acid by means of a food frequency questionnaire without any validation that the instrument could accurately measure the intake of a specific nutrient. Problems of recall bias and errors in self-reporting, both sources of error that could be associated with the method of data collection, were not addressed in either study. In addition, many foods rich in folate are also rich in other nutrients such as vitamina A, C and E.

Prospective Studies

Although maintaining control of variables in a study is important, researchers conducting studies on the effects of vitamin intake on NTD need to have greater control over the vitamin intake of their subjects while maintaining ethical standards. In two prospective, but morally questionable studies in Cuba and Great Britain, (5,6) respectively, suggested that folic acid supplementation might be an effective method of primary prevention of NTD.

Vergel et al (5) recruited women who had previous NTD pregnancies and divided the subjects into three groups: fully-supplemented with folic acid (5 mg daily), partially-supplemented (undefined) and unsupplemented. The subjects were told to try and conceive and to return to the researchers after the first missed menstrual period. Among the fully-supplemented (n=81) and partially-supplemented (n=20) folic acid groups, there were no recurrences of NTD births. In the other 114 women who became pregnant without folic acid supplementation, there were four NTD recurrences (3.5%). Folate concentrations were significantly different (p=0.001, paired t-test) for serum red-cell folates between fully supplemented and unsupplemented women. However, the numbers 0 in 81 fully-supplemented, 0 in 20 partially-supplemented and 4 in 114 unsupplemented were not significantly different by Chi-square test, which can indicate no significant differences existed in the incidence of NTD between supplemented and unsupplemented groups. The researchers attribute the lack of significance to the small sample size and suggest that folic acid supplementation may be effective in primary prevention of neural tube defects.

The study of Vergel et al (5) however, raises ethical questions because folic acid was denied to pregnant women controls who may have needed it to prevent another birth defect in their fetuses.

A study by (Czeizel and Dudas (17) 4,783 women planning a pregnancy were randomly assigned to receive a single table of a vitamin supplement (containing 12 vitamins, including 0.8 mg of folic acid, 4 minerals, 3 trace elements) or a trace element supplement daily for at least one month before conception and until the date of the second missed menstrual period or later. The outcome of pregnancy was known in 2104 women who received the vitamin supplement and in 2052 who recieved the trace

element supplement. There were six cases of neural tube defects in the group receiving the trace element supplement, as compared with none in the vitamin supplement group the authors concluded that periconceptional vitamin use decreases the incidence of a first occurrence of neural tube defects.

In a highly acclaimed randomized, double-blind human study on the association of folic acid intake and NTD recurrence, the MRC Vitamin Study Group (6) of the Medical College of St. Bartholomew's Hospital in London undertook a prospective study. However, the data monitoring committee stopped the trial due to the high incidence of NTD pregnancies occurring among unsupplemented mothers. Similar to the study of Vergel et al (5) the sample consisted of high risk women with previous histories of NTD pregnancies. The MRC study collected a large sample of women (n=1817) from seven different countries; they were divided into four groups: group 1, supplemented with 4 mg. of folic acid; group 2, supplemented with other vitamins (A, D, B-1, B-2, B-6, C, nicotinamide); group 3, supplemented with 4 mg of folic acid and the same vitamins as in group 2; group 4, unsupplemented. The vitamins were consumed by subjects from the day of randomization to 12 weeks of pregnancy, but there was no control over the womens' diets. Neural tube defects were defined as any occurrence of anencephaly, spina bifida or encephalocele.

The results of the study showed that the prevalence of NTD was 1% if folic acid supplements were taken and 3.5% if no folic acid was taken. Relative risk was calculated to be 0.28 (95% C.I., 0.12 to 0.71), indicating that 72% of NTD ocurrences were prevented by folic acid supplementation.

While prospective studies avoid the problems of recall bias associated with retrospective studies, they encounter new problems involving ethics. It is well known that women with a previous history of NTD are at an increased risk of NTD recurrence. Laurence (23) reported that the risk for repeat NTD was as much as 10 times greater than the risk of NTD for women with no previous occurrences. He suggested that increased folic acid intake decreases the risk of NTD by as much as one-third. In addition, Smithells et al (24) supports supplementation of folic acid starting before conception not only for women with a history of NTD, but for women with a family history of the defects.

Even though the results of previous studies indicated that a relationship may exist between folic acid intake and NTD, the MRC researchers chose to put women with a history of NTD ocurrence at risk of recurrence by restricting folic acid supplementation. Although the monitoring committee stopped the trial, 1817 women had already passed the critical first 6 weeks of pregnancy when the neural tube is formed and thus were knowingly deprived of the benefit of folate supplemention. This particular practice common in double blind trails has been questioned by various researchers. In addition, questions arise as to a generalization of the results, because the study included only families with a history of NTD, while 95% of NTD occurrences are in families with no history of NTD (11). The MRC study did not address the large population of women of child-bearing age with no history of NTD.

The MRC researchers reported that there were no effects of the other vitamins on folic acid availability or on the decreased risk of NTD, but admit that the power of the study to detect interaction effects was limited. Questions have been raised concerning the interpretation of the results. Crawford (25) suggested that vitamins other than folic acid may be associated also with prevalence rates of NTD, pointing out that both folic acid and vitamin B-12 are required for neural protein synthesis; however, vitamin B-12 was not included in the supplements. It has been questioned whether the study showed a pharmacological effect of folic acid, a correction in a folic acid deficiency or a mass effect on the low levels of vitamin B-12 found in mothers of anencephalics (25,26). Yates et al (27) determined serum and erythrocyte folate levels in women who had two or more pregnancies resulting in infants with NTD and matched controls women with a history of infants with NTD had lower erythrocyte folate levels than did controls, but there were no differences in folate serum levels. Red cell folate was lowest in women who had had three or more infants with NTD.

Evidence against a protective effect of folate

Only one fairly recent investigation reported no correlation between maternal folic acid intake and NTD. Mills et al (27) examined the use of vitamin supplements containing folic acid by women who had a history of NTD (n=571), women who had stillbirths or other fetal malformations (n=546), and women who had normal infants (n=573). This study differed from the other studies discussed above in that spina bifida was not included in the definition of NTD, but anencephaly, meningocele, myelomeningocele, encephalocele, rachischisis, iniencephaly and lipomeningocele were included.

Case and control mothers were matched by geographical location. All mothers were contacted by telephone not less than once month or more that three months after delivery or after the anomaly was identified. A standard questionnaire was used to interview the mothers about their use of vitamins, diet, drug exposure, demographics and medical history. It was stated that recall bias was kept to a minimum by completing the interviews within five months of the birth of an infant or diagnosis of a birth

defect. Subjects were classified as; (a) fully-supplemented if they took a multivitamins containing the RDA of at least four vitamins at least six days per week, or (b) partially supplemented if they consumed less that the RDA, and (c) unsupplemented if they did not consume vitamin supplements. The amounts of folate were calculated on the basis of brand names, vitamin labels and the ingredients listed on cereal boxes.

Pairwise comparisons of the socio-economic status of the mothers of normal infants with mothers of abnormal control infants revealed no significant differences. However, mothers of babies with NTD had significantly less education (p=0.002) and were less likely to be employed (p=0.003) than mothers of normal infants. The mothers of infants with NTD were less likely to take multivitamins (p=0.39, not statistically significant) and the percentage of fully-supplemented mothers of infants with NTD (15.4%) was not significantly different from the percentage of fully-supplemented mothers with abnormal control infants (15.5%). Mills et al (28) reported that for multivitamin users, the odds ratio for having a child with NTD remained very close to one, regardless of whether the abnormal or normal control group was used for comparison, whether the RDA or any amount of vitamin was considered, and whether or not vitamin,containing cereals were included. Therefore, it was concluded that periconceptional use of folate-containing multivitamins by American women does not decrease the risk of having an infant with NTD.

The interpretation of the results of the above study is further complicated in view of the different definition of NTD given by Mills et al as compared to other investigators in the field. Comparing studies is extremely difficult when some researchers include spina bifida in the definition of NTD (3,4,6), while others exclude this defect from the definition (28). In the study by Milusnky et al (4), spina bifida alone accounted for one third of the NTD cases. The exclusion of such a large group could have a significant effect on the results of the Mills et al study. We believe that results obtained by Czeizel and Dudas (17) in which the perioconceptional intake of folate reduced the incidence of neural tube defects are in the right track.

Discussion

National committees on nutrition differ in their support of folic acid supplementation during pregnancy. The RDA for folate intake during pregnancy has been set at 400 ug per day. The National Research Council (29) reports that this level is sufficient to build or maintain maternal stores, and can be met through diet without supplementation. However, the U.S. Centers for Disease Control have made interim recommendations for folic acid supplementation (30). The IOM cautions that recommendations on multivitamin use during pregnancy may divert attention from other dietary, environmental or social factors that may contribute to the prevalence of NTD (9). There some concerns about excessive folate intake; extremely high levels of folic acid have been shown to be neurotoxic and to produce convulsions in animals (31). However in humans, no adverse effects were found in doses of 10 mg of folic acid/day for 5 years (32). Presently, the IOM makes no recommendations on folate supplementation in pregnancy.

Hibbard (33) believes that folic acid supplementations for pregnant women is valuable only in selected patients and that most women in Western society can get the necessary nutrients from a good mixed diet. He adds that folic acid intake is hindered by ignorance, inertia or a lifetime of bad habits, therefore, healthy eating, not supplementation, should be stressed during pregnancy.

Contrary to the view of Hibbard, Horn (34) argues that most women are not able to meet the demands of expanding red cell mass and fetal growth in pregnancy through diet alone. It is known that folate circulation in tissues is reduced during pregnancy; many pregnant women become anemic. The need for folate supplementation is strengthened by the report of Worthington-Roberts and Williams (19) that although leafy-green vegetables, yeast, legumes, nuts, and whole grains are all good sources of folic acid, up to 80% of the nutrient can be lost during cooking and storage.

Conclusion

Folic acid studies, like other studies of nutrients, are plagued by the lack of appropriate controls. In order to control the independent variable (folic acid intake), researchers must conduct prospective studies so as to avoid the use of food frequency surveys and the existence of recall bias. However, prospective studies are plagued by ethical problems like those encountered by Vergel et al (9) and the MRC vitamin group (6). Despite the apparent weaknesses, the recent studies reviewed here in have addressed a significant public health issue and have raised serious questions as to the importance of folic acid supplementation for pregnant women. It has been established that a daily dose 4mg folic acid is effective in reducing the risk of (NTD) affected pregnancies by 70% among women who have had a previously affected pregnancy (recurrence risk) (6). We also know that a supplement of 0.8 mg folic acid is effective in reducing the risk of a first ocurrence (17). Hence, the FDA has proposed folic acid fortification of foods this probably

due to the fact that neural tube defects occur in pregnancy before most women even know that they are pregnant, putting all unplanned pregnancies at risk. We believe that these recommendations for supplementation are a step in the right direction and should be given to women in childbearing age but also accompanied by guidelines for healthy eating during pregnancy.

Fortification has been thought as a principal strategy for increasing folic acid intake (35). It is in the public health tradition to treat the whole population for the benefit of a group. This has been done previously such as in adding iodine to table salt and adding flouride to the water supply. Nevertheless, these fortification efforts might provide the population with other benefits since there are several chronic degenerative diseases that might be prevented by the addition of folate (36-38). It is also prudent to complement this approach by adding some B-12 because of the obvious risk of potentially masking the early diagnosis of pernicious anemia due to cobalamin (B-12) deficiency (37). By a verting costly birth defects, folic acid fortification may yield a substancial economic benefit (40).

Recently it has been suggested that placental transfer of folate is not likely to be involved in pathogenesis of NTD and that fetal folate status in NTD fetuses should be investigated during the first trimester since normal fetal folate levels have been observed later in gestation (39). These authors also suggest that the decreased maternal RBC folate methylation rate observed in the NTD group could be a potential marker of a genetic abnormality of folate metabolism; which may increase the risk of women having children with NTD. At the same time this problem may also increase the need for higher doses of folate.

Resumen

La deficiencia de folato hoy en día es la deficiencia vitamínica más común en mujeres. Las mujeres que consumen niveles bajos de folato durante el embarazo están a riesgo de problemas como defectos del tubo neural, sin embargo otros factores como herencia, clase social, edad, orden de nacimiento, alimentación, período de tiempo entre embarazos, estatus de zinc, uso de medicamentos anticonvulsivos, metabolismo anormal de homocisteina y uso de contraceptivos orales han sido implicados como causantes de defectos del tubo neural. Estudios en animales han demostrado que los fetos son altamente dependientes del estatus de folato de la madre. También varios estudios retrospectivos y prospectivos en humanos evidencian que el folato disminuye la incidencia del tubo neural.

Acknowledgements

The authors will like to thank Mrs. Aida Font for her excellent secretarial service.

References

1. Subar AF, Block G, James LD. Folate intake and food sources in the U.S. Population. Am J Clin Nutr 1989;50:508-516.
2. Blocker DE, Ausman LM, Meadows CA, Thenen SW. Experimental maternal and neonatal folate status relationships in nonhuman primates. Am J Clin Nutr 1989;50:120-128.
3. Bowers C, Stanley FJ. Dietary folate as a risk factor for neural-tube defects: evidence from a case-control study in Western Australia. Med J Aust 1989;150:613-617.
4. Milunsky A, Hershel J, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ, Willett W. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. JAMA 1989;262:28-47-2852.
5. Vergel RG, Sánchez LR, Heredero PL, Rodríguez PL, Martínez AJ. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. Prenat Diagn 1990; 10:149-152.
6. Medical Research Council Vitamin Study Group. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study Group. Lancet 1991;338:131-137.
7. Anonymous. Folate supplementation prevents recurrence of neural tube defects. Nutr Rev 1992;50:22-24.
8. Copp AJ, Brook FA, Estibeiro JP, Shum AS, Crockroft DL. The embryonic development of mammalian neural tube defects. Prog Neurobiol 1990;35:363-403.
9. Institute of Medicine. Subcommittee on Nutritional Status and Weight Gain during Pregnancy. Nutrition During Pregnancy. National Academy of Sciences, Washington, D.C. 1990.
10. Rose NC, Meunuti MT. Periconceptional folate supplementation and neural tube defects. Clin Obstet Gynecol 1994;37:695-320.
11. Hobbins JC. Diagnosis and management of neural-tube defects today. New Engl J Med 1991;324:690-691.
12. Mann J. Dietary folate and neural-tube defects. Med J Aust 1989;150:609.
13. Steegers-Theunissen RP, Boers GH, Trijbels FJ, Eskes TK. Neural tube defects and derangement of homocysteine metabolis (letter). New Engl J Med 1991;324:199-200.
14 Davis RE, Smith BK. Pyridoxal, vitamin B-12 and folate metabolism in women taking oral contraceptive agents. S Afr Med J 1974;48:1937-40.
15. Eichman E, Gesenius H. Vitamin supplementation and prevention. Br Med J 1983;282:1509-1511.
16. Stein Z, Susser M. Maternal starvation and birth defects. In Birth Defects: Risks and Consequences. New York, N.Y. Academic Press, Inc. 1976.
17. Czeizel AE, Dudás I. Prevention of the first occurence of neural tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832-1835.
18. Mason JB. Folate, colitis, dysplasia, and cancer Nutr Rev 1989; 47:314-317.
19. Worthington-Roberts B, Williams SR. Nutrition in Pregnancy and Lactation. St. Louis, MO., 1989.
20. Herbert V, Colman N. Folic acid and vitamin B-12. In M.E. Shils and V. Young, eds. Modern Nutrition in Health and Disease, 7th ed. Lea and Febiger, Philadelphia, 1989.
21. Mulinare J, Cordero JF, Erickson JD, Berry RJ. Periconceptional use of multivitamins and the occurrence of neural tube defects.

JAMA 1988;260:3141-3145.
22. Werler M, Shapiro S, Mitchell A. Periconceptional Folic acid exposure and risk of occurent neural tube defects. JAMA 1993; 269:1257-1262.
23. Laurence KM. Folic acid to prevent neural tube defects (letter). Lancet 1991;338:379.
24. Smithells RW, Sheppard S, Schorah CJ, Wild J. Folic acid to prevent neural tube defects (letter). Lancet 1991;338:380.
25. Crawford MA. Folic acid to prevent neural tube defects (letter). Lancet 1991;338:380.
26. Anonymous. Evidence for and association between periconceptional use of multivitamins and neural tube defects. Nutr Rev 1990;48: 15-19.
27. Yates JRW, Ferguson-Smith MA, Schenkin A, Guzman-Rodríguez R, White M, Cark B. Is disordenel folate metabolicm the basis for the genetic predisposition to neural tube defects? Clin Genet 1987; 31:279-287.
28. Mills JL, Rhoads GG, Simpson JL, Cunningham GC. The absence of a relation between the periconceptional use of vitamins and neural-tube defects. New Engl J Med 1989;321:430-435.
29. National Research Council. Recommended Dietary Allowances. Subcommittee on the Tenth Edition of the RDAs. Food and Nutrition Board. Commission on Life Sciences. Washington, D.C., 1989.
30. U.S. Centers for Disease Control. Use of folic acid for prevention of spina bifida and other neural tube defects. MMWR 1991;40:513-516.
31. Scott JM, Kirke P, O'Broin S, Weir DG. Folic acid to prevent neural tube defects (letter). Lancet 1991;338:505.
32. Spies T, Hillman R, Cohlan S, Kramer B, Kanof A. Disease of Metabolism. G. Duncan ed., Saunders, Philadelphia, 1959.
33. Hibbard BM. Iron and folate supplements during pregnancy: supplementation is valuable only in selected patients. Br Med J 1988;297:1324-1325.
34. Horn E. Iron and folate supplements during pregnancy: supplementing everyone treats those at risk and is cost effective. Br Med J 1988;297:1325-1326.
35. Anonymous. Food Labelling: health claims and label statements, Folate and neural tube defects. Fed Regis., 1993;58:53254-53295.
36. Beresford SAA. Annotation: How do we get enough folic acid to prevent some neural tube defects? Am J Public Health 1994;84:348-349.
37. Boushey CJ, Beresford SAA, Onenn FS, Motulsky AG. A quantitative Assessment of plasma homocyteine as a risk factor for vascular disease, JAMA 1995;274:1049-1057.
38. Oakley GPJr, Adams MJ, Dickinson CM. More folic acid for every one, now. J Nutr 1996;126:751s-755s.
39. Rayburn WF, Stanley JR, Garrett ME. Periconceptional Folate intake and neural tube defects. J Am Coll Nutr 1996;15:121-125.
40. Romano PS, Weitzman NJ, Sheffler RM, Pi RD. Folic acid fortification of grain: an economic analysis. Am J Public Health 1995, 85:667-676.