ELSEVIER

The Scientific Basis for Eliminating Folic Acid–Preventable Spina Bifida: A Modern Miracle from Epidemiology

GODFREY P. OAKLEY, JR., MD, MSPM

One of the most remarkable successes of epidemiology was the demonstration in the late twentieth century that spina bifida and anencephaly–two of the most common and severe birth defects–are caused primarily by folate deficiency. This article reviews the descriptive epidemiological studies that began when we did not have a clue about etiology. The paper tells the success story of the trials that proved that folic acid would prevent folic-acid-preventable spina bifida. Finally, it will tell how difficult it is to get prevention policy implemented, even when the scientific evidence is compelling. It concludes by noting that the inaction or inappropriate actions of food regulatory bodies in so many countries means that only 10% of folic-acid-preventable spina bifida is actually being prevented–a serious failure of public health policy.
Ann Epidemiol 2009;19:226–230.

KEY WORDS: Birth Defects/Congenital Anomalies, Prevention, Translation, Public Health Policy, Pediatrics, Nutrition.

"…folic acid fortification may be the most important science-driven intervention in nutrition and public health in decades." (1)

A MIRACLE

Finding a simple, cheap, and effective public health intervention that keeps thousands of children around the world, each year, alive and out of wheelchairs is a humanitarian miracle. The work of scientists such as Sir Nicholas Wald (MRC Vitamin Study Research Group [2]), Smithells and colleagues (3), and Czeizel and Dudas (4) provided the clinical trials that ultimately proved that a simple, well-known vitamin could prevent most children from having spina bifida and anencephaly (2–4). These epidemiological studies provided the key science that allowed folic acid fortification, which "may be the most important science-driven intervention in nutrition and public health in decades." (1) The science in "science-driven" was epidemiology.

The reason we do epidemiology is the expectation that we will improve the health of the public. Spine bifida and anencephaly are two of the most common and severe birth defects. Their elimination is a noble goal. Just as a vaccine to prevent polio was hailed as a miracle, so was the result from epidemiological studies that unequivocally proved that folic acid would prevent spina bifida and anencephaly.

BEFORE THE CORRECT HYPOTHESIS—OBSERVATIONAL EPIDEMIOLOGY

Before we learned that folate deficiency was the main cause of spina bifida and anencephaly, observational epidemiology sought a clue to the etiology of these diseases when we hardly "had a clue." Ian Leck from Manchester, England, summarized the literature in a review in 1974 entitled, "Causation of Neural Tube Defects: Clues from Epidemiology." Much of this early work came from investigators from the United Kingdom because these birth defects were well known there, common, and severe. Leck (5) stated in his introductory paragraph.

The most dramatic of all errors of nervous system development are those that arise in the embryo when the closure of the neural tube and its submergence within the mesoderm are disrupted. The epidemiological study of the resulting defects—anencephaly, encephalocele, myelocele and meningocele—was pioneered a quarter of a century ago by Record and McKeown (1949). Their studies, and others during the ensuing decade (reviewed by Penrose, 1957; McKeown, 1960), showed that the frequency of these defects varied with many factors—time, place, sex, ethnic group, family, social class, and maternal age and parity—and that most of these variations involved both anencephaly and the other neural tube defects. It thus became fairly clear that the two groups of defects must have similar causes, and that these causes must be in part environmental—in contrast to the position for other common defects, notable cleft lip, for which the only demographic variables with the well-established effects on frequency are the ones with genetic implications: sex, ethnic group, family, and parental age.

Leck (5) begins the final paragraph: "It may seem paradoxical that we should know so much about how the prevalence of these defects varies and so little about the causes of these

From the Department of Epidemiology, Rollins School of Public Health of Emory University, Atlanta, GA.

Address correspondence to: Dr. Godfrey P. Oakley, Rollins School of Public Health of Emory University, Department of Epidemiology, Atlanta, GA. Tel.: (678) 613 6918. E-mail: gpoakley@mindspring.com.

Received January 27, 2009; accepted January 30, 2009.

1047-2797/09/$–see front matter
doi:10.1016/j.annepidem.2009.01.016

variations." Later he notes in the paragraph that, if then published preliminary links of these birth defects to high vitamin A, to folate depletion or to antagonism of plasma albumin to insulin were causal, "it might become possible to prevent these defects by giving treatment to compensate for the underlying abnormalities during the critical stage of pregnancy." Folate/folic acid was given little attention in the article and simply noted in a long list of possible associations that had been examined.

THE RIGHT HYPOTHESIS: SMITHELLS TO THE RESCUE

Leck cited a short paper by Hibbard and Smithells (6) published in the "Preliminary Communications" section of *Lancet* in 1965 entitled: "Folic acid metabolism and human embryopathy." The paper showed that women near delivery who had babies with neural tube defects (NTDs) and those who had babies with other birth defects had a five-fold increase in an abnormal formiminoglutamic acid excretation test (FIGLU). However, they concluded that an abnormal FIGLU test "may indicate defective absorption or metabolism rather than a deficient intake of folate." Smithells, an academic pediatrician who worked with a birth defects registry and who had seen and cared for many children with spina bifida, thought it worth pursuing a lead that folic acid might prevent these birth defects. The clue was modest, but Smithells thought it worth exploring.

THE NON-RANDOMIZED TRIAL OF SMITHELLS AND OTHERS MAKES A VITAMIN DEFICIENCY A TOP ETIOLOGICAL HYPOTHESIS

The main contribution of all the early descriptive epidemiology was to show that something in the broadly defined environment must play a major role in the etiology of NTDs. It was Smithells who thought that it might be nutritional, primarily because poor women had a higher risk and he thought poor diets might be the cause. He then organized a randomized controlled trial (RCT) to test whether or not a vitamin similar to a daily multivitamin would prevent or lower the risk in the pregnancies of women who had previously had a child with an NTD. One of the sad chapters in ethical reviews of study protocols is that three of the four hospitals where he planned to do the study refused permission for a randomized study. These decisions forced Smithells to undertake a nonrandomized study, which undermined confidence in the inferences that could be drawn from his results. This unfortunate chapter delayed by more than a decade the public health policies to prevent folic acid–preventable spina bifida and anencephaly.

In spite of being denied the possibility of a randomized study, Smithells and colleagues (3) eventually conducted a large trial that involved nearly 1000 women who had previously had a child with an NTD. They found that women who consumed the multivitamin for 1 month before pregnancy through the second missed period had a recurrence of 0.7%; those who did not consume supplements had a recurrence of 4.7%—a highly statistically significant and remarkable seven-fold reduction. The key question was whether or not this was a causal association. Like the observational studies on smoking and lung cancer of Doll and Hill, Smithells and colleagues cut their data many ways trying to identify a non-causal explanation and could find none:

> We may conclude that none of the known or suspected factors which might influence recurrence rates contributed substantially to the differences between FS [fully supplemented sic] fully supplemented and US [unsupplemented sic] un-supplemented groups. The presumptive evidence of a protective effect of vitamin supplementation remains very strong. Theoretically, our supplementation regimen carries little risk since it uses "physiological" amounts of vitamins. In practice, an assiduous search for evidence of harm, which we shall continue, has so far revealed none. However, the possibilities that other amounts or combinations of vitamins might be more effective or less safe are very real and need urgent investigation. Further studies are a prerequisite for policy decisions designed to reduce the occurrence of NTDs (3).

This remarkable paper deserves careful review. In many ways Smithells and colleagues got the "right" answer (vitamin supplementation) using the "wrong" design (non-randomized). We conclude that smoking causes lung cancer without RCTs, but we were reluctant to conclude that a multivitamin would prevent spina bifida.

I was personally not convinced by the data of Smithells and colleagues, thinking that there was a perfectly reasonable non-causal explanation. My colleagues and I wrote a letter to *Lancet* (7) recounting that recent evidence had suggested a recurrence rate of about 2.5%, not the 5% reported in the control group of Smithells and colleagues. If one looked at the total recurrence rate in the Smithells study it was about 2.5%, which certainly left room for some non-causal explanation. We were wrong and Smithells was right.

OTHER OBSERVATIONAL DATA

I spent a great deal of time in the 1980s seeking to find resources to conduct a RCT to test the link between vitamins and the recurrence of neural tube defects. If a causal relationship could be demonstrated, the fortification (as with milk and vitamin D) of a food staple could prevent these serious birth defects efficiently and cheaply.

AEP Vol. 19, No. 4
April 2009: 226–230

Unfortunately, neither the National Institutes of Health (NIH) nor the March of Dimes nor the Congress would provide the Centers for Disease Control and Prevention (CDC) the resources to conduct such a study. Not until late in the 1980s did the Spina Bifida Association of America convince Congress to give the CDC sufficient resources to conduct an RCT in China.

In the meantime, concern that Vietnam veterans had an increased risk of having babies with birth defects was a sufficiently politically visible issue that Congress provided the CDC with resources for a very large case-control study to evaluate these concerns. Given the available evidence, it was important to include vitamin consumption as a possible confounder. Indeed, results from this study showed a relative risk of about 0.5, suggesting that in the general population of Atlanta, pre-conception and early pregnancy consumption of a multivitamin similar to the one in the study by Smithells and colleagues was associated with a 50% reduction in the incidence of spina bifida and anencephaly (8).

Investigators in Perth, Australia, also reported that vitamins protected against neural tube defects (9).

Our requests for NIH to sponsor an RCT were fruitless, but prompted a case-control study—surprising, given NIH's history of sponsoring RCTs for evaluating drugs to prevent cardiovascular disease and to cure cancer (10). Their study conducted in California and Illinois was negative for reasons that are not clear to this day and are a reminder that, even when there is causality, there can be negative studies.

Support from NIH and Health Resources and Services Administration to look at a cohort of women having serum alpha-fetoprotein testing provided Milunsky and colleagues (11) the opportunity to obtain vitamin exposure early in pregnancy before the woman knew whether or not she had an affected child. This study showed a marked degree of protection for folic acid users (prevalence ratio, 0.27; 95% confidence interval of 0.12 to 0.59). A particular note was that the prevalence of NTDs among those who took multivitamins without folic acid was close to those who never took multivitamins. The authors concluded: "We believe that the combined data from this and other studies ([sic] Smithells and Mulinare) provide good evidence that folic acid-containing multivitamins taken during the first 6 weeks of pregnancy will prevent, by more than 50% the occurrence of NTDs."

RANDOMIZED CONTROLLED TRIALS TO THE RESCUE

After Smithells published his first study, Laurence et al. (12) from Wales reported a study of large-dose folic acid that had appeared to be negative, but when reanalyzed using only those who he confirmed took their medicine, the authors found a protective effect. Wald and colleagues conducted a 2×2 factorial RCT to determine whether 1) a high dose of folic acid (4000 μg daily, 10 times the then current recommended daily allowance) alone; 2) in combination with other vitamins and minerals; or 3) the other vitamins and minerals alone would prevent neural tube defects among women who had already had an affected child (2). This key study, generally called the MRC Study, found that only the folic acid arms were associated with a statistically significant 72% reduction, remarkably similar to the findings of Smithells and colleagues (3). Given that the study was randomized, it was presumed that the relationship was causal. At the CDC, we were able in short order to obtain recommendations for women who previously had an affected child and published them in our widely read publication, *Morbidity and Mortality Weekly Report* (MMWR) (13).

POPULATION RECOMMENDATION MORE DIFFICULT

Having wide agreement that folic acid was the vitamin that prevented NTDs capped by the findings of the MRC study, CDC scientists pushed for a population recommendation. Recurrent NTDs, the outcome studied in the MRC study, accounted for no more than 5% of the births of babies with NTDs. If one wanted to prevent most NTDs, one needed a strategy for all women of reproductive age. The data existing in the observation literature, especially in Smithells et al. (3), Mulinare et al. (8), and Milunsky et al. (11), suggested that 400 μg would be a sufficient dose. We began a process to obtain a CDC recommendation that all women of reproductive age should consume 400 μg of folic acid a day to prevent spina bifida and other NTDs. We argued for this idea from late 1991 through the spring of 1992 and reached CDC agreement on such a recommendation. During our deliberations, we became aware of the results of Czeizel and Dudas (4), constituting a second, confirmatory, folic acid RCT and, much to the credit of the *New England Journal of Medicine*, were able to use and quote these results in the Public Health Service recommendation months before the publication of the paper.

It was not easy to obtain the Public Health Service recommendation because the vitamin industry had persuaded Congress to require that the FDA reach an opinion on whether or not folic acid prevented NTDs. In a process parallel to that of the CDC, the FDA concluded in June 1992 that folic acid did not prevent NTDs. These two differing conclusions reached the desk of an official on the Assistant Secretary for Health's staff who realized his boss could not sign two conflicting conclusions. Following

significant discussions in the summer, CDC arguments prevailed and the Public Health Service, FDA included, agreed that folic acid did prevent spina bifida and recommended that all women capable of becoming pregnant consume 0.4 mg (400 μg) to lower the risk of spina bifida and anencephaly. These results were published in the Sept. 11, 1992 issue of the *MMWR* and provided the basis for our current population prevention programs (14).

CHINA STUDY AFFIRMS PUBLIC HEALTH SERVICE RECOMMENDATION

When we learned of the MRC results, we concluded it was no longer ethical to conduct the RCT that we and our Chinese collaborators were about to initiate. We did, however, conduct a large community trial that used 400 μg of folic acid. That study showed that, in the highest risk areas of China, the intervention was associated with an 85% reduction in the incidence of spina bifida and anencephaly and, in lower risk areas, there was about a 40% decrease (15).

FOLIC ACID FORTIFICATION HAPPENS AFTER A STRUGGLE

Folic acid fortification of food staples, such as flour, is a readily available way to achieve the goal of consuming 400 μg of folate per day. Given the seriousness and frequency of the birth defects and the low cost and effectiveness of fortification of food staples, one would think, at least in developed countries, that folic acid fortification would have been a top priority among food regulators. But this has not been the case. Although required folic acid fortification is being practiced by about 40 countries some 17 years after the RCTs were published, no food regulatory body has moved swiftly and the majority of countries still do not have required fortification. This inaction contrasts with the response of developed countries to adopt a new *Haemophilus* influenza vaccine that was proven effective at about the time the MRC paper was published. Within 2 years of the availability of the vaccine, universal, country-wide immunization programs were implemented, resulting in the virtual disappearance of invasive *Haemophilus* disease. The vaccine-industrial complex works very smoothly compared to a rather dysfunctional food regulatory process in almost all countries. Food regulatory bodies seem able to move swiftly when the news about a food or additive is harmful, no matter how weak the data, but are almost paralyzed when there is proof that a food or additive is beneficial.

The first country to require folic acid fortification was the United States; the effort required was near herculean. The FDA held three public hearings and then continued to delay the decision for 3 years. It required strong and consistent political leadership and pressure, primarily from the March of Dimes, to persuade the FDA to require the folic acid fortification. Canada followed quickly, not because the food regulators wanted to improve the public health, but because the Canadian millers took political steps to force their food regulators to use the North American Free Trade Agreement as a rationale to require mandatory fortification. Most of the flour milled in Canada is sold in the United States, and the millers did not want the cost of having to mill flour for the U.S. market different from that for the Canadian market.

Even though no country has yet to demonstrate elimination of folic acid–preventable spina bifida and anencephaly, mandatory folic acid fortification of flour has been associated with a marked decrease in the rates of spina bifida and anencephaly (16–21). Increasing the concentration of folic acid in fortified foods and fortifying more foods is likely to move countries that currently have required folic acid fortification closer to elimination of these birth defects (22)." Folic acid fortification has had benefits beyond birth defects prevention; it raised serum folates to such levels that it is unlikely there is any folate deficiency anemia in countries that fortified foods (23). Serum homocysteine concentrations have been lowered (24). While there remains uncertainty of the full benefit of this reduction in concentrations of homocysteine, there is substantial data showing that folate reduces deaths from stroke. If true, the number of stroke deaths prevented by folic acid would be about 10 times the number of birth defects prevented (25).

GLOBAL PREVENTION OF FOLIC ACID–PREVENTABLE SPINA BIFIDA AND ANENCEPHALY ONLY 10% DONE

We must now demonstrate the progress, or lack thereof, toward elimination of folic acid–preventable spina bifida and anencephaly. My colleague Karen Bell and I have used available data to estimate how well the prevention is going. So far, we estimate that only about 10% of the birth defects that can be prevented are actually being prevented (26). This is a reminder that having the science for prevention is necessary, but it is seldom sufficient. There must be science-based advocacy to create the political will to actually make prevention happen.

CONCLUSIONS

Epidemiology provided unequivocal evidence that folic acid will prevent folic acid–preventable spina bifida and anencephaly. Required folic acid fortification of flour has been shown to be a safe, highly effective way to prevent these

birth defects. It has also virtually eliminated folate deficiency anemia and reduced population average homocysteine concentrations. It is no wonder that Rosenberg said: "…folic acid fortification may be the most important science-driven intervention in nutrition and public health in decades." (1) May the day soon come when no new children bear the consequences of their governments' failures to implement fully effective prevention programs based on scientific evidence.

REFERENCES

1. Rosenberg IH. Science-based micronutrient fortification: which nutrients, how much, and how to know? Am J Clin Nutr. 2005;82:279–280.
2. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet. 1991;338:131–137.
3. Smithells RW, Nevin NC, Seller MJ, Sheppard S, Harris R, Read AP. Further experience of vitamin supplementation for prevention of neural tube defect recurrences. Lancet. 1983;1:1027–1031.
4. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med. 1992;327:1832–1835.
5. Leck I. Causation of neural tube defects: clues from epidemiology. Br Med Bull. 1974;30:158–163.
6. Hibbard ED, Smithells RW. Folic acid metabolism and human embryopathy. Lancet. 1965;1254.
7. Oakley GP Jr, Adams MJ Jr, James LM. Vitamins and neural tube defects. Lancet. 1983;2:798–799.
8. Mulinare J, Cordero JF, Erickson JD, Berry RJ. Periconceptional use of multivitamins and the occurrence of neural tube defects. [see comment]. JAMA. 1988;260:3141–3145.
9. Bower 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;150:613–619.
10. Mills JL, Rhoads GG, Simpson JL, Cunningham GC, Conley MR, Lassman MR. The absence of a relation between the periconceptional use of vitamins and neural-tube defects. National Institute of Child Health and Human Development Neural Tube Defects Study Group. [see comment]. N Engl J Med. 1989;321:430–435.
11. Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. [see comment]. JAMA. 1989;262:2847–2852.
12. Laurence KM, James N, Miller MH, Tennant GB, Campbell H. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. Br Med J (Clin Res Ed). 1981;282:1509–1511.
13. Centers for Disease Control and Prevention. Use of folic acid for prevention of spina bifida and other neural tube defects–1983-1991. MMWR Morb Mortal Wkly Rep. 1991;40:513–516.
14. Centers for Disease Control and Prevention. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morb Mortal Wkly Rep. 1992;41(RR-14):1–7.
15. Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. N Engl J Med. 1999;341:1485–1490. Erratum in: N Engl J Med. 1999;341:1864.
16. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. JAMA. 2001;285:2981–2986.
17. De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, et al. Reduction in neural tube defects after folic acid fortification in Canada. N Engl J Med. 2007;357:135–142.
18. Castilla EE, Orioli IM, Lopez-Camelo JS, Dutra Mda G, Nazer-Herrera J. Latin American Collaborative Study of Congenital Malformations (ECLAMC). Preliminary data on changes in neural tube defect prevalence rates after folic acid fortification in South America. Am J Med Genet A. 2003;123A(2):123–128.
19. Erickson JD. Folic acid and prevention of spina bifida and anencephaly. 10 years after the U.S. Public Health Service recommendation. MMWR Recomm Rep. 2002;51(RR-13):1–3.
20. Hertrampf E, Cortes F, Erickson JD, Cayazzo M, Freire W, Bailey LB. Consumption of folic acid-fortified bread improves folate status in women of reproductive age in Chile. J Nutr. 2003;133:3166–3169.
21. Sayed AR, Bourne D, Pattinson R, Nixon J, Henderson B. Decline in the prevalence of neural tube defects following folic acid fortification and its cost-benefit in South Africa. Birth Defects Res. 2008;82:211–216.
22. Oakley GP Jr. When will we eliminate folic acid-preventable spina bifida? Epidemiology. 2007;18:367–368.
23. Lawrence JM, Petitti DB, Watkins M, Umekubo MA. Trends in serum folate after food fortification. Lancet. 1999;354:915–916.
24. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. N Engl J Med. 1999;340:1449–1454.
25. Yang Q, Botto LD, Erickson JD, Berry RJ, Sambell C, Johansen H. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation. 2006;113:1335–1343.
26. Bell KN and Oakley GP Jr. Update on prevention of folic acid-preventable spina bifida and anencephaly. Birth Defects Research Part A. 2009;85:102–107.