Use of Multivitamins and Folic Acid in Early Pregnancy and Multiple Births in Sweden

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Folic acid is recommended to reduce the risk of neural tube defects and other congenital malformations. Data from the Swedish Medical Birth Registry were used to study frequency of twinning in women who in early pregnancy reported the use of folic acid. Women (n = 2,569) who in early pregnancy reported the use of folic acid had an increased rate of twin deliveries after consideration of maternal age and of length of involuntary childlessness, both variables being significant confounders. The effect of folic acid was seen also in women who did not report involuntary childlessness. A similar but not statistically significant trend was seen after use of multivitamins without simultaneous use of folic acid tablets (n = 1,979). The increased risk seems to be limited to dizygotic twinning (relative risk = 2.13, 95%CI 1.64–2.74). If this association is causal, wide-spread supplementation with folic acid may represent a hazard larger than the postulated beneficial effect on neural tube defects, at least in low-risk areas.

During the past decades, interest has been paid to vitamin deficiency as a cause of certain congenital malformations, notably neural tube defects, and to the possibility to overcome such deficiencies and reduce the risk of a congenital malformation with vitamin and especially folic acid supplementation. It has become generally accepted that folic acid supplementation before conception and into early pregnancy reduces the risk of a neural tube defect and also for many other congenital malformations, notably heart defects (Botto et al., 1996, 2000; Hall & Solehdin, 1998; Scanlon et al., 1998). Most of the evidence is based on interviews in case-control situations (Milunsky et al., 1989; Mulinare et al., 1988) and — for neural tube defects — on a small randomized study (Czeizel & Dudás, 1992) and on results of intervention programs, notably the recently published study from China (Berry et al., 1999). In other such studies, no effect of intervention could be seen (Kadir et al., 1999; Rosano et al., 1999).

Possible other effects of vitamin supplementation have received less notice. Already in the randomized study, mentioned above, an excess of multiple births was noted (Czeizel et al., 1994) and in a study based on five different US populations, an association between folic acid supplementation and multiple birth was found in four (Werler et al., 1997).

This paper presents a prospective study of the use of multivitamins and/or folic acid in early pregnancy and the occurrence of multiple births.

Material and Method

Two study designs were used.

Study 1. This study aimed at a detailed description of vitamin use. Questionnaires were given to women in early pregnancy in order to get information on vitamin usage before and during early pregnancy. This study was made in one region of Sweden (Skåne) in 1997 and comprised 2,022 women. The women were asked to specify what type of vitamin supplementation they had used in a form listing all available vitamin preparations and also if they had used the vitamins already before becoming pregnant.

Study 2. This study was specifically directed towards the question on vitamin use and multiple births, using data from the Swedish Medical Birth Registry (Cnattingius et al., 1990). This register covers the whole of Sweden and contains detailed information on prenatal care, delivery, and paediatric investigation of the newborn. Data are collected from copies of the original medical records which have the same structure in all of Sweden. At the first antenatal care visit (usually around week 10) of the pregnant woman (and nearly all pregnant women attend the free antenatal centres), an interview is performed by the attending midwife and answers are recorded on a standardized form. Among other things, the woman is asked about possible drugs she has used since she became pregnant and drug names are recorded and later computerized as ATC codes. Also information on maternal age and length of possible involuntary childlessness (in years) is recorded together with numerous other items. Delivery outcome is also recorded on a standardized form and so are the results of the paediatric examination of the newborn.

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Computerization of drug codes began in July 1994 and the first infants born with records containing information on maternal use of drugs were thus born during 1995. Women who reported the use of multivitamins but not of separate folic acid or had used folic acid (possibly together with multivitamins) were identified. Folic acid is mainly used in preparations of 0.4 mg — a few women used high dosage (5 mg) preparations. The ATC code cannot discriminate between multivitamin preparations with and without folic acid added. In the most popular brand each tablet contains 0.2 mg of folic acid (with B12) and the suggested dosage is one tablet a day.

Statistics. Presence of twin births in Study 2 was compared with that in all deliveries in Sweden 1995–1999 (n = 442,906). The odds ratio (OR) for a woman to have used multivitamins or folic acid was studied with consideration to two maternal characteristics of importance for twinning: age and period of involuntary childlessness. The risk of having a multiple birth delivery was estimated after stratification for the year of birth, maternal age (5-year classes), and period of involuntary childlessness (0–9+ years) as odds ratios (OR), determined with Mantel-Haenszel technique. Confidence intervals (95%) were estimated with a test-based method. No stratification for parity or maternal education was made because these variables had no significant impact on twinning rate.

In order to estimate the proportion of monozygotic and dizygotic twinning, the numbers of like-sexed and unlike-sexed twin pairs were determined and compared with the corresponding expected numbers, calculated from all births, stratifying for year of birth, maternal age, and period of involuntary childlessness. Observed and expected numbers were compared as a risk ratio (RR) and 95% confidence intervals were based on exact Poisson distributions. The number of monozygotic twin pairs was determined as the difference between the number of like-sexed and unlike-sexed pairs (according to Weinberg´s law).

Results

Study 1

Among women who, in the regional study, completed questionnaires on vitamin use prior to conception and in early pregnancy 643 reported any use of vitamins, that is, about 32%. Among these, 370 used vitamins before pregnancy (18%). Only about 8% then used a supplementation which contained folic acid or folic acid tablets. Among the latter, about 70% had started with the vitamin supplementation before conception. Among all 2,022 women, 73 had a preconceptional use of less than 0.4 mg/day (3.6%), 87 used at least 0.4 but not 4 mg/day (4.3%), and 5 used 4 mg or more a day (0.2%). Thus, 1,857 (92%) did not get any folic acid supplementation prior to conception.

Study 2

In the material from the Medical Birth Registry, 2,569 women reported the use of folic acid (0.6%) and 1,971 women reported the use of multivitamins but not of separate folic acid tablets (0.4%). Among the former, 72 twin births occurred (2.8%), among the latter 37 (1.9%). In the total population, the twinning rate was 1.5%.

Table 1 summarizes data from this study. Vitamin use increases with maternal age and so does twinning. Reporting of a period of involuntary childlessness was associated with a small but not statistically significant increase in the odds ratio for having used multivitamins and a marked increase in the odds ratio for having used folic acid. If the analysis is restricted to women reporting 5 years of unwanted childlessness or more, only slightly higher odds ratios were found.

Dizygotic twinning increases with maternal age and twin births are a common result of in vitro fertilization or ovulation stimulation at subfertility. Among the folic acid users, only 20 women reported the use of ovulation stiumlation (2 twin pairs). Therefore, the analysis of multiple births was made with stratification for year of birth, maternal age, and length of involuntary childlessness as recorded at the first antenatal visit.

Table 2 summarizes the findings on an association between vitamin use and twinning. Use of multivitamins (without extra folic acid use) did not significantly increase the twinning rate, but use of folic acid did. The same effects were seen when women did not report any subfertility problems.

Among the 72 twin deliveries after folic acid use, the sex of both infants was known in 69. Among them, 38 were like-sexed and 31 unlike-sexed. The expected numbers (stratified for year of birth, maternal age, and length of involuntary childlessness) was 27.9 and 14.5, respectively. The estimated observed number of monozygotic twin pairs is then 7 and the expected number 13.4, RR = 0.52 (95% confidence interval 0.21–1.08). Corresponding numbers

Table 1

Odds Ratio (OR) with 95% Confidence Interval (95%CI) for Vitamin Use and for Twinning According to Maternal Age and Length of Involuntary Childlessness

Maternal characteristic	Multivitamin use		Folic acid use		Twinning	
	OR	95%CI	OR	95%CI	OR	95%CI
<25 years age	0.89	0.79–1.00	0.67	0.50–0.75	0.58	0.53–0.63
35+ years age	1.13	1.00–1.19	1.26	1.14–1.39	2.05	1.85–2.28
Any involuntary childlessness	1.15	0.96–1.37	2.23	1.99–2.51	4.19	3.94-4.46
5+ years of involuntary childlessness	1.26	0.90–1.76	2.28	1.84–2.81	7.85	7.23–8.52

Note: Stratification for year of birth, maternal age (in all analyses except maternal age), length of involuntary childlessness (0–9+ years, in all analyses except involuntary childlessness). In the analysis of twinning, stratification was also made for maternal smoking in early pregnancy.

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Table 2

Odds Ratio (OR) with 95% Confidence Intervals (95%CI) for Twin Deliveries After the Use of Vitamins in Early Pregnancy

	All women		Women not reporting unwanted childlessness	
Vitamin use	OR	95%CI	OR	95%CI
Multivitamins	1.26	0.91–1.70	1.23	0.85–1.28
Folic acid	1.46	1.15–1.87	1.45	1.06–1.98

Note: Stratification for year of birth, maternal age, and length of involuntary childlessness (in the analysis of "all women").

for dizygotic twin pairs are 62 and 29.0, RR = 2.13 (95% confidence interval 1.64–2.74). The increase in twinning rate thus seems restricted to dizygotic twinning.

Discussion

The analysis of twin births after vitamin use was based on data obtained by routine prospective information on vitamin use, obtained by interview of the women in early pregnancy. There are some weaknesses in these data.

First of all, it is likely that some women who had used vitamins did not report it or the midwife did not record it because it was thought to be of little interest. This means that more women than appear from the data set have actually used vitamins. This also explains the higher rate of folic acid use in Study 1 (8%) than in Study 2 (0.6%). As recording was made early in pregnancy, the loss of data must be unrelated to pregnancy outcome. This will bias the estimated odds ratios towards 1.0 and the effects seen will be under-estimated.

Second, it is not known in Study 2 how many women used folic acid preconceptionally. From the detailed vitamin usage study, this seemed to be the case for about 70%. If vitamins should have an effect on multiple births, it is possible that they should be used before conception. If so, this error will also lead to an underestimation of effects.

Third, two possible confounders were identified as is apparent from Table 1. Vitamin use increases with maternal age and so does the risk of dizygotic twinning. This problem was overcome by stratification for maternal age (5-year classes). The truncation of maternal age may not completely remove the age effect but as it is not very strong for either variable, the procedure is probably sufficient.

The second confounder is subfertility and fertility treatments. Women with fertility problems may be more apt to use vitamins than other women, and folic acid supplementation is often given, notably at in vitro fertilization which like ovulation stimulation carries a marked increase in the risk of multiple births. A spurious association between folic acid use and multiple births could therefore be obtained. This problem was to some extent overcome by stratification for period of involuntary childlessness and by a separate analysis of women who did not report any involuntary childlessness. The latter group showed approximately the same OR as the total sample.

The information on unwanted childlessness is imperfect. It is recorded by the midwife in early pregnancy and is based on an interview with the pregnant woman. Checks of stated lengths of involuntary childlessness (Ghazi et al., 1991) showed a good validity, but absence of such information may be misleading. We know that about 40% of women who actually underwent IVF did not report a period of involuntary childlessness and the same appears to be true also for women who have had ovulation stimulation (unpublished data). In Sweden during the years of the study, less than 2% of all deliveries were the result of IVF (EpC Report, 2000). It can be estimated that the inclusion of women treated with IVF into the group reporting no involuntary childlessness will result in an OR of 1.2 for (mainly dizygotic) twinning and thus cannot explain the high ORs observed after folic acid.

A third possible confounder is maternal smoking. Only 3% of women reporting the use of folic acid were smokers in early pregnancy which is much less than in the general population these years (16%), probably mainly due to an association with subfertility. As maternal smoking in early pregnancy is associated with an increased rate of dizygotic twinning (Källén, 1998), a further stratification for maternal smoking would increase the association between folic acid use and twinning.

The increase in twinning rate after the use of multivitamins without simultaneous use of folic acid may be random or could be an expression of the same phenomenon seen after folic acid use — some but not all of the multivitamin preparations in Sweden contain folic acid and a popular bland supplies only 0.2 mg per day.

The increased risk of twinning after folic acid which supports observations published previously may still be due to unidentified confounding. As long as the analysis is not carried out in a large enough randomized study, analyzed after "intention to treat", the presence of undetected confounders can always explain a difference between groups. It is also possible, however, that causality exists. If so, the biological explanation is unclear. One possibility is that an increased level of folic acid reduces the spontaneous abortion rate, an effect which could be more marked for multiple pregnancies than for singleton pregnancies. One would then expect a similar increase in risk of monozygotic twinning and dizygotic twinning but this was not seen. On the other hand, prevention of the fetal death of one of dizygotic twins ("vanishing twin") could selectively increase dizygotic twinning. However, the majority of the "vanishing twins" seem to represent blighted ova (Robinson et al., 1977) and their survival to birth seems unlikely. A further possibility is that an increase in folic acid level could increase the probability for multiple ovulation or implantation of more than one egg.

If causality exists, a widespread supplementation with folic acid could significantly increase the twinning rate in the population which would represent a burden for neonatal care and an increase in the occurrence of infants with long term sequels. This possibility should be weighed against the possibility that folic acid supplementation would decrease the number of infants born with neural tube defects. If 30% of 100,000 women used folic acid supplementation, the number of twin births would increase with 15%, that is 225 extra twin pairs, which is equivalent to nearly half of the impact from IVF pregnancies (25% of

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2,000 = 500) — the latter are of major concern in society among other things because of the increased risk of cerebral palsy and other long-term effects. Against this hazard should be put the suggested 50% decrease of spina bifida rate - given the present Swedish rate of about 3 per 10,000 births, a 30% supplementation rate should perhaps result in a reduction with 4–5 infants with spina bifida per 100,000 births. These calculations invalidate — at least for a region with a low risk of neural tube defects — the conclusion previously drawn that an effect on multiple births should not interfere with programs for general folic acid supplementation (Werler et al., 1997).

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