Plasma concentration of folate as a biomarker for the intake of fruit and vegetables: the Hordaland Homocysteine Study[1–3]

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ABSTRACT

Background: Nutritional biomarkers may be used to assess dietary exposure without the errors commonly associated with self-reported dietary data.

Objective: The objective was to examine the association between plasma folate and intake of folate, fruit, and vegetables in a large cohort of healthy adults consuming foods that had not been fortified with folic acid.

Design: The present study population included 5533 middle-aged (47–49 y) and old (71–74 y) subjects from the Hordaland Homocysteine Study. The participants completed a food-frequency questionnaire and provided blood samples for chemical analyses.

Results: We observed a significant difference in plasma concentrations of folate across increasing quartiles of fruit, vegetable, and orange juice consumption. The difference in plasma folate between the highest and lowest quartiles was 1.97 (95% CI: 1.86, 2.07) nmol/L for fruit intake, 1.79 (95% CI: 1.69, 1.89) nmol/L for vegetable intake, and 2.69 (95% CI: 2.51, 2.87) nmol/L for orange juice intake. A significant inverse relation was observed across increasing quartiles of milk and bread intakes. The difference between the highest and lowest quartiles was −1.03 (95% CI: −1.13, −0.92) nmol/L for milk and −1.60 (95% CI: −1.69, −1.50) nmol/L for bread.

Conclusion: Plasma folate concentration may be a useful biomarker for the intake of fruit and vegetables in populations consuming unfortified food products. The association can be attenuated by and should be corrected for individual intake of folic acid supplements. *Am J Clin Nutr* 2005;81:434–9.

KEY WORDS Folate, Hordaland Homocysteine Study, biomarkers, fruit, vegetables

INTRODUCTION

Inaccurate methods of estimating food and nutrient intakes have restricted progress in research on dietary determinants of disease risk (1, 2). Traditionally, intake of fruit and vegetables has been estimated with the use of food-frequency questionnaires (FFQs), dietary records, and dietary recalls. Because of the errors associated with this type of dietary assessment, such methods have been validated by and complemented with measurement of biomarkers. Plasma carotenoids have been widely investigated as potential biomarkers for intake of fruit and vegetables (3–10). However, fruit and vegetables are also rich sources of folate, and plasma concentrations of folate may be another potential biomarker for the intake of these food items.

Total folate intake includes natural folates from foods together with synthetic folic acid from supplements and fortified food. Foods have generally not been enriched with folic acid in Norway because of strict public regulations. Consequently, most of the folate intake probably derives from natural food products. In a preliminary analysis of a nationwide survey among Norwegian adults (Norkost II; $n = 2672$), folate from fruit and vegetables accounted for 27% of the total folate intake, whereas bread and cereals accounted for 18% (EB Loeken, unpublished observations, 2004). In the present study, we examined the association between the intake of folate, fruit, and vegetables and the plasma folate concentration in a large cohort of middle-aged and old subjects.

SUBJECTS AND METHODS

Subjects

The Hordaland Health Study 1997–1999 was conducted from 1997 to 1999 as a collaboration between the National Health Screening Services, University of Bergen, and local health services. The present study population included a subsample of 1344 men and 1782 women aged 47–49 y and 1348 men and 1671 women aged 71–74 y who had participated in the Hordaland Homocysteine Study in 1992–1993 (11). The participation rate in the present subsample from the Hordaland Health Study 1997–1999 follow-up was 77%. All participants had a medical examination, donated a blood sample, and completed a food-frequency questionnaire (FFQ). The FFQs were distributed during the medical examination. Participants who did not return their questionnaires on time received one additional postal reminder.

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TABLE 1
Descriptive variables for the study population and its diet

	Middle-aged (47–49 y)		Old (71–74 y)		P^1		
	Men (n = 1216)	Women (n = 1622)	Men (n = 1211)	Women (n = 1487)	Sex effect	Age effect	Interaction
Multivitamin intake (%)	15	21	10	15	<0.001	<0.001	NS
Fish-oil intake (%)	38	33	40	33	<0.001	<0.355	NS
Smokers (%)	31	33	16	13	<0.697	<0.001	NS
BMI (kg/m^2)	26.1 ± 3.2[2]	24.9 ± 4.1	26.0 ± 3.2	26.2 ± 4.4	<0.001	<0.001	<0.01
Energy (MJ)	10.1 ± 2.2	7.6 ± 1.7	8.7 ± 2.0	6.6 ± 1.7	<0.001	<0.001	<0.01
Fat (% of energy)	32.1 ± 4.9	31.6 ± 5.1	31.1 ± 5.1	29.9 ± 5.6	<0.001	<0.001	<0.05
Protein (% of energy)	15.8 ± 2.2	16.4 ± 2.4	16.0 ± 2.3	16.4 ± 2.4	<0.001	<0.063	<0.05
Carbohydrates (% of energy)	49.6 ± 5.7	50.3 ± 5.9	50.8 ± 6.0	52.8 ± 6.2	<0.001	<0.001	<0.01
Sugar (% of energy)	7.1 ± 4.3	6.7 ± 4.4	7.0 ± 4.2	6.8 ± 4.2	<0.008	<0.299	NS
Alcohol (% of energy)	3.5 ± 2.6	1.6 ± 2.3	2.1 ± 3.5	0.8 ± 1.7	<0.001	<0.001	<0.01
Fiber (g/10 MJ)	27.5 ± 12.9	17.8 ± 8.5	22.8 ± 11.5	14.8 ± 7.9	<0.001	<0.001	NS
Folate intake (μg/d)[3]	240 ± 80	209 ± 69	213 ± 64	187 ± 65	<0.001	<0.001	NS
Plasma folate (nmol/L)	7.3 ± 3.9	8.5 ± 6.1	7.5 ± 5.0	9.6 ± 8.5	<0.001	<0.001	<0.01
Plasma homocysteine (μmol/L)	10.8 ± 3.5	9.1 ± 3.4	12.8 ± 4.0	11.4 ± 3.8	<0.001	<0.001	NS

[1] Two-factor ANOVA.
[2] $\bar{x}$ ± SD (all such values).
[3] Includes intake from supplements (minus the loss from cooking).

Participants with reported energy intakes below the 5th% percentile (3621 kJ for women; 5307 kJ for men) and above the 95th% percentile (11 266 kJ for women; 14 925 kJ for men), ie 372 men and 344 women, were excluded from further analyses, which limited the total number of eligible subjects to 5533.

The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate. All subjects gave their written consent to participate in the study.

Food-frequency questionnaire

In the present study, we used a 169-item FFQ, which is a slightly modified version of an FFQ previously described in detail (12, 13). This FFQ aimed to capture habitual diet. The FFQ includes frequency alternatives (from once a month to several times per day) and the number of units eaten and portion sizes (household units, eg, slices, glasses, cups, pieces, spoons, and deciliters). The FFQ included 27 questions about the intake of fruit and vegetables and 9 questions about the most commonly used brands of multivitamin supplements on the market. Subjects using multivitamin supplements were classified as folate supplement users.

Daily food and nutrient intakes were computed from the FFQ by using a food database and software system developed at the Department of Nutrition, University of Oslo (KBS, version 3.2). The food database is mainly based on the official food-composition table (14) but is supplemented with an update on folate content from 2001 (15). Losses during the household cooking of foods were subtracted. Estimated intake of folate includes folic acid from supplements.

Clinical examination

The examination included measurements of height, weight, and blood pressure. Blood was drawn from nonfasting subjects and was stored at −80 °C until biochemical analyses were performed within 12 mo.

Analytic procedures

Folate was determined by microbiological assay with the use of a chloramphenicol resistant strain of *Lactobacillus casei* (16). The folate assay was adopted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland). Plasma samples of folate were obtained for 99.2% of the participants. Plasma total homocysteine (tHcy) was determined by HPLC and fluorescence detection (17, 18). Plasma samples of tHcy were obtained for 92.5% of the participants. The precision (between-day CV) of the assay was <3%. The MTHFR 677C→T genotyping was performed by real-time polymerase chain reaction (19).

Statistical analyses

Differences between mean values of descriptive variables were tested by using a two-factor ANOVA. Differences between mean intakes of folate-containing foods were tested by using an independent-samples Student's t test.

Spearman's correlation coefficients were calculated between estimated dietary intake of various food groups and plasma concentration of folate. Intake of foods was categorized in quartiles. Multiple linear regression analysis was used to assess the simultaneous relation between various predictors of plasma folate concentration. Plasma folate concentration was the dependent variable, whereas the independent variables were represented in the model with indicator variables for each of the 3 nonreference quartiles. Thus, the regression coefficient was used to estimate the difference in mean plasma folate concentration between the reference and each quartile of the individual variables. Plasma folate concentration across quartiles was tested for homogeneity of means and for linear trend. All calculations were performed by using SPSS 10.0 (SPSS Inc, Chicago).

RESULTS

Characteristics of the study population and their dietary intake are listed in **Table 1**. The 71–74-y-olds had significantly lower

TABLE 2
Relative contribution of different food groups to total folate intake

	Men (n = 2695)		Women (n = 3450)		
	Food intake	Folate intake[1]	Food intake	Folate intake[1]	P[2]
	g/d	*μg/d (% of total)*	*g/d*	*μg/d (% of total)*	
Vegetables	191 ± 15[3]	47 (19)	202 ± 149	50 (22)	<0.002
Bread	206 ± 84	51 (20)	151 ± 59	37 (16)	<0.001
Fruit[4]	193 ± 177	23 (9)	191 ± 181	25 (11)	<0.243
Potato	147 ± 79	31 (12)	98 ± 61	20 (9)	<0.001
Dairy products	358 ± 255	21 (8)	276 ± 200	18 (8)	<0.001
Supplements[5]	1 ± <1	11 (4)	1 ± <1	17 (8)	<0.001
Orange juice	46 ± 81	11 (4)	52 ± 94	12 (5)	<0.018
Meat	117 ± 66	13 (5)	84 ± 52	9 (4)	<0.001
Egg	18 ± 12	11 (4)	15 ± 10	9 (4)	<0.001
Fish and seafood	101 ± 65	9 (4)	73 ± 52	7 (3)	<0.001
Cheese	30 ± 29	7 (3)	30 ± 26	7 (3)	<0.426
Cereals[6]	40 ± 36	8 (3)	30 ± 28	6 (3)	<0.001
Cake	30 ± 33	6 (2)	23 ± 26	5 (2)	<0.001
Liver pâté	4 ± 7	6 (2)	3 ± 6	4 (2)	<0.001
Total	1528 ± 525	253 (100)	1281 ± 490	226 (100)	<0.001
FV[7]	430 ± 307	81 (32)	446 ± 325	87 (38)	<0.003

[1] Because of limitations in our food calculation system, SDs could not be calculated for folate intake.
[2] Independent-samples t test comparing the daily intake of food of men and women.
[3] $\bar{x}$ ± SD (all such values).
[4] Includes berries and conserved fruit.
[5] Values are given as the number of units/d.
[6] Dry weight.
[7] Sum of fruit, berries, vegetables, and orange juice.

energy intakes than did the 47–49-y-olds, and, within both age groups, women had significantly lower energy intakes than did men. Within the same age group, men had a significantly lower plasma concentration of folate than did women and a higher plasma concentration of homocysteine. Total folate intake from food was significantly higher among men than women, whereas the use of supplements was higher among women (Table 1). For men and women in both age groups, bread, fruit, and vegetables represented the most important sources of folate intake, followed by potatoes and dairy products (**Table 2**). Folate intake from supplements and orange juice represented 4–8% each of the mean estimated total intake of folate among men and women. The recommended Norwegian daily intake of folate is 300 μg (400 μg for pregnant women), and only 10% of the study population met this recommendation.

Spearman's correlation coefficients between plasma concentration of folate and several groups of food categorized by supplement use are shown in **Table 3**. The highest correlations were observed between plasma concentrations of folate and total intakes of vegetables, fruit, and orange juice. This finding was observed in both age groups and both sexes (data not shown). Significant negative correlations were found between plasma folate concentration and the intake of milk and bread in both groups. On the basis of food tables, the plasma concentration of folate was more strongly associated with the intake of vegetables, fruit, and orange juice than with estimated total folate intake. Moreover, the positive correlation between plasma folate and intake of food folate was only significant for nonsupplement users.

The increase in plasma concentration of folate categorized by quartiles of foods consumed is shown in **Table 4**. Data from both age groups and both sexes are presented together, because the observed trends were present among men as well as women and within both age groups. There was a significantly increased plasma concentration of folate with increasing intake of vegetables, fruit, and orange juice. A decrease in plasma folate concentration was observed with increasing intake of the other food items presented in Table 4. Adjustment for energy intake did not change the associations (data not shown).

TABLE 3
Spearman's correlation coefficients between plasma folate concentration and intake of various foods items and the estimated intake of folate

	Non-folate-supplement users (n = 4629)	Folate-supplement users (n = 861)
Total food folate intake[1]	0.13[2]	0.06
Plasma homocysteine	−0.37[2]	−0.35[2]
Vegetables	0.18[2]	0.11[2]
Fruit[3]	0.16[2]	0.13[2]
Orange juice	0.15[2]	0.17[2]
FV[4]	0.22[2]	0.18[2]
Bread	−0.10[2]	−0.15[2]
Milk[5]	−0.09[2]	−0.08[6]
Potato	−0.05[2]	−0.06

[1] Estimated from the Norwegian food-composition table (15).
[2] $P < 0.01$ (independent-samples t test).
[3] Includes berries and conserved fruit.
[4] Sum of fruit, berries, vegetables, and orange juice.
[5] Includes milk, sour cream, yogurt, and ice cream.
[6] $P < 0.05$ (independent-samples t test).

TABLE 4
Estimated change in plasma folate concentration by intake of various folate-containing foods, in quartiles

	No. of subjects	Plasma concentration					
		Adjusted for age and sex			Multiple adjustments[1]		
		$\bar{x}$	(95% CI)	P^2	$\bar{x}$	(95% CI)	P^2
		nmol/L	*nmol/L*		*nmol/L*	*nmol/L*	
Sex							
Male	3109						
Female	2424	1.63	(1.61, 1.66)	<0.001	1.63	(1.57, 1.70)	<0.001
Age							
Young	2698						
Old	2835	0.75	(0.71, 0.80)	<0.001	0.76	(0.68, 0.83)	<0.001
Smoking							
No	4228						
Yes	1305	−1.25	(−1.30, −1.20)	<0.001	−1.25	(−1.33, −1.16)	<0.001
Supplements							
No	4666						
Yes	867	2.90	(2.83, 2.96)	<0.001	2.89	(2.82, 2.96)	<0.001
Intake[3]							
Folate (μg/d)[4]							
30–161 (133)	1368						
162–200 (181)	1391	0.26	(0.19, 0.34)		0.28	(0.18, 0.39)	
201–246 (222)	1397	0.72	(0.64, 0.79)		0.73	(0.63, 0.84)	
247–1169 (305)	1377	1.11	(1.04, 1.19)	<0.001	1.11	(1.00, 1.22)	<0.001
Vegetables (g/d)							
0–104 (66)	1384						
105–166 (134)	1381	0.59	(0.52, 0.66)		0.61	(0.51, 0.71)	
166–251 (205)	1384	1.18	(1.11, 1.25)		1.26	(1.16, 1.36)	
251–1858 (380)	1384	1.81	(1.75, 1.88)	<0.001	1.79	(1.69, 1.89)	<0.001
Fruit (g/d)[5]							
0–128 (79)	1381						
129–207 (167)	1386	0.66	(0.59, 0.72)		0.66	(0.56, 0.76)	
208–315 (256)	1384	1.32	(1.26, 1.39)		1.35	(1.25, 1.45)	
316–2202 (462)	1382	1.98	(1.91, 2.05)	<0.001	1.97	(1.86, 2.07)	<0.001
Potato (g/d)							
0–68 (43)	1381						
68–116 (92)	1385	−0.38	(−0.45, −0.32)		−0.41	(−0.51, −0.30)	
116–142 (133)	1384	−0.37	(−0.43, −0.30)		−0.37	(−0.47, −0.26)	
142–557 (211)	1383	−0.95	(−1.01, −0.89)	<0.001	−0.95	(−1.06, −0.85)	<0.001
Orange juice (g/d)[6]							
0 (0)	2159						
1–96 (40)	2470	0.80	(0.75, 0.86)		0.87	(0.79, 0.95)	
108–192 (140)	674	1.97	(1.89, 2.05)		1.98	(1.86, 2.10)	
216–1500 (345)	230	2.78	(2.65, 2.90)	<0.001	2.69	(2.51, 2.87)	<0.001
FVJ (g/d)[7]							
0–286 (194)	1382						
287–432 (358)	1385	0.84	(0.77, 0.91)		0.87	(0.77, 0.96)	
433–626 (520)	1382	1.74	(1.68, 1.81)		1.79	(1.69, 1.89)	
627–3431 (874)	1384	2.54	(2.48, 2.61)	<0.001	2.53	(2.43, 2.63)	<0.001
Bread (g/d)							
0–125 (96)	1382						
125–164 (148)	1379	−0.43	(−0.48, −0.37)		−0.39	(−0.49, −0.29)	
164–212 (187)	1382	−0.87	(−0.92, −0.81)		−0.85	(−0.95, −0.76)	
212–605 (270)	1390	−1.60	(−1.66, −1.55)	<0.001	−1.60	(−1.69, −1.50)	<0.001
Milk (g/d)[8]							
0–153 (71)	1382						
153–278 (209)	1383	−0.21	(−0.27, −0.15)		−0.13	(−0.23, −0.03)	
279–431 (351)	1385	−0.51	(−0.58, −0.45)		−0.46	(−0.57, −0.36)	
432–1670 (602)	1383	−1.04	(−1.10, −0.97)	<0.001	−1.03	(−1.13, −0.92)	<0.001

[1] Adjusted for age, sex, smoking, and use of supplements.
[2] Calculated from a linear regression analysis using indicator variables for the quartiles as independent variable.
[3] Values in parentheses are mean intakes.
[4] Estimated intake from the Norwegian food-composition table (15).
[5] Include berries and conserved fruit and berries.
[6] Groups do not represent quartiles but rather typical intakes.
[7] Fruit, berries, vegetables, and orange juice.
[8] Includes yogurt, cream, sour cream, and ice cream.

DISCUSSION

We observed a positive and significant association between plasma folate concentration and the dietary intake of vegetables, fruit, and orange juice among folate-supplement users as well as nonsupplement users. These associations were consistent among men and women in both age groups. The folate intake of the study population was markedly lower than the recommended daily intake and may be related to high incidence of cardiovascular disease as well as intestinal cancers in Norway.

The association between plasma concentration of folate and estimated total intake of food folates was weaker than the association between plasma folate and the intake of fruit, vegetables, and orange juice. This may indicate an over- or underreporting of important folate-containing foods by the FFQ, inadequate folate values in food tables, different bioavailability of folate from different food products, or perhaps a combination of these conditions.

Although highly significant, the observed correlation between plasma concentration of folate and the intake of fruit and vegetables was moderate. However, our correlations are in the same range as reported for the association between dietary intake of fruit and vegetables and vitamin C and carotenoids (8, 20–22).

Folate sources are not restricted to fruit and vegetables, and folate may be more bioavailable when given as a folic acid supplement (23–26). Among the middle-aged subjects, 15% of the men and 21% of the women reported the use of multivitamin supplements. Among the older subjects the corresponding prevalences were 10% and 15%, respectively. Because of the large variety of supplements and possible confusion about brands and vitamin contents, we cannot rule out the possibility that the self-reported use of folate supplements may have been underestimated in this study, although supplement users had a markedly higher plasma concentration of folate than did the nonsupplement users (Table 4). Thus, the use of folate as a biomarker for the intake of folate-rich food items requires that the use of supplements be accurately assessed and corrected for.

The difference in plasma folate concentration between the lowest and the highest quartiles of total intake of fruit, vegetable, and juice was 37%. Several studies have investigated how dietary interventions may increase folate concentrations in various fractions of the blood. In a controlled crossover feeding study, Broekmans et al (4) showed that plasma folate concentration could be increased 15% by increasing the intake of fruit and vegetables from 100 to 500 g. In another controlled crossover feeding study, a 13% increase in red blood cell folate was achieved by increasing the intake of fruit and vegetables from 1 to 7 servings (27). By feeding healthy subjects 300 g chopped spinach for 4 d, van het Hof et al (28) observed a 23% increase in plasma folate concentration. In addition, Appel et al (29) reported a 27% higher serum concentration of folate in an intervention group consuming 10 servings compared with a control group consuming 4 servings of fruit and vegetables.

In our study, orange juice was an important determinant of plasma folate concentrations (Table 4). Correlations for orange juice were slightly stronger in the older age group (data not shown). Rhode et al (30) previously reported that folate from orange juice was just as bioavailable as was folic acid. It has been suggested that an assortment of fruit and vegetables will provide dietary folate with 60–90% bioavailability relative to folic acid (23). Conversely, in an in vitro model system, Wei and Gregory (31) found that polyglutamate hydrolase, an enzyme required for folate polyglutamate deconjugation before absorption, was inhibited by compounds in orange juice, which suggests an impaired folate absorption of folate from orange juice. Nevertheless, in assessing total folate intake in US citizens before the folic acid fortification of flour, orange juice was found to be one of the most important folate sources (32), although this study did not take folate bioavailability into account. It was shown previously that the polyglutamate chain of folates in the diet reduces their bioavailability (33), but there is insufficient knowledge on the relative content of the different forms of folate in various food items. The bioavailability and bioefficacy of folate is strongly influenced by cooking procedures that can damage the vitamin (34). Compared with other folate sources fruit, orange juice, and many vegetables do not require prolonged heat treatment to be palatable and may thus be subject to less degradation.

As shown in Table 2, bread is quantitatively one of the most important food contributors to total folate intake (Table 2). Yet a surprising inverse trend was observed between the intake of bread and plasma folate concentration in the present study. In the diet of middle-aged and senior citizens, bread may replace other folate-containing foods, such as fruit and vegetables that would otherwise have provided more bioavailable folate (23). The intake of bread was negatively correlated with the intake of vegetables among the middle-aged participants ($r = -0.085$, $P < 0.001$), but this association was not present among the oldest participants. Nevertheless, the inverse trend between the intake of bread and plasma folate concentration was present in both age groups. Some studies have observed overreporting of bread intake on FFQs (35, 36). To a certain extent, overreporting of bread intake could have attenuated the association observed between the intake of bread and plasma folate concentration in the present study, but it seems unlikely that the inverse association could have been caused exclusively by overreporting of bread intake.

We also observed an inverse association between plasma folate and the intake of milk. The inverse association with milk, together with the positive association with orange juice, may indicate that preferences with respect to beverages are an important determinant of plasma folate in populations with low folate intake. Furthermore, there was a moderate inverse association between plasma folate and the intake of potatoes. This may suggest that potatoes replaced vegetables in the diet of the present population. Fruit and vegetables contain more folate per calorie than do potatoes (15). However, adjustment for the intake of fruit, vegetables, and orange juice in the regression model did not remove the significant inverse associations between plasma concentration of folate and bread, potato, or milk (data not shown).

In contrast with red blood cell folate concentration, which is a good measure of long-term folate status, plasma folate concentration probably reflects more recent folate uptake (24). Because food folate accumulates in red blood cells (37), it is possible that the associations we observed for plasma folate may be even stronger for red blood cell folate.

Folate has a key role in homocysteine metabolism, and high plasma homocysteine concentrations can be lowered by folic acid supplementation (38) or food folates (39). Regressions of past diet on homocysteine concentrations did show opposite but slightly weaker associations than those for folate (data not shown). Folate intake is an important predictor of plasma homocysteine, but elevated homocysteine concentrations can also be the result of low intakes of vitamin B-12, poor kidney function,

smoking, coffee consumption, high body weight, or mutations in the folate-metabolizing enzymes (33, 40).

The fact that folate is present in several types of food products and that most subjects eat a large variety of food items makes it unlikely that strong correlations will be found between plasma folate and single food items. Nevertheless, our data show that in a population consuming a nonfortified diet, plasma folate concentration was positively associated with the intake of fruit, vegetables, and orange juice.

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LFA, AB, and CAD contributed to the conception of the study. CAD developed the FFQ. AB prepared the first version of the manuscript. SEV, GST, HR, and PMU were responsible for the study design and data collection in the Hordaland Homocysteine Study. EBL assisted in estimating folate intake. All authors contributed to writing of the paper. None of the authors have any financial conflicts related to this work.

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