Folate is absorbed across the colon of adults: evidence from cecal infusion of $^{13}$C-labeled [6S]-5-formyltetrahydrofolic acid$^{1-3}$

Susanne Aufreiter, Jesse F Gregory III, Christine M Pfeiffer, Zia Fazili, Young-In Kim, Norman Marcon, Patarapong Kamalaporn, Paul B Pencharz, and Deborah L O’Connor

ABSTRACT

Background: Folate deficiency increases the risk of several human diseases. Likewise, high intakes of folate, particularly synthetic folic acid intake, may be associated with adverse health outcomes in humans. A more comprehensive understanding of the “input side” of folate nutrition may help to set dietary recommendations that strike the right balance between health benefits and risks. It is well known that the microflora in the colon produce large quantities of folate that approach or exceed recommended dietary intakes; however, there is no direct evidence of the bioavailability of this pool in humans.

Objective: The objective was to determine whether, and to what extent, the natural folate vitamer 5-formyltetrahydrofolic acid is absorbed across the intact colon of humans.

Design: During screening colonoscopy, 684 nmol (320 μg) $[^{13}$C]glutamyl-5-formyltetrahydrofolic acid was infused directly into the cecum of 6 healthy adults. Three or more weeks later, each subject received an intravenous injection of the same compound (172 nmol). Blood samples were collected before and after each treatment. The ratio of labeled to unlabeled folates was determined in plasma by tandem mass spectrometry.

Results: The apparent rate of folate absorption across the colon of a bolus dose of [13C]5-formyltetrahydrofolic acid infused into the cecum was 0.6 ± 0.2 nmol/h, as determined by the appearance of $[^{13}$C]5-methyltetrahydrofolic acid in plasma. In comparison, the rate of appearance of $[^{13}$C]5-methyltetrahydrofolic acid after an intravenous injection of $[^{13}$C]5-formyltetrahydrofolate was 7 ± 1.2 nmol/h.

Conclusion: Physiologic doses of natural folate are absorbed across the intact colon in humans.

INTRODUCTION

Folate deficiency has been implicated in numerous negative health outcomes, including neural tube defects (NTDs) and other congenital defects, vascular disease, neuropsychiatric disorders, and cancer (1). The case for folate in reducing the risk of NTDs led to mandatory folic acid fortification of the North American food supply in 1998. A subsequent decrease in the incidence of NTDs was observed (2, 3). Researchers have called for the level of folate fortification to be increased to prevent a further 25% of NTDs suspected to be folate related (4, 5). However, concern remains that high folic acid intakes could delay diagnosis of vitamin B-12 deficiency and lead to the onset and progression of potentially irreversible neurologic damage (1, 6, 7). In addition, many other health risks associated with folic acid fortification and supplementation have been reported (6). For example, it has been suggested that high folic acid intakes are associated with decreased natural killer cell toxicity, a reduction in the effectiveness of antifolate drugs used against malaria, rheumatoid arthritis, psoriasis, and cancer and in pregnant women, an increased incidence of obesity and insulin resistance in their offspring (6, 8–13). High folic acid intakes have also been shown to facilitate the progression of preneoplastic cells to cancer in animals (14). These observations are supported by results from human intervention and epidemiologic studies of folate and colorectal cancer (CRC) (14–16).

To date, calculation of folate intake in the examination of the relation between exogenous folate supply and health outcomes has been based solely on oral intake (1). Another potential source of folate is the depot synthesized by microflora residing in the colon. It is known that the intestinal microflora produce large quantities of folate that approach or exceed dietary intakes of the vitamin (17–19). Recently, we showed that the forms of folate synthesized are available for absorption, at least if present in the small intestine (19). If a significant proportion of folate from the colon can be absorbed, it should be considered when examining the relation between folate supply and health, particularly given its proximity to the colonocytes and our understanding that both low and high levels of exogenous folate may influence the development and progression of CRC (6, 15, 16). Data from ani-

1 From the Departments of Nutritional Sciences (SA, Y-IK, DLO, and PBP), Medicine (Y-IK and NM), and Paediatrics (PBP), University of Toronto, Toronto, Canada; the Research Institute, The Hospital for Sick Children, Toronto, Canada (SA, DLO, and PBP); the Division of Gastroenterology, St Michael’s Hospital, Toronto, Canada (Y-IK, NM, and PK); the Food Science and Human Nutrition Department, University of Florida, Gainesville, FL (JFG); and the Centers for Disease Control and Prevention, Atlanta, GA (CMP and ZF).

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3 Address correspondence to DL O’Connor, Department of Nutritional Sciences, University of Toronto, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8. E-mail: deborah_l.o’connor@sickkids.ca.

mal studies suggest a relation between intestinal folate bio-
synthesis and folate status (20–24). However, in many studies,
coprophy could have facilitated absorption of microbially syn-
thesized folates across the small intestine, despite efforts to prevent
it. Whereas in vitro data are suggestive, there is no direct evidence
to support that folate is absorbed across the intact human colon
(25–27). The objectives of this study, then, were to determine
whether, and to what extent folate is absorbed across the colon
of humans. A physiologic dose of $[^{13}C]$-5-formyltetrahydrofolic
acid was infused into the cecum of humans undergoing colo-
noscopy. Here we report results of plasma analyses of $^{13}C$-
labeled and unlabeled folates.

SUBJECTS AND METHODS

Study population and recruitment

Healthy men and nonpregnant women with normal blood
chemistry and folate status were recruited between February and
May 2007 from patients waiting to undergo screening colo-
noscopies at St Michael’s Hospital, Toronto, Canada. Folate
status was assessed by measuring red blood cell (RBC) folate
concentrations by microbial assay (28). Blood chemistry in-
cluded complete blood counts and measurement of serum
electrolytes, glucose, renal function, and liver enzymes. In-
dividuals who consumed >1 alcoholic drink/day, had a known
sensitivity to leucovorin (5-formyltetrahydrofolic acid), or had
documented or suspected gastrointestinal disease that could in-
terefere with folate absorption and metabolism (eg, celiac disease
and inflammatory bowel disease) were excluded. Additional
exclusion criteria were pregnancy, use of oral contraceptives,
and medications known to affect folate metabolism (eg, dilantin,
phenytoin, primidone, metformin, sulfasalazine, triamterene,
or methotrexate). All subjects were screened for the 5,10-
methylenetetrahydrofolate reductase (MTHFR) 677C
or methotrexate). All subjects were screened for the 5,10-
acid was infused into the cecum of humans undergoing colo-
noscopy (25–27). The objectives of this study, then, were to determine
whether, and to what extent folate is absorbed across the colon
of humans. A physiologic dose of $[^{13}C]$-5-formyltetrahydrofolic
acid was infused into the cecum of humans undergoing colo-
noscopy. Here we report results of plasma analyses of $^{13}C$-
labeled and unlabeled folates.

Study protocol

An indwelling catheter was inserted in one arm of each subject
to collect a baseline fasting blood sample (5 mL) shortly before
colonoscopy and for blood sampling after cecal infusion. After
confirmation via colonoscopy that the colon was free of any lesions,
including polyps proximal to the rectum (polyps in the rectum were
allowed), 684 nmol (320 µg) $[^{13}C]$-5-formyltetrahydrofolic acid
in 100 mL physiologic saline was delivered to the cecum of each
subject via an irrigation catheter in the biopsy channel of the
colonoscope (CF 160L; Olympus America Corporation, Center
Valley, PA). The ileocecal valve of each subject was intubated
before cecal infusion of the labeled folate to rule out subclinical
Crohn disease or other mucosal diseases involving the terminal
ileum. After it was confirmed that the ileocecal valve was normal
and was competent, we infused the labeled folate in the
cecum via spray catheter at the opposite side of the ileocecal
valve and kept the ileocecal valve in the superior position (ie,
11–12 o’clock position) to prevent reflux of the test dose into the
terminal ileum. The translocation of the infused labeled folate in
the cecum was observed for ≥5 min, and no obvious reflux of the
cecal content into the terminal ileum was noted. After cecal
infusion, blood (5 mL) was taken at 30-min intervals for 4 h.

Three or more weeks after colonoscopy in the Clinical In-
vestigation Unit at The Hospital for Sick Children, fasting blood
samples were collected from each subject and an intravenous
injection of 172 nmol (80 µg) $[^{13}C]$-5-formyltetrahydrofolic acid,
dissolved in 1 mL sterile saline, was administered. After in-
jection, blood samples (5 mL) were collected from each subject
at 30-min intervals for 4 h via an indwelling catheter inserted in
the arm not used for injection of the test dose. For 4 h after both
the cecal infusion and the IV injection, subjects were provided
with beverages and snacks that we confirmed to be low in folate
content by direct analyses in our laboratory.

Blood samples throughout the study were collected into EDTA-
treated tubes and processed within 2 h of collection. For mea-
 surement of RBC folate (baseline only), 100-µL aliquots of whole
blood were diluted 10-fold with ascorbic acid and deionized water
(1% wt:vol) and incubated at 37°C for 30 min to convert folates
into their microbiologically assayable form. Plasma at all col-
lection times was separated from whole blood by centrifugation
(1500 × g for 20 min at 4°C) and portioned for future folate
analyses with added sodium ascorbate (1% wt:vol) to prevent the
oxidation of folate. All samples were frozen on dry ice immedi-
ately after processing and stored at −80°C.

Biochemical and mass spectrometry analyses

The total folate content of plasma samples was determined by
the standard microbial assay according to the method of Molloy
and Scott by using the test organism Lactobacillus rhamnosus
(ATCC7664; American Type Tissue Culture Collection, Mana-
sas, VA) (28). The folate content of foods consumed during the
4-h after cecal infusion and IV injection was determined by microbiological assay after tri-enzyme treatment of samples as described by Hyun and Tamura (30). The accuracy and reproducibility of these assays were assessed by using a whole-blood control standard with a certified value (29.5 nmol/L; whole blood 95/528; National Institute of Biological Standards and Control, Hertfordshire, United Kingdom). Analysis of the whole-blood control standard in our laboratory yielded a folate content of 31.7 ± 1.0 nmol/L, with an interassay CV of 4.6%. RBC folate was calculated by subtracting plasma total folate from whole-blood folate with correction for RBC volume.

Plasma enrichment of the infused $[^{13}\text{C}]$5-formyltetrahydrofolic acid and its metabolite $[^{13}\text{C}]$5-methyltetrahydrofolic acid was determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) at the Centers for Disease Control and Prevention (Atlanta, GA) as previously described (31). Before LC-MS/MS analysis, folates were extracted from plasma (275 μL) that was diluted with formate buffer (825 μL, pH 3.2) and ascobic acid (1 g/L) by using phenyl solid phase extraction (SPE) cartridges. Extracts (20 μL) were then loaded onto a Luna C-8 analytic column for chromatographic separation by using an isocratic mobile phase. Mass-to-charge ratios of transitions of interest [(M+0) and (M+5)] were monitored in positive ion mode via turbo ion electro spray on a Sciex API 4000 triple quadrupole MS system (Applied Biosystems).

To verify that area ratios were reproducible and matched theoretical molar ratios, we prepared standard curves at 3 folate concentrations (20, 60, and 100 nmol/L) with various proportions of $[^{13}\text{C}]$-labeled and -unlabeled 5-formyl- and 5-methyltetrahydrofolic acid (0–50% label; standard compounds obtained from Merck Eprova AG). The standard mixtures were prepared over multiple days and analyzed either directly by LC-MS/MS or after carrying them through SPE cartridges. Because the $[^{13}\text{C}]$5-formyltetrahydrofolic acid used in this study contained a small portion of the (M+4) label (9% of the (M+5) label), we also monitored this transition to ensure that we were capturing the entire signal produced by this compound. As expected, measured area ratios (expressed as the sum of [M+0] and [M+5] over [M+0]) were very reproducible from day-to-day (2–4% CV), were not affected by the SPE cartridges, and matched theoretical molar ratios within ±5%.

After these initial experiments, we only used areas from the (M+5) channel for 5-formyl- and 5-methyltetrahydrofolic acid to calculate area ratios ($[M+5]/[M+0]$) because there is no metabolic conversion from (M+5) to (M+4), and (M+4) areas were typically small and might therefore increase imprecision. No correction for natural abundance of isotopes was necessary because the contribution at (M+5) due to the presence of $^{13}\text{C}$, $^{15}\text{N}$, and $^{17}\text{O}$ in the unlabeled species is negligible. The measured area ratio is therefore equivalent to the molar ratio. When the area ratio is calculated as labeled divided by total folate ($[M+5]$ divided by sum of [M+5] and [M+0]), it is equivalent to the enrichment level. Plasma samples collected directly after IV injection showed >30% enrichment for $[^{13}\text{C}]$5-formyltetrahydrofolic acid. Samples collected after cecal infusions showed <30% enrichment.

Quantification of the plasma folate response

In addition to reporting our data as molar ratios of 5-formyl- and 5-methyltetrahydrofolic acid, we chose to quantify the plasma folate response and pharmacokinetic data to produce the lexicon most familiar to readers without a background in stable isotopes, ie, sum of peak areas and nmol folate/person. This was done with a number of important caveats. Most importantly, to calculate the sum of all peak areas, we added the peak areas for labeled $(M+5)$ and unlabeled $(M+0)$ 5-formyl- and 5-methyltetrahydrofolic acid. This required adjustment of the peak areas for 5-formyltetrahydrofolic acid (divided by 2.3) to account for the differences in the LC-MS/MS signal between 5-formyltetrahydrofolic acid and 5-methyltetrahydrofolic acid. Second, to quantify the total amount (nmol/L) of labeled $(M+5)$ 5-formyltetrahydrofolic acid or 5-methyltetrahydrofolic acid, we took the peak area for each labeled metabolite and converted it to nmol folate/L plasma using the total plasma folate concentration determined by microbial assay for each subject (ie, either cecal infusion or IV injection) as shown in the following equation:

$$X = \frac{(M+5) \text{ peak area} \times \text{total plasma folate determined by microbial assay (nmol/L)}}{(M+5) \text{ peak areas}_{(M+5)} + (M+0) \text{ peak areas}_{(M+0)}}$$

where $X$ is the concentration (nmol/L) of labeled 5-formyltetrahydrofolic acid or 5-methyltetrahydrofolic acid.

The lowest plasma folate concentration determined by microbial assay for each subject and treatment was used, rather than simply the baseline concentration, to avoid the distortion in blood folate content induced by interruption of bile flow during fasting (32). Finally, to express our data on a whole-body basis (ie, convert from nmol/L to nmol/person), we determined the total plasma volume of each subject. Blood volumes were estimated by using the values 75 mL/kg for males and 66.5 mL/kg for females of normal weight (33). Plasma volumes were then calculated from the estimated blood volume by correcting for RBC volume (hematocrit). To account for the different hydration of lean and fat mass, we adjusted the blood volume for each individual using the relation of blood volume and deviation from ideal weight described by Feldschuh and Enson (34).

**Statistical analysis**

SAS for Windows (version 9.1; SAS Institute Inc, Cary, NC) was used to generate descriptive statistics (ie, mean, SEM). Changes in the total plasma folate concentration determined by microbial assay or molar ratios of either 5-formyltetrahydrofolic acid or 5-methyltetrahydrofolic acid were determined by repeated-measures ANOVA (PROC MIXED) using sample as the main effect and quadratic sample or cubic sample as necessary. These analyses included baseline RBC folate concentration in the statistical model. Given the number of assumptions that were made in producing the sum of peak areas and nmol folate/person from the LC-MS/MS results, we did not perform statistical analyses on these data. The apparent plasma half-life (one-phase exponential decrease over time) of $[^{13}\text{C}]$5-formyltetrahydrofolic acid after IV injection for each subject was determined by using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) by using the slope of the descending portion of each plasma response curve. The rate of appearance of $[^{13}\text{C}]$5-methyltetrahydrofolic acid in plasma over time, after cecal infusion, was determined from the linear slope of the ascending portion of each plasma response curve.
RESULTS

Subject characteristics

Subject characteristics are summarized in Table 1. Ten subjects were recruited from colonoscopy screening patients at St Michael’s Hospital. Two were excluded because they were homozygous for the MTHFR 677C→T variant, and 2 were excluded because of the detection of polyps proximal to the rectum (both cecal polyps) at the time of colonoscopy. All 6 remaining participants were white and 50–63 y of age. BMI folate concentrations of the subjects varied widely but were well above a cutoff of 360 nmol/L associated with tissue folate depletion (35). Three of the 6 participants were heterozygous for the MTHFR 677C→T polymorphism. One subject had suboptimal vitamin B-12 status (127 pmol/L; normal: ≥150 pmol/L) (36). Three of the subjects were obese as defined by a body mass index (BMI; in kg/m²) >30. Two participants regularly consumed folic acid-containing vitamin supplements before enrollment; the first and second discontinued supplementation ≥5 and 2 wk, respectively, before the study intervention. Rectal polyps in 3 subjects were excised in a standard fashion (one microadenoma, one adenoma, and one hyperplastic polyp) and, as allowed for in the inclusion criteria, these individuals were included in the study. Mean (±SD) dietary intakes of folate during the 4-h blood collection period after IV injection and cecal infusion were 14.1±3.7 and 14.8±4.0 μg, respectively. All participants completed both clinic visits with complete blood sample collection.

Plasma folate response

The plasma folate responses to IV injection and cecal infusion of [13C5]5-formyltetrahydrofolic acid, as determined by microbial assay and LC-MS/MS, are shown in Figure 1. The change in the plasma folate concentration over time, as determined by microbial assay after IV injection of the test compound, was statistically significant (P = 0.0005). As described in the Statistical Analysis section, where LC-MS/MS data were converted to the sum of peak areas or nmol, we did not perform statistical analyses. Nonetheless, the pattern for total folate (sum of peak areas) calculated by adding both labeled (M+5) and unlabeled (M+0) peak areas for 5-formyl- and 5-methyltetrahydrofolic acid generally followed the same trend as shown for the folate concentration determined by microbial assay after IV injection. As illustrated, plasma folate rose briefly after IV injection of [13C5]5-formyltetrahydrofolic acid and returned to baseline within the 4-h observational period. The change in plasma folate concentration, as determined by microbial assay, was not statistically significant after cecal infusion.

The molar ratios (M+5/M+0) for 5-formyltetrahydrofolic acid and 5-methyltetrahydrofolic acid after administration of [13C5]5-formyltetrahydrofolic acid are found in Figure 2. There was a statistically significant change after IV injection in the molar ratios for 5-formyltetrahydrofolic acid (P = 0.0037). The changes in molar ratios for 5-methyltetrahydrofolic acid after IV injection were not statistically significant (P = 0.0974).

After cecal infusion of the test dose, there was no statistically significant change in the molar ratios for 5-formyltetrahydrofolic acid but there was for 5-methyltetrahydrofolic acid (P < 0.0001). We did not observe detectable levels of [13C5]5-formyltetrahydrofolic acid or [13C5]5-methyltetrahydrofolic acid after cecal infusion of the test compound for 3 and 1 of the 6 subjects, respectively.

We converted the LC-MS/MS data for [13C5]5-formyltetrahydrofolic acid (M+5), unlabeled 5-formyltetrahydrofolic acid (M+0), and [13C5]5-methyltetrahydrofolic acid (M+5) to nmol/person, as shown in Figure 3. The amount of labeled 5-formyltetrahydrofolic acid rose after IV injection of the test dose to a maximum of 12 ± 1.2 nmol, which was followed by a rapid decline to the baseline value 4 h after injection. The [13C5]5-methyltetrahydrofolic acid content also increased rapidly in plasma after IV injection of the test dose, which was followed by a slight decline and then maintenance at a mean incremental increase of ≈6 nmol per person at the end of the 4-h study period. After cecal infusion of the test dose, the mean [13C5]5-formyltetrahydrofolic acid content showed a very minimal increase, whereas the mean [13C5]5-methyltetrahydrofolic acid content showed an approximately linear increase with no decrease over the 4-h blood sampling period. The mean plasma unlabeled 5-formyltetrahydrofolic acid content remained constant for 1.5 h after cecal infusion and rose linearly thereafter with no decrease over the 4-h blood sampling period.

Pharmacokinetics

A summary of the pharmacokinetic data is shown in Table 2. The mean (±SEM) maximal concentration (Cmax) of [13C5]5-formyltetrahydrofolic acid after IV injection was 12 ± 1.2 nmol. The concentration of labeled 5-formyltetrahydrofolic acid in plasma decreased from its maximum after IV injection, with a half-life of 0.3 ± 0.04 h. The mean Cmax for [13C5]5-methyltetrahydrofolic acid was 9 ± 0.8 nmol per person. After cecal infusion, [13C5]5-methyltetrahydrofolic acid increased after a mean delay of 0.8 ± 0.3 h, at a mean rate of 0.6 ± 0.2 nmol/h, reaching a mean Cmax of 1.7 ± 0.3 nmol per person 4 h after cecal infusion. A table summarizing the characteristics of the plasma response of the unlabeled folates determined, after

### Table 1

<table>
<thead>
<tr>
<th>Subject characteristics</th>
<th>Value (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (y)</td>
<td>56</td>
</tr>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>4/2</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>96.2 ± 9.8 (60.5–128.4)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>33.3 ± 3.1 (23.1–43.7)</td>
</tr>
<tr>
<td>**Plasma volume (L)**²</td>
<td>3.44 ± 0.3 (2.37–4.29)</td>
</tr>
<tr>
<td><strong>Blood screening data</strong></td>
<td></td>
</tr>
<tr>
<td>MTHFR⁵ 677C→T genotype</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>3</td>
</tr>
<tr>
<td>CT</td>
<td>3</td>
</tr>
</tbody>
</table>

¹ Mean ± SD; range in parentheses (all such values).
² Whole blood volume was calculated by using an estimate of 75 mL/kg for men and 66.5 mL/kg for women of normal weight (33). Plasma volume was estimated from the whole blood volume on the basis of hematocrit values and were corrected for the deviation of each subject’s actual weight from their ideal body weight as described by Feldschuh and Enson (34).
⁵ MTHFR, 5,10-methylenetetrahydrofolate reductase.
cecal infusion of $^{13}$C-$^5$-formyltetrahydrofolic acid, is provided elsewhere (see Table S1 under “Supplemental data” in the online issue).

DISCUSSION

The results of this study provide the first direct in vivo evidence that folate can be absorbed across the colon in humans. We predict the rate of folate absorption across the colon to be $0.6 \pm 0.2$ nmol/h based on the appearance of $^{13}$C-$^5$-methyltetrahydrofolic acid in plasma after cecal infusion of 684 nmol $^{13}$C-$^5$-formyltetrahydrofolic acid. In comparison, the rise in $^{13}$C-$^5$-methyltetrahydrofolic acid after IV injection of 172 nmol of $^{13}$C-$^5$-formyltetrahydrofolic acid was $7 \pm 1.2$ nmol/h. The apparent rate of folate absorption across the colon reported herein is considerably lower than that reported in the literature for the small intestine (37, 38). Extrapolating from the data published by Wright et al (37, 38) in which the appearance of $^{13}$C-$^5$-methyltetrahydrofolic acid in plasma was monitored after an oral dose of 431–569 nmol $^{13}$C-$^5$-formyltetrahydrofolic acid, we estimated the rate of folate absorption across the small intestine to be 34 nmol/h. It is important to acknowledge, however, that whereas the rate of folate absorption across the colon appears to be much slower than across the small intestine, the transit time in the small intestine ($3 \pm 1$ h) is considerably shorter than that in the colon (24–72 h), which allows for a greater opportunity for absorption to occur in the distal portion of the gastrointestinal tract (39–42). Furthermore, whereas folate absorption across the small intestine relies on oral intake and hence is intermittent, the availability of folate for absorption across the colon is continuous as microbial synthesis of folate occurs 24 h per day. Earlier reports confirm that the colon of adults, with an intact microbiota, contains folate well in excess of the test dose administered herein (17, 18).

Our observation that folate absorption occurs across the colon is consistent with our earlier work in which we injected $^3$H-
Para-aminobenzoic acid, a precursor of bacterially synthesized folate, into the cecum of 11-d old piglets and were able to extract titrated folates from the liver, kidney, and urine over the subsequent 3 d (22). In this latter study, we predicted that \( 18\% \) of the dietary requirement for the piglet could be met by folate absorption across the colon. Similarly, Rong et al (21) injected \( [3H] \)-para-aminobenzoic acid into the cecum of rats and observed that bacterially synthesized tritiated folate was incorporated into the liver of rats despite the prevention of coprophagy.

Secondary analyses of data collected from an observational and an intervention human study are similarly supportive of the findings in the present study (43, 44). We reported pre-fortification of the food supply in Canada that the consumption of dietary fiber was positively associated with serum folate concentrations in women \( (n = 224) \), even after statistically controlling for folate intake \( (P, 0.001) \) (43). In fact, serum folate increased by 1.8% with each gram of dietary fiber ingested. Likewise, in a randomized controlled trial of type 2 patients with diabetes, investigators showed that serum folate was significantly higher in subjects treated with miglitol than in those treated with metformin (44). Miglitol is an \( \alpha \)-glucosidase inhibitor that improves glycemic control by competitively inhibiting carbohydrate digestion. In contrast, metformin promotes glycemic control by affecting insulin sensitivity and hepatic glucose output. In both studies, increased colonic bacterial growth secondary to increased availability of fermentable substrate was proposed as the mechanism for the observed increase in serum folate content among high fiber consumers and miglitol users.

We did not assess what percentage of the test dose was metabolized within the colonocyte; however, it is known that a significant fraction of other nutrients are absorbed at the level of the small intestine. For example, select amino acids and folate are metabolized within the enterocyte and the liver by so-called first-pass splanchnic metabolism (38, 45–47). Given the proximity of colonocytes to the depot of folate in the colon, it is enticing to

### Table 2

**Pharmacokinetic data after intravenous injection and after cecal infusion of [\( ^{13}C_5 \)]-formyltetrahydrofolic acid**

<table>
<thead>
<tr>
<th>Subject</th>
<th>( C_{max} ) nmol</th>
<th>( t_{1/2} ) h</th>
<th>( C_{max} ) nmol</th>
<th>Rate of appearance ( \text{nmol/h} )</th>
<th>Plasma volume ( L )</th>
<th>( t_{delay} ) h</th>
<th>( C_{max} ) nmol</th>
<th>Rate of appearance ( \text{nmol/h} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>13.5</td>
<td>0.315</td>
<td>12.3</td>
<td>3 ± 1</td>
<td>4.29</td>
<td>2</td>
<td>2.22</td>
<td>0.98 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>9.75</td>
<td>0.174</td>
<td>5.35</td>
<td>10.87</td>
<td>3.82</td>
<td>1</td>
<td>1.53</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>C</td>
<td>11.5</td>
<td>0.342</td>
<td>8.11</td>
<td>3 ± 1</td>
<td>3.08</td>
<td>0.5</td>
<td>2.13</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>D</td>
<td>15.9</td>
<td>0.358</td>
<td>8.06</td>
<td>7 ± 4</td>
<td>3.63</td>
<td>0.5</td>
<td>1.98</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>E</td>
<td>13.1</td>
<td>0.285</td>
<td>10.24</td>
<td>10 ± 4</td>
<td>2.37</td>
<td>0.5</td>
<td>1.76</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>F</td>
<td>7.83</td>
<td>0.467</td>
<td>9.30</td>
<td>9 ± 1</td>
<td>2.98</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>12 ± 1</td>
<td>0.3 ± 0.04</td>
<td>9 ± 1</td>
<td>7 ± 1</td>
<td>3.44 ± 0.3</td>
<td>0.8 ± 0.3(^3)</td>
<td>1.7 ± 0.3(^3)</td>
<td>0.6 ± 0.2(^3)</td>
</tr>
</tbody>
</table>

\(^1\) \( C_{max} \), maximal concentration; \( t_{1/2} \), apparent plasma half-life; \( t_{delay} \), time lag before detection of the labeled vitamer.

\(^2\) Determined by the ascending slope of the appearance of [\( ^{13}C_5 \)]-5-methyltetrahydrofolic acid in plasma.

\(^3\) A zero value was used for subject F because [\( ^{13}C_5 \)]-5-methyltetrahydrofolic acid was undetected after cecal infusion of the test dose.
generalizability of our findings from 6 subjects to the larger exceeded, we could not rule it out. Last, we appreciate that the cecum back into the small intestine and believe that we suc-

we measured folate absorption after bowel cleansing in prepa-

much longer than 4 h. The second limitation of this study is that Unlike folate absorption across the small intestine, the calcula-

methyltetrahydrofolic acid concentration in plasma had not re-

These data serve as a caution in interpreting folate absorption studies using “unlabeled” folates, because the portion of the plasma response due to the test dose cannot be distinguished from that due to displacement of endogenous folate and, hence, would lead to overestimation of folate bioavailability.

The proposed potential mechanisms for how folate is absorbed across the colon include passive diffusion and active transport by 2 solute carriers—reduced folate carrier and proton-coupled folate transporter (50–52). Reduced folate carrier is widely ex-

pressed in the body, including in the small intestine and the colon, and has an optimal pH of 7.4 (51). The proton-coupled folate transporter is expressed in the colon at concentrations lower than in the small intestine, with an optimal pH of 5.5 (52). The pH of the colon varies along its length across the optimal pH of these 2 carriers, from about 5.2 in the proximal colo

To 6.4–6.9 in the midcolon, to >7 in the distal colon (53).

We acknowledge several limitations of this work. First, we originally planned to compare the 2 areas under the curve produ-

ced by measuring \([^{13}C]5\)-formyltetrahydrofolic acid in plasma after cecal infusion compared with that after IV infusion of the test dose. This would allow us to determine the percentage bioavailability of our test dose. However, at the end of the blood collection phase after cecal infusion, the \([^{13}C]5\)-methylytetrahydrofolic acid concentration in plasma had not re-

turned to baseline, but, rather, continued to rise (Figure 3B). Unlike folate absorption across the small intestine, the calcula-

tion of percentage bioavailability across the colon via the area under the curve approach would require blood sampling for much longer than 4 h. The second limitation of this study is that we measured folate absorption after bowel cleansing in prepara-

ation for colonoscopy (54). It is possible that both of these factors influenced our study results. Third, although we took every precaution to avoid reflux of our test compound from the cecum back into the small intestine and believe that we suc-

ceeded, we could not rule it out. Last, we appreciate that the generalizability of our findings from 6 subjects to the larger popula-

tion is limited. All of our subjects were \(\geq 50\) y of age, 3 were obese, 1 took folic acid supplements 16 d before the study, 1 had a low vitamin B-12 value, and, as a group, had high blood folate concentrations, at least compared with concentrations before fortification of the food supply. For the most part, it does not appear that aging in the absence of disease affects folate ab-

sorption (1, 55); however, evidence in the literature indicates that adipsosity may affect folate metabolism (56, 57) and high levels of folate exposure may down-regulate folate absorption (58). To account for this, some investigators allow for a 5–6-wk washout of supplements (59, 60). Finally, severe vitamin B-12 deficiency is known to block the uptake of folate into tissues (35), although the appearance and disappearance of \([^{13}C]5\)-methyltetrahydrofolic acid in the plasma of our subject with moderate vitamin B-12 deficiency was remarkably similar to that of others.

In conclusion, the results of the present study provide the first direct in vivo evidence that physiologic concentrations of folate can be absorbed across the colon in humans. The effect of the large depot of folate found in the colon on whole-body folate status, and perhaps more importantly on colonic health, is worthy of future investigation.

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