Biochemical and Molecular Actions of Nutrients

The Binding of Folic Acid and 5-Methyltetrahydrofolate to Folate-Binding Proteins during Gastric Passage Differs in a Dynamic In Vitro Gastrointestinal Model

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ABSTRACT Despite its low natural folate concentration, milk is responsible for 10–15% of the daily folate intake in countries with a high dairy consumption. Milk products can be considered as a potential matrix for folate fortification, e.g., with synthetic folic acid, to enhance the daily intake of folate. In untreated milk, the natural folate, 5-methyltetrahydrofolate (5-CH3-H4folate), is bound to folate-binding proteins (FBP). In this study, the extent of binding to FBP for folic acid and 5-CH3-H4folate was investigated in a dynamic in vitro model simulating human gastric passage. Protein binding of folic acid and 5-CH3-H4folate was characterized using gel-exclusion chromatography. Before gastric passage, folic acid and 5-CH3-H4folate were bound mainly to FBP (76–79%), whereas 7% was free. Folic acid remained bound to FBP to a similar extent after gastric passage. For 5-CH3-H4folate, the FBP-bound fraction gradually decreased from 79 to 5% and the free fraction increased from 7 to 93%. Although folic acid enters the proximal part of the intestine bound to FBP, 5-CH3-H4folate appears to be present mainly as free folate in the duodenal lumen. The stability of FBP was similar in both folate/FBP mixtures, i.e., 70% of the initial FBP content was retained after gastric passage. This study indicated that FBP are partly stable during gastric passage but have different binding characteristics for folic acid and 5-CH3-H4folate in the duodenal lumen. This could result in different bioavailability from folic acid- and 5-CH3-H4folate-fortified milk products.


KEY WORDS: folate-binding protein • 5-methyltetrahydrofolate • folic acid • gastric passage • milk products

Prevention of neural tube defects (1,2) and reducing the risk for cardiovascular disease (3,4) and colon cancer (5,6) can be achieved by an adequate folate intake. This can be reached by a high consumption of folate-rich food products or by the intake of supplements or fortified food products (7–9). The dominant folate compound in natural food products is 5-methyltetrahydrofolate (5-CH3-H4folate), whereas in supplements and fortified products, primarily folic acid is used. Although the natural folate concentration in milk is low compared with folate-rich food products such as vegetables and citrus fruit, milk is responsible for 10–15% of the daily folate intake in European countries with a high milk consumption, such as The Netherlands (10) and Sweden (11). Milk can be considered as a potential matrix for folate (folic acid or 5-CH3-H4folate) fortification because it is widely consumed and might enhance folate bioavailability from the diet (12); in addition, folic acid and 5-CH3-H4folate appear to be highly bioaccessible from the milk matrix (13).

In untreated milk, 5-CH3-H4folate occurs bound to folate-binding proteins (FBP) (14–16). The role of FBP in folate bioavailability is unclear. It has been suggested that FBP protects folate from bacterial uptake and degradation (17,18) or may play a role in sequestering folate from the blood plasma into the mammary glands, thereby supplying folate to the newborn (19). FBP could also affect mucosal folate transport, although both inhibition and enhancement have been reported (20–23). The influence of FBP on folate absorption might depend on its binding to folate after gastric passage. In a previous study, the effect of FBP on the absorption of folic acid was investigated in rats (22) that were administered free folic acid or folic acid bound to bovine milk FBP. Under acidic gastric conditions (pH < 4.5), folic acid was released from FBP and recombined in the small intestine (pH 6–7). In a study (24) with 6-d-old goat kids, who were bottle-fed FBP-bound folic acid in goat’s milk, it was shown that gastric acidity and gastrointestinal digestive enzymes had little effect on the binding characteristics of FBP for folic acid. In our previous studies (13,25) using an in vitro dynamic gastrointestinal model, it was found that FBP partly survives gastrointestinal passage and...
lowers the folate bioaccessibility; these effects appeared to be higher in folic acid–fortified milk products compared with 5-CH$_3$-H$_4$folate–fortified milk products. This suggests a different extent of binding to FBP for folic acid and 5-CH$_3$-H$_4$folate.

The present study was performed to investigate the binding characteristics of FBP for folic acid and 5-CH$_3$-H$_4$folate and to establish the effect of FBP stability on folate binding during gastric passage in a controlled in vitro model simulating human gastric conditions. The binding of folic acid and 5-CH$_3$-H$_4$folate to an equimolar amount of FBP during gastric passage was compared to examine the effect of FBP on the bioavailability of folic acid and 5-CH$_3$-H$_4$folate from milk products.

**MATERIALS AND METHODS**

**Chemicals.** 5-CH$_3$-H$_4$folate and folic acid were studied as a mixture of radio-labeled and unlabeled compounds. Radio-labeled folate compounds, [H]-folic acid (888 GBq/mmol; 37 GBq/L) and [14C]- (6-RS)-5-CH$_3$-H$_4$folate (2.11 GBq/mmol; 3.7 GBq/L) were obtained from Amersham (Buckinghamshire, UK) and examined for their purity. The unlabeled standard solutions of folic acid and (6-RS)-5-CH$_3$-H$_4$folate were obtained from Schircks’ Laboratories (Zutphen, the Netherlands) and Sigma (St. Louis, MO), respectively. The 0.1 mol/L phosphate buffer (pH 7.2) used for gel filtration contained 13.4 g/L Na$_2$HPO$_4$, 3.5 g/L NaH$_2$PO$_4$, 8.3 g/L NaCl and 0.02 g/L NaN$_3$ (all pGibson Pharmacia. The gastric enzymes pepsin (2200 U/mg, P7012) and lipase (150 kU/g; Rhizopus lipases F-AP 15) were obtained from Sigma (St. Louis, MO) and Amano (Amano, Denmark). Sephadex G75 Superfine powder, scintillation liquid (Superphase 2) and the low-molecular-weight gel filtration calibration kit were obtained from Amersham Pharmaceuticals. The gastric enzymes pepsin (2200 U/mg, P7012) and lipase (150 kU/g; Rhizopus lipases F-AP 15) were obtained from Sigma (St. Louis, MO) and Amano Pharmaceutica (Nagoya, Japan), respectively. The 0.1 mol/L phosphate buffer (pH 7.2) used for gel filtration contained 13.4 g/L Na$_2$HPO$_4$, 3.5 g/L NaH$_2$PO$_4$, 8.3 g/L NaCl and 0.02 g/L Na-azide (all from Sigma). For SDS-PAGE with immunoblotting, Tris-Glycine gel (Pager Gold Precast Gels, 8 x 10 cm, 12%, Sanvertech, Heerhugowaard, The Netherlands), nitrocellulose membranes (Protran, Schleicher & Schuell, Dassel, Germany) and goat-anti-rabbit IgG alkaline phosphatase conjugate (Dako, Glostrup, Denmark) were used. All other chemicals were obtained from Sigma.

**Folate binding to FBP during gastric passage under static experimental conditions.** First, the binding characteristics of FBP were studied under static experimental conditions, i.e., in test tubes for 1–2 h. Whey powder was used as the source of FBP. The FBP suspensions were made by mixing 50 mg whey powder (~1 nmol FBP) with 6 mL 0.1 mol/L phosphate buffer (pH 7.2). An equimolar FBP-folate suspension was made by adding [H]-folic acid or [14C]- (6-RS)-5-CH$_3$-H$_4$folate combined with unlabeled folic acid or 5-CH$_3$-H$_4$folate to reach 1 nmol folate per 6 mL suspension (167 nmol/L). The FBP and folate concentrations used in this study were comparable to the natural FBP and folate concentrations in milk, i.e., 110–220 nmol folate/L and 160–210 nmol FBP/L, respectively (27). The mixtures of folic acid, 5-CH$_3$-H$_4$folate or a combination of both folate compounds with the FBP suspensions were incubated for 1 h at pH 7.2 at ~20°C to allow association. Various test conditions were simulated in the incubation experiments (n = 2) (Table 1). In addition to the incubation at pH 7, an additional incubation period of 1 h at pH 3 (gastric pH conditions) with or without pepsin was tested. The pH of all mixtures was adjusted to pH 7 before elution over the Sephadex column to study the binding profile of the whey proteins.

**Folate binding to and the fate of FBP during gastric passage under dynamic experimental conditions.** After the static experiments, the binding characteristics of FBP for 5-CH$_3$-H$_4$folate and folic acid were studied in a dynamic in vitro gastrointestinal model (TIM) as described previously (13,25,28–30). This model has compartments for the stomach, duodenum, jejunum and ileum. Each compartment has a flexible inner wall surrounded by water at 37°C to squeeze the walls and mix the food with the “secreted” electrolytes and enzymes and to transport the chyme by peristaltic movements. The pH values as well as the gastric emptying and small intestinal passage of the food are controlled according to preset curves. Conditions were simulated according to the in vivo situation in adult humans.

In this model, we studied the fate of the FBP-folate complex during gastric passage and the possible recombination or stability of the FBP-folate complex in the duodenum. The folic acid–fortified and the 5-CH$_3$-H$_4$folate–fortified FBP suspensions were tested separately in duplicate experiments. Artificial oral and gastric juices with lipase and pepsin were gradually added into the gastric compartment. The pH was measured continuously and regulated by the addition of HCl to follow the preset pH curve. This curve corresponded to the in vivo pH drop in the stomach of adults after consumption of milk products. In the duodenal compartment, the pH was controlled at 6.5 by the addition of sodium bicarbonate.

The FBP suspension was made by dissolving 2.75 g whey powder in 330 mL of 0.01 mol/L phosphate buffer. This resulted in a final FBP content of ~55 nmol. An equimolar folate:FBP mixture was obtained by adding 55 nmol folic acid or 5-CH$_3$-H$_4$folate, as a mixture of [H]-folic acid or [14C]- (6-RS)-5-CH$_3$-H$_4$folate with unlabeled folic acid and [14C]-5-CH$_3$-H$_4$folate.

### TABLE 1

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Folate fortificant</th>
<th>pH 7</th>
<th>pH 3</th>
<th>pH 3 + pepsin</th>
<th>pH 7</th>
</tr>
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<tr>
<td>kDa</td>
<td></td>
<td></td>
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<tr>
<td>&gt;60</td>
<td>Folic acid</td>
<td>11 ± 1</td>
<td>13 ± 0</td>
<td>10 ± 4</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>30–40</td>
<td></td>
<td>79 ± 0</td>
<td>78 ± 0</td>
<td>78 ± 6</td>
<td>65 ± 1</td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>10 ± 1</td>
<td>9 ± 0</td>
<td>12 ± 2</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>&gt;60</td>
<td>5-CH$_3$-H$_4$folate</td>
<td>14 ± 0</td>
<td>16 ± 0</td>
<td>10 ± 8</td>
<td>6 ± 0</td>
</tr>
<tr>
<td>30–40</td>
<td></td>
<td>79 ± 0</td>
<td>79 ± 0</td>
<td>27 ± 1</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>7 ± 0</td>
<td>5 ± 0</td>
<td>63 ± 7</td>
<td>56 ± 2</td>
</tr>
</tbody>
</table>

1 Values are means ± range, n = 2.
2 The pH was adjusted to pH 7 before application to the Sephadex column.
3 Folic acid and 5-CH$_3$-H$_4$folate were added as an equimolar mixture to the whey suspension.
Characterization of FBP in bovine whey

**Gel filtration.** The distribution of [3H]-folic acid or [14C]-5-CH3-H4folate over the whey proteins was studied by gel filtration on a Sephadex G75-column (2.6 cm × 30 cm). The column was equilibrated with 0.1 mol/L phosphate buffer (pH 7.2). The [3H]-folic acid or [14C]-5-CH3-H4folate-fortified FBP suspensions (3 mL), whether digested or not, were eluted with the phosphate buffer at a flow rate of 25 mL/h. The eluant was measured spectrophotometrically with an UV detector at 280 nm, as an indicator of protein content, and subsequently collected as fractions at 8-min intervals during a total run time of 800 min. Portions (200–500 μL) of these fractions were quantified for their [3H]-folic acid or [14C]-5-CH3-H4folate content by measuring radioactivity with a scintillation counter after the addition of 4 mL of scintillation suspension.

The column was calibrated with the elution volumes of proteins within a low-molecular-weight gel filtration calibration kit, including blue dextran 2000 (200 kDa), albumin (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa) and ribonuclease A (14 kDa). The exact elution volume of FBP was determined by quantification of the FBP content in the collected fractions over time.

**ELISA.** The FBP content of the samples from the experiments with the gastrointestinal model and the gel filtration samples was analyzed by a two-site ELISA according to Højer-Madsen et al. (31) to determine the FBP stability after gastric passage and the elution volume of FBP, respectively. To 1 g of sample 0.09 g of Triton X-100 was added. The sample was put on a shaking device and incubated for 45 min at ~20°C. After this extraction, the sample was applied to a microtiter plate followed by the ELISA procedure as described previously (32).

**SDS-PAGE and immunoblotting.** The whey suspensions, before and after incubation with pepsin at pH 3, were subjected to 12% acrylamide gel filtration within 5 d. After 120 min, the stomach was almost completely emptied, according to the preset curve for gastric emptying in humans, and the residual contents in the stomach and duodenum were collected for calculation of the mass balance of folate. The folate content of the collected samples was determined by radioactivity measurements with a scintillation counter (Wallac 1409, PerkinElmer, Boston, MA).

**RESULTS**

Characterization of FBP in bovine whey. The whey suspension was analyzed via molecular size exclusion (Sephadex G75) at UV absorbance of 280 nm (Fig. 1A). The proteins were separated into two visible peaks. Based on calibration proteins, the first peak (elution volume ~ 50 mL) corresponded to proteins larger than ~60 kDa and the second peak (elution volume ~ 75 mL) to proteins between 30 and 40 kDa. The ELISA analysis showed a maximum FBP content at an elution volume of ~75 mL (Fig. 1B), corresponding to the elution volume of the second peak, i.e., proteins between 30 and 40 kDa (Fig. 1A). No FBP was detected at the elution volume of the first peak of the UV-chromatogram, indicating that the proteins larger than 60 kDa did not contain ELISA-detectable FBP.

The extent of protein-bound folate was studied after incubation of the FBP suspension with radiolabeled 5-CH3-H4folate or folic acid (Fig. 1C). Based on the folate content, three folate peaks were visible, successively corresponding to compounds with a molecular weight of FBP, folate, and folic acid (i.e., free folate). The distribution of [3H]-folic acid or [14C]-5-CH3-H4folate over the three fractions was 11, 79 and 10%, 14, 79, and 7%, respectively (Table 1).

Folate binding to FBP during gastric passage under static experimental conditions. The extent of binding to FBP for folic acid and 5-CH3-H4folate was studied under static experimental conditions simulating gastric passage. Incubation of the FBP suspension with folic acid or 5-CH3-H4folate at pH 7 showed that the major part (79%) of both folate compounds was initially bound to FBP before the incubation period at pH 3 (Fig. 1C, Table 1). The amount of bound folic acid (78%)
did not differ after incubation at pH 3 with or without pepsin (Table 1). Incubation at pH 3 without pepsin did not affect the extent of binding to FBP for 5-CH$_3$-H$_4$folate (79%). However, the FBP-bound fraction of 5-CH$_3$-H$_4$folate decreased from 79 to 27% after incubation at pH 3 with pepsin for 1 h. At the same time, the fraction of free 5-CH$_3$-H$_4$folate increased from 7 to 63%. This indicated that a major portion of 5-CH$_3$-H$_4$folate could occur freely in the duodenal lumen.

The whey proteins were also incubated with a mixture of folic acid and 5-CH$_3$-H$_4$folate (both in a 1:1 mol/L ratio with FBP) and the FBP-bound fractions were compared with those after the incubation with the single folate compounds. In this mixture of folic acid and 5-CH$_3$-H$_4$folate, there was a small decrease in FBP-bound folic acid (from 79 to 65%) and a pronounced decrease in FBP-bound 5-CH$_3$-H$_4$folate (from 79 to 38%).

FBP in the whey suspension showed two clear bands between 30 and 40 kDa with SDS-PAGE combined with immunoblotting. After pepsin incubation at pH 3, the intensity of the bands was lowered.

**Folate binding to and fate of FBP during gastric passage under dynamic experimental conditions.** The extent of binding of folic acid and 5-CH$_3$-H$_4$folate to FBP was investigated in duplicate experiments in the gastrointestinal model. The mass balance of folate in these experiments was 102 ± 1% ($n = 4$). The gel filtration analyses of the whey suspension (gastric intake) and the samples of the duodenal lumen gave an analytical recovery of 98 ± 2% ($n = 20$). The gastric passage of folic acid and 5-CH$_3$-H$_4$folate over time as measured in the duodenal compartment showed that most of the folate entered the proximal part of the intestine within 30–90 min after the start of the experiment (Fig. 2). The distribution of folic acid and 5-CH$_3$-H$_4$folate over the protein fractions was determined in the gastric intake (0 min) and in the duodenal samples collected during 0–30, 30–60, 60–90 and 90–120 min (Fig. 3). At initial test conditions, the major part of folic acid was bound to FBP and this fraction (76–81%) remained constant in the five successive samples over time during gastric passage (Table 2). The binding of folic acid to proteins larger than 60 kDa and the free folic acid fraction also remained unchanged over time. A similar initial FBP-bound fraction (79%) was observed for 5-CH$_3$-H$_4$folate before digestion. However, during gastric passage, the FBP-bound 5-CH$_3$-H$_4$folate
H₄folate fraction declined from 79 to 5% in 2 h. Consequently, the fraction of free 5-CH₃-H₄folate increased from 7% in the initial whey suspension before gastric passage to 93% in the duodenal sample collected between 90 and 120 min. The data from the duplicate experiments show a very low variation, which allows evaluation of the difference in the binding of folic acid and 5-CH₃-H₄folate to FBP during gastric passage.

The initial FBP concentration in the whey suspensions added to the gastric compartment was 158 ± 44 nmol/L (n = 4). During gastric passage (0–120 min), 70% of the initial amounts of FBP in both folic acid- and 5-CH₃-H₄folate-fortified whey suspensions were recovered in the duodenal lumen (Table 3).

### DISCUSSION

The present study was performed to investigate the stability and binding characteristics of FBP for folic acid and 5-CH₃-H₄folate during gastric passage. Before digestion of the folate-fortified FBP suspensions from whey, the major part of both labeled folate compounds (76–79%) appeared to be bound to proteins with a molecular weight of 30–40 kDa (Tables 1 and 2). ELISA analysis of the collected fractions (Fig. 1B) showed that the fractions between 30 and 40 kDa contained FBP. This was confirmed by SDS-PAGE electrophoresis combined with immunoblotting. A molecular weight of FBP between 30 and 40 kDa agrees with the previous reported studies (35–37). It appeared that folic acid and 5-CH₃-H₄folate were initially bound to FBP to a similar extent. Only a minor part of folic acid and 5-CH₃-H₄folate was present as free folate (7–10%) or was bound to proteins larger than 60 kDa (11–17%). Interestingly, no FBP was detected in the protein fraction larger than 60 kDa with the immunoblot assay, which indicated that dimers/polymers of FBP were not present in the whey suspensions.

Exposing FBP in a whey suspension to an equimolar mixture of folic acid and 5-CH₃-H₄folate resulted in a low binding of 5-CH₃-H₄folate (38%) and a relatively high binding of folic acid (65%) to FBP (Table 1), indicating that FBP has a higher affinity for folic acid than for 5-CH₃-H₄folate. This agrees with the results from previous studies in which FBP binding characteristics were investigated in in vitro experiments at pH 5.0 and 7.4 (38, 39). This difference in affinity for FBP between folic acid and 5-CH₃-H₄folate was found to vary within the pH range of 7.4 to 10.1 (40).

The present study also showed that incubation at pH 3 had no effect on the extent of binding of folic acid and 5-CH₃-H₄folate to FBP, once the pH of the incubation medium was returned to 7, reflecting the actual pH changes occurring during gastric and duodenal passage. An explanation may be that at low pH, dissociation of folate takes place, followed by a reassociation of folate to FBP at neutral pH. This is in line with other studies (22, 35, 36) showing that the dissociation of folic acid from FBP is completely reversible, even after pepsin treatment (22). We also found that incubation of the folic acid-FBP suspension at pH 3 with pepsin had no effect on the extent of binding of folic acid to FBP (remained 78%). However, the FBP binding characteristics for folic acid are apparently different from the binding to 5-CH₃-H₄folate because there was a marked decrease in FBP-bound fraction (27%) after pepsin incubation of the 5-CH₃-H₄folate-FBP suspension. This different effect of pepsin on binding of folic acid and 5-CH₃-H₄folate to FBP suggests a difference in FBP binding characteristics for the folate vitamers.

### TABLE 2

Distribution of [14C]-folic acid and [14C]-5-CH₃-H₄folate over the collected whey protein fractions after gel filtration of the [14C]-folic acid or [14C]-5-CH₃-H₄folate fortified whey suspensions before (gastric intake) and after gastric passage (duodenal samples) in the dynamic in vitro gastrointestinal model

<table>
<thead>
<tr>
<th>Protein fraction (kDa)</th>
<th>Folate fortificant</th>
<th>Distribution of folic acid or 5-CH₃-H₄folate over protein fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–30 min</td>
<td>30–60 min</td>
</tr>
<tr>
<td>0 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>Folic acid</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>30–40</td>
<td></td>
<td>76 ± 2</td>
</tr>
<tr>
<td>&lt;10</td>
<td>5-CH₃-H₄folate</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>&gt;60</td>
<td></td>
<td>14 ± 0</td>
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<tr>
<td>30–40</td>
<td></td>
<td>79 ± 0</td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>7 ± 0</td>
</tr>
</tbody>
</table>

1 Values are means ± range, n = 2.

### TABLE 3

The folate binding protein (FBP) content given as a percentage of the initial amount in the whey suspension in the duodenal samples collected from the dynamic in vitro gastrointestinal model

<table>
<thead>
<tr>
<th>Folate fortificant</th>
<th>0–30 min</th>
<th>30–60 min</th>
<th>60–90 min</th>
<th>90–120 min</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid</td>
<td>2 ± 1</td>
<td>24 ± 2</td>
<td>28 ± 2</td>
<td>16 ± 1</td>
<td>70 ± 1</td>
</tr>
<tr>
<td>5-CH₃-H₄folate</td>
<td>3 ± 1</td>
<td>25 ± 2</td>
<td>33 ± 3</td>
<td>9 ± 1</td>
<td>70 ± 7</td>
</tr>
</tbody>
</table>

1 The values are means ± range, n = 2.
2 Quantified by ELISA.
In addition to the experiments under static conditions, experiments in a gastrointestinal model were performed because this model simulates the kinetic digestion and passage of the whey suspension from the stomach into the duodenum. These studies show that the amount of FBP-bound folate remained constant during the gastric passage from 0 to 120 min, indicating no change in the extent of folate acid binding to FBP (Table 2, Fig. 3). In contrast, the FBP-bound 5-CH$_3$-H$_4$folate fraction gradually decreased during gastric passage from 79 to 5% within 120 min. The results obtained with these static and dynamic in vitro experiments simulating gastric conditions give the first evidence that the extent of binding to FBP is higher for folic acid than for 5-CH$_3$-H$_4$folate after gastric passage. It should be noted that these FBP binding characteristics for folate acid and 5-CH$_3$-H$_4$folate were established for FBP in whey powder. To draw conclusions about the FBP binding characteristics in milk products, results obtained in the present study should be extrapolated with caution. Direct extrapolation of the binding characteristics of FBP in whey protein concentrate to those in milk products might not be feasible because a previous study (41) showed different binding properties of FBP in raw milk, pasteurized milk and whey protein concentrate. Nevertheless, this difference between folate acid and 5-CH$_3$-H$_4$folate in extent of binding to FBP is also supported by our previous studies (13,25) in which the effect of FBP on the bioaccessibility of folate acid and 5-CH$_3$-H$_4$folate from fortified dairy products was investigated in the in vitro gastrointestinal model (bioaccessibility is, in these studies, defined as the free folate fractions that are available for absorption during gastrointestinal passage). The bioaccessibility of folic acid from folic acid-fortified milk and yogurt was lower ($P < 0.05$), i.e., 11–14 and 47%, respectively, after the addition of FBP to the fortified milk (13) and yogurt (25). However, FBP did not lower the bioaccessibility of 5-CH$_3$-H$_4$folate from fortified milk (13) and lowered the bioaccessibility of 5-CH$_3$-H$_4$folate from fortified yogurt by 26% (25). These findings indicate that FBP in whey powder, milk and yogurt have different binding characteristics for folate acid and 5-CH$_3$-H$_4$folate.

In this regard, one point to consider is the presence of endogenous folate in the whey protein concentrate (~4 µg 5-CH$_3$-H$_4$folate/g whey powder). This endogenous folate could compete with the added (exogenous) folic acid and 5-CH$_3$-H$_4$folate and as a result influence the extent of binding to FBP. However, this does not alter our general conclusions on the extent of binding to FBP because we measured the relative binding of folic acid and 5-CH$_3$-H$_4$folate to FBP before and after gastric passage rather than focusing on the absolute quantification of the binding activity of FBP. Because both folate compounds could be used for the fortification of dairy products, already containing endogenous FBP and 5-CH$_3$-H$_4$folate, this study provides information about the extent of binding of folic acid and 5-CH$_3$-H$_4$folate to FBP in the duodenal lumen after consumption of fortified dairy products.

An in vivo study (24) with 6- to 7-d-old goat kids supports our in vitro studies because it showed that folate acid remained bound to FBP throughout the stomach and small intestine. Analysis of the goat’s jejunal and ileal contents with gel filtration showed that a major part of the labeled folic acid (85–90%) was eluted in fractions corresponding to a molecular weight of 39 kDa (i.e., FBP-bound folic acid). On the basis of these results, the authors suggested that goat’s milk FBP is resistant to digestion by gastric and intestinal enzymes. However, the fact that folic acid was bound to FBP in the goat’s intestine, does not necessarily mean that FBP was completely resistant to degradation. The stability of FBP can be investigated only by quantitative determination of FBP before and after exposure to gastric and/or intestinal enzymes. Therefore, we studied the extent of binding to FBP in parallel with the quantitative determination of FBP. In contrast to the observed difference in FBP’s binding characteristics for folic acid and 5-CH$_3$-H$_4$folate, FBP stability in the 5-CH$_3$-H$_4$folate/FBP and folic acid/FBP mixtures did not differ after gastric passage on the basis of the ELISA measurements. From both mixtures, 70% of the initial amount of FBP was recovered in the duodenum after gastric passage for 120 min. In our previous studies, in which folic acid- and 5-CH$_3$-H$_4$folate–fortified dairy products were tested in the gastrointestinal model (13,25), the FBP content was quantified by ELISA in the samples collected after passage through the stomach and small intestine. It appeared that bovine FBP in a dairy matrix was less stable in combination with 5-CH$_3$-H$_4$folate (0–17%) than with folic acid (13–34%). Thus, a major portion of FBP passed through the stomach intact and was largely digested by pancreatic enzymes along the passage through the small intestine. Apparently, this further digestion of FBP in the small intestine was dependent on the folate compound, folic acid or 5-CH$_3$-H$_4$folate, present in the dairy matrix.

We conclude that a major part of folic acid is still bound to FBP after gastric passage, whereas a large portion of 5-CH$_3$-H$_4$folate is released from FBP. This difference in extent of binding to FBP for the two folate compounds can influence the folate bioavailability (i.e., release from the food matrix and intestinal transport) from milk products. To examine this further, studies are underway in our laboratory concerning the effect of FBP on intestinal transport of folic acid and 5-CH$_3$-H$_4$folate.

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LITERATURE CITED

FOLATE BINDING TO FBP DURING GASTRIC PASSAGE


