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Fucose as a Cleavage Product of 2'Fucosyllactose Does Not Cross the Blood-Brain Barrier in Mice

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Scope: To further examine the role of the human milk oligosaccharide 2'fucosyllactose (2´FL) and fucose (Fuc) in cognition. Using ^{13}C-labeled 2'FL,thestudy previously showed in mice that ^{13}C-enrichment of the brain is not caused by $^{13}C_1$-2´FL itself, but rather by microbial metabolites. Here, the study applies $^{13}C_1$-Fuc in the same mouse model to investigate its uptake into the brain.

Methods and Results: Mice received $^{13}C_1$-Fuc via oral gavage (2 mmol $^{13}C_1$-Fuc/kg^{-1} body weight) or intravenously (0.4 mmol/kg^{-1} body weight). ^{13}C-enrichment is measured in organs, including various brain regions, biological fluids and excrements. By EA-IRMS, the study observes an early rise of ^{13}C-enrichment in plasma, 30 min after oral dosing. However, ^{13}C-enrichment in the brain does not occur until 3-5 h post-dosing, when the ^{13}C-Fuc bolus has already reached the lower gut. Therefore, the researcher assume that ^{13}C-Fuc is absorbed in the upper small intestine but cannot cross the blood-brain barrier which is also observed after intravenous application of $^{13}C_1$-Fuc.

Conclusions: Late ^{13}C-enrichment in the rodent brain may be derived from $^{13}C_1$-Fuc metabolites derived from bacterial fermentation. The precise role that Fuc or 2´FL metabolites might play in gut-brain communication needs to be investigated in further studies.

1. Introduction

Breastfeeding supports the healthy growth and development of infants.[1,2] Among multiple benefits, improved postnatal cognitive development in children is increasingly discussed to be favored by not yet identified factors in human milk.[2–6] The question of whether sialylated or fucosylated human milk oligosaccharides (HMOs) are involved in these processes has been researched at length.[7–14] Regarding sialic acid and sialyllactose (SL) and its potential effects on the brain we refer to a recent review being in favor of a direct incorporation of these milk components into brain glycoproteins and glycolipids.[15] The authors underline the importance of pig models to address such questions. For example, Obelitz-Ryom and coworkers presented data showing preterm piglets fed SL-supplemented milk had improved learning skills and cognition compared to non-supplemented formula-reared counterparts; however, SL supplementation did not increase the sialic acid (SA) content in the hippocampus or change magnetic resonance imaging (MRI) endpoints, although these pigs upregulated genes related to sialic acid metabolism, myelination and ganglioside biosynthesis in the hippocampus.[10] In contrast, Mudd et al. applied MRI in young pigs and identified effects in various parts of the brain, which led the authors to conclude that these parts may be differentially sensitive to dietary SL supplementation.[8] In a previous

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study using a mouse model, however, we did not find a direct incorporation of ^{13}C-SL or its constituent ^{13}C-SA into the brain; these molecules were not able to cross the blood-brain barrier.[16] There, we also discussed various factors for the divergent opinions on a direct link between milk oligosaccharides and the brain.

Fucosylated HMOs such as 2´fucosyllactose (2´FL) have been the subject of extensive investigation in recent years. The biological importance of fucosylation on host microbe interactions, leukocyte trafficking, cancer metastasis and learning, memory and cognitive processes has been summarized elsewhere.[17] Fucose (Fuc), a major monosaccharide building block of 2´FL and α1-2-fucosylated glycans, is an integral part of many glycoconjugates in the brain. This suggests that α1-2-fucosylation is important in modulating neuronal communication in the brain.[18–21] Additional data indicates that protein fucosylation is regulated in response to synaptic activity.[18] Both task-specific learning and long-term potentiation (LTP), the latter being closely associated with learning and memory,[22] have been shown to induce the fucosylation of proteins at the synapse.[23] The addition of Fuc or 2´FL, but not 3FL, to hippocampal slices of rats was found to enhance LTP in hippocampal tissue. LTP was also found to be enhanced in rats receiving oral 2´FL, but not after Fuc application.[24,25] Recently, Tosh and coworkers reported the in vivo assignment of seven Fuc-α1-2-galactosylated glycans and free L-Fuc to the human brain.[21] Fuc is part of the synapsin proteins which are considered to regulate the release of neurotransmitters at the synapse; the rapid degradation of synapsins seems to be prevented by its fucosylation as suggested by Murrey et al.[18] Some studies also linked the Fuc-α(1-2)Gal modification of neuronal glycoproteins to cognitive processes and suggested previously unknown molecular mechanisms of neuronal plasticity.[20]

These observations and others lead to the intriguing hypothesis that brain composition and brain activity may be influenced by dietary means. Of particular importance is the infant gut associated microbiota; their properties and the communications among them as well as with other microorganisms have been thoroughly described.[26–28] Interactions between the gut microbiota and the central nervous system comprise a proposed signaling network known as the "gut-brain axis". HMOs are important factors in early nutrition and brain development, and may provide numerous benefits through the modulation of the gut-brain axis (for reviews see.[29–32])

2´FL is a particularly interesting oligosaccharide as it is one of the major HMOs in women with an active fucosyltransferase-2 (*FUT2)* gene (70–80% of the European population). 2´FL is absorbed in infants and circulates in the blood, making it potentially available to organs and tissues including the brain.[33–37]

To investigate a potential link between HMOs and brain functions, a clear understanding of their metabolic fate is required. A general assumption is that HMOs may affect the brain either through the transport of components via blood or through interactions with the vagus nerve.

By using ^{13}C-labeled 2´FL and subsequent elemental analysis isotope ratio mass spectrometry (EA-IRMS) of biological fluids and tissues, we recently demonstrated that a direct incorporation of 2´FL into the brain of wild type mice does not occur.[14] We concluded that the ^{13}C-enrichment found in the brain after oral application of $^{13}C_1$-2´FL is likely either derived from ^{13}C-Fuc being cleaved from 2´FL within the gastrointestinal tract or even further metabolized, e.g., by fermentation through gut microbes which may then be incorporated directly into the brain. A direct influence on the brain by Fuc or its metabolites requires that the blood-brain barrier can be overcome. We addressed the questions of whether Fuc can be intestinally absorbed, released into the blood stream, and transported to tissues and organs including the brain (**Figure 1**). In addition, Fuc was also applied intravenously to directly investigate whether it can pass through the blood-brain barrier and be retained in the brain.

2. Experimental Section

2.1. Materials

The study used L-Fucose (Fuc) labeled with the stable isotope ^{13}C at C_1 [1-$^{13}C_1$-Fuc] with a ^{13}C-enrichment of 99% (Elicityl, Crolles, France).

2.2. Dosage Information

In a previous study, physiological doses of the fucosylated oligosaccharide 2´FL were used in the same mouse model.[14] Thus, isomolar doses of ^{13}C-Fuc, i.e., 2 mmol/kg^{-1} body weight was used for oral and 0.4 mmol/kg^{-1} body weight for intravenous applications.

2.3. Animal Models

2.3.1. Intravenous Application of ^{13}C-Labeled Fuc to Wild-Type NMRI Mice

Male NMRI mice (8-weeks-old, 39 ± 2 g body weight) were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed in groups of five animals with free access to water and food (Altromin Spezialfutter GmbH & Co KG, Lage, Germany). On the day of experiments, animals (n = 5 treated) received 66 mg ^{13}C-Fuc/kg^{-1} body weight) divided into three equivalent doses every 6 h through the tail vein. Controls (n = 3) received 0.9% saline in the same way. From the time of injection, animals were individually housed in metabolic cages until the end of the experiment 24 h after the first injection.

2.3.2. Oral Application of ^{13}C-Fuc

Male NMRI mice (8-weeks-old, 38 ± 2 g body weight) were housed as described above. On the day of the experiment, animals received either a single dose of 0.33 g ^{13}C-Fuc/kg^{-1} body weight (treated, n = 5 per time point) or saline as the vehicle (controls, n = 3 for the time points 0.5/5 h, 1/3 h, 9, and 15 h) via oral gavage. Time points of controls were consolidated in case the treatments were done on the same day to save animals. As for the intravenous application, the gavage dose was calculated to

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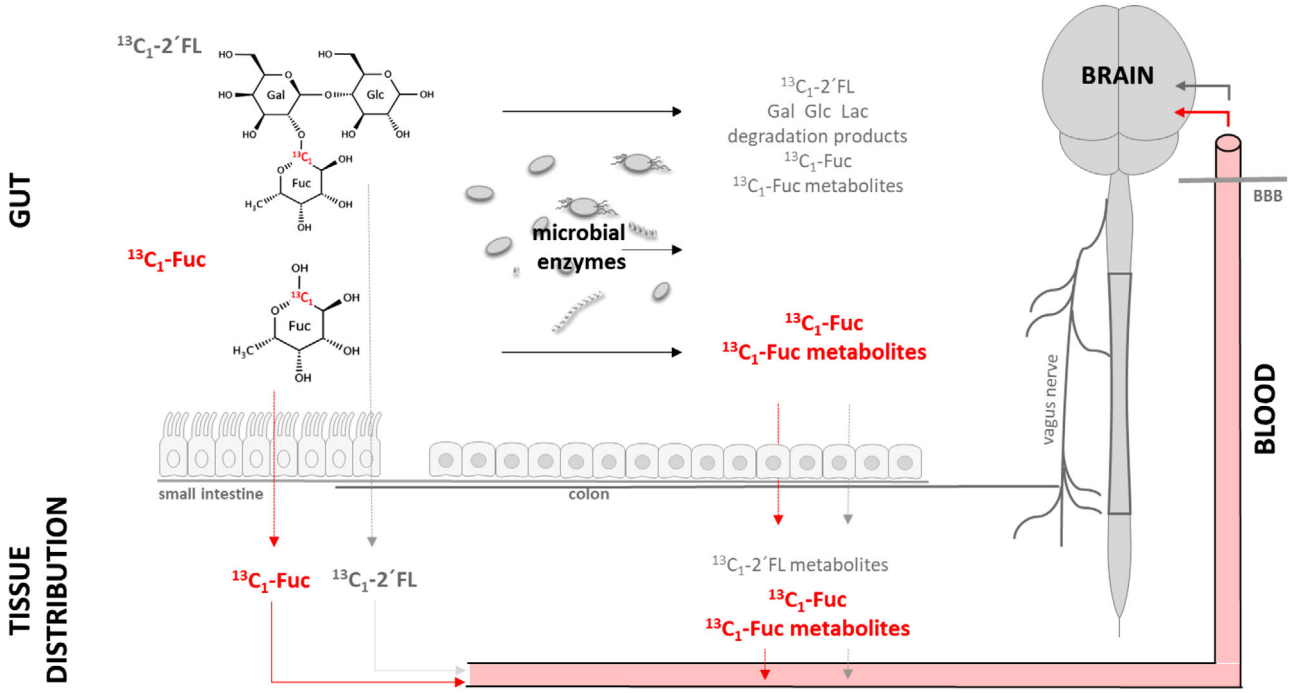

Figure 1. Potential pathways of 2´FL and Fuc metabolism and their link to the brain. After oral intake, Fuc or 2´FL is transported through the gut where (i) they may be taken up into the intestinal cells and released intact into the blood to be transported to organs and tissues or (ii) they are subjected to intracellular degradation resulting in various metabolic products which may be further used from the intestinal cells themselves or released to the blood or (iii) Fuc and 2´FL are fermented by gastrointestinal microorganisms leading to microbial metabolites with a high potential for local or systemic effects including effects on the vagus nerve and, hence, influencing brain activity. A direct influence on the brain by Fuc or its metabolites requires that the blood-brain barrier can be overcome. (Images from Motifolio Toolkit (Motifolio Inc, Ellicott City, MD, USA).

be isomolar to the dose of ^{13}C-2'FL given in a previous study.[14] Animals were kept individually in metabolic cages and sacrificed after 0.5, 1, 3, 5, 9, and 15 h.

All experiments were carried out by individuals with appropriate training and experience according to the requirements of the Federation of European Laboratory Animal Science Associations and the European Communities Council Directive (Directive 2010/63/EU). Experiments were approved by the regional authority (Regional Authority Darmstadt; V54 – 19 c 20/15 – FU/1056).

2.3.3. Sample and Tissue Collection

At the end of the experiments, the treatment of the animals was done as described previously with a modification of the euthanasia protocol.[14] Briefly, animals were killed individually with CO_2 (flux rate 1.4 L/min^{-1}) until the intertoe reflex and respiration ceased completely. From each animal, a blood sample was taken from the retrobulbar plexus and centrifuged at 1000 x *g* at 4 °C for 10 min to obtain plasma. The abdomen was immediately opened and animals were perfused with saline to avoid plasma contamination of organs. Then, organs were quickly removed (liver, heart, spleen and kidney), the brain was placed on ice while separating the stem, cerebellum and cerebrum. Furthermore, the small intestine (SI) was cut into three pieces of equal length; the large intestine (LI) was taken separately. Intestinal content was collected from each segment. Urine left in the metabolic cages was collected. All samples were snap-frozen in liquid nitrogen and kept at -80 °C until analysis.

2.4. Analytical Methods

The biological samples were subjected to Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) as described previously.[14] Isotope ratio calculations were done using Elementar Software (IonVantage and Ionos; Elementar UK, Stockport UK) and results were expressed as $\delta^{13}C_{VPDB}$ enrichment with VPDB being the international standard Vienna Pee Dee Belemnite from the International Atomic Energy Agency IAEA (Vienna, Austria). It is notable that the natural abundance of ^{13}C reveals negative values for the baseline ^{13}C-enrichment between -23 and -26 in biological fluids and tissues of mice or humans, when the isotope ratios as δ^{13}C are standardized for VPDB.

2.5. Statistical Analysis

Statistical analyses were carried out using GraphPad Prism 6.0.7 (GraphPad Software Inc, La Jolla, U.S.A.). Results were expressed as box plots with medians and min to max whiskers. Data were analyzed by ANOVA with multiple comparison test or Student *t*-test as group comparison between treated animals versus controls for the respective time points. Differences were considered significant at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

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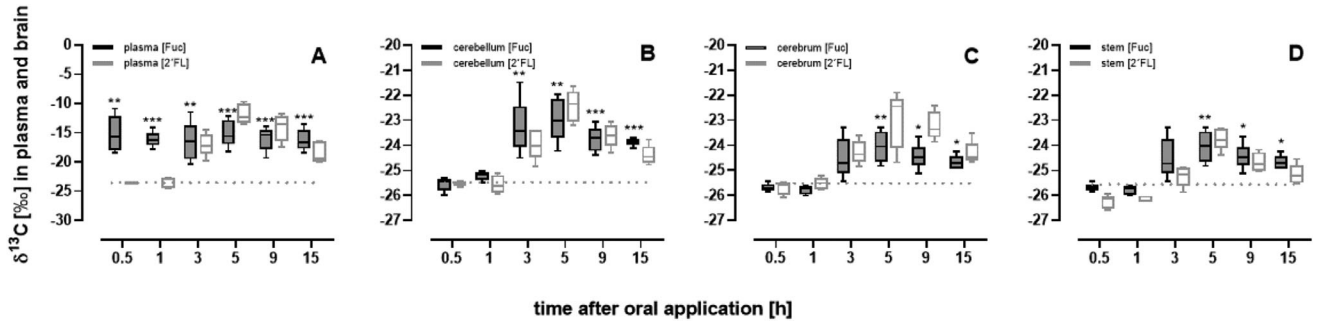

Figure 2. ^{13}C-enrichment (δ^{13}C in $^{0}/_{00}$) in plasma (A) and in brain sections (brain stem, cerebellum, cerebrum; B-D) of wild-type mice receiving an oral dose of ^{13}C-labeled Fuc (black boxes). For comparison, data from our previous study using an isomolar dose of ^{13}C-labeled 2´FL (grey boxes) have been added.[14] Data are depicted as box plots with median and min-max whiskers; controls δ^{13}C of the Fuc study are indicated as dotted line. Differences were calculated between the groups receiving an oral dose of ^{13}C-Fuc and their saline controls for the same time points; they were considered significant at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

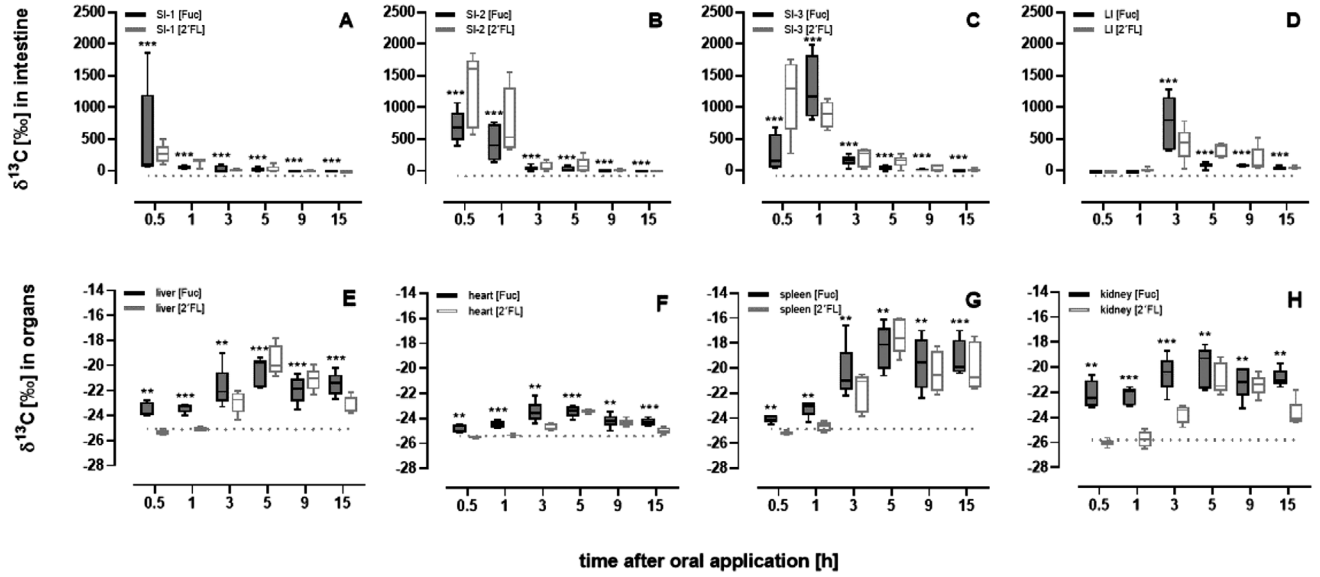

Figure 3. ^{13}C-enrichment (δ^{13}C in $^{0}/_{00}$) in luminal content of intestinal segments (upper panel, A-D) and organs (liver, heart, spleen, kidney) (lower panel, E-H) in wild-type mice receiving an oral dose of ^{13}C-labeled Fuc (black boxes). For comparison, data from our previous study using an isomolar dose of ^{13}C-labeled 2´FL (grey boxes) have been added.[14] Data are depicted as box plots with median and min-max whiskers; controls δ^{13}C of the Fuc study are indicated as dotted line. Differences were calculated between the groups receiving an oral dose of ^{13}C-Fuc and their saline controls for the same time points; they were considered significant at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. (LI, large intestine; SI, small intestine with section 1, 2, 3).

3. Results and Discussion

3.1. Oral Application of ^{13}C-Fuc

After oral application of ^{13}C-Fuc labeled at the same C-atom as ^{13}C-2´FL, namely C_1, EA-IRMS analysis revealed an immediate ^{13}C-enrichment of plasma at the earliest time point samples were collected, 30 min after dosing (**Figure 2**A, black boxes). This ^{13}C-enrichment remained at the same level at all time points. When compared to the data from our previous study using ^{13}C-2´FL[38] (also refer to **Figure** 2A, grey boxes), ^{13}C-enrichment in plasma rose at 2 h after dosing which is in line with findings by Vazquez and coworkers in rat pups receiving a single dose of unlabeled 2´FL;[24] however, ^{13}C-enrichment of plasma reached its maximum only at 5 h, indicating that at these later time points, an additional uptake of fermentation products of 2´FL carrying the ^{13}C-label occurred since the ^{13}C-2´FL dose had already reached the lower gut as discussed previously.[38] In addition, Vazquez and co-workers detected Fuc in serum and found it remained at stable levels in pups and adult rats.[24] With ^{13}C-labeled Fuc, orally applied Fuc was rapidly absorbed and the ^{13}C-enrichment levels remained high which may be explained by the absorption of Fuc metabolites at later time points (Figure 2A).

Most interestingly, the immediate rise in the ^{13}C-enrichment of plasma after ^{13}C-Fuc application was not associated with a ^{13}C-enrichment in the tissues, including the brain sections

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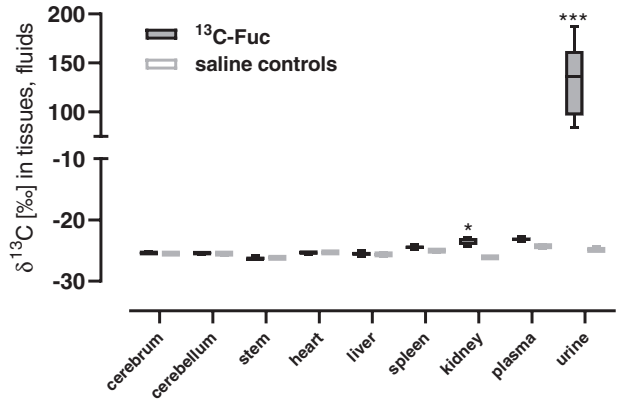

Figure 4. ^{13}C-enrichment (δ^{13}C in $^{0}/_{00}$) in brain sections, various organs, plasma and urine in mice receiving an intravenous dose of ^{13}C-labeled Fuc (0.4 mmol/kg^{-1} body weight; n = 5) or saline (controls; n = 3). Twelve hours after the last of three partial dosages, mice were sacrificed and organs and urine were collected as described in the Experimental Section. Data are depicted as boxes with median and min-max. Differences to corresponding controls were significant at ***$p < 0.001$.

cerebellum, cerebrum and stem (**Figure** 2B-D). The ^{13}C-enrichment in brain increased only at later time points (>3 h) very similar to the course seen for ^{13}C-2´FL (compare black and grey boxes in **Figure** 2B-D).

The ^{13}C-enrichment observed in the brain at these later time points; however, was not organ-specific. Concomitant with the transport of the ^{13}C-Fuc into lower gut segments (compare ^{13}C-enrichment shown in **Figure 3**A-D), a similar ^{13}C-enrichment pattern as for the brain segments was seen for the liver, heart, spleen and kidney (**Figure** 3E-H). These data indicate that the immediate rise in the ^{13}C-enrichment of plasma after ^{13}C-Fuc application was not associated with a ^{13}C-enrichment in these tissues, but did occur later (>3 h) and was similar to what has been seen for ^{13}C-2´FL (compare black and grey boxes for Fuc and 2´FL, respectively, in **Figure** 3). In contrast to these previous observations, an early ^{13}C-enrichment was observed in all organs except the brain (**Figure** 3E-H) in parallel to the fast ^{13}C-enrichment in plasma even at the first time point, i.e., 0.5 h after oral application of the dose (**Figure** 2A).

3.2. Intravenous Application of ^{13}C-Fuc

To prove whether Fuc was able to cross the blood-brain barrier, ^{13}C-Fuc was applied intravenously to bypass the gastrointestinal barrier and to avoid microbial Fuc degradation at the same time. 12 h after the last intravenous dose of ^{13}C-labeled Fuc, small amounts of ^{13}C-Fuc were still found in the plasma, but the majority of ^{13}C-Fuc was excreted via the urine (**Figure 4**). Most importantly, there was no ^{13}C-enrichment in brain sections as well as in liver, heart and spleen and a low enrichment in kidney which might be due to urinary remnants not taken care of during the tissue preparation (**Figure** 4).

From the observations described above, we suggest that Fuc was not able to cross the blood-brain barrier since intravenous application of ^{13}C-Fuc did not lead to a ^{13}C-enrichment of brain tissue (**Figure** 4). In addition, the fast, initial rise in plasma ^{13}C-enrichment after oral ^{13}C-Fuc application may be due to the absorption of ^{13}C-Fuc starting in the small intestine (**Figure** 3). At later time points (> 3 h), however, ^{13}C-enrichment in plasma may derive from both intestinal absorption of intact Fuc and its fermentation products carrying the ^{13}C-label. Since Fuc was not able to cross the blood-brain barrier as described above (**Figure** 2), the ^{13}C-enrichment observed in brain tissue was most likely due to ^{13}C-labeled fermentation products similar to what we have seen after ^{13}C-2´FL application. The small ^{13}C-enrichment we had observed at early time points in other organs (**Figure** 3), however, may reflect a minor uptake of intact Fuc to be metabolized or used for glycoconjugate synthesis. We are aware that data regarding the metabolic fate of Fuc and 2´FL are urgently needed to answer the question whether brain composition and/or activity through signaling processes can be influenced by dietary means. In this context, various short chain fatty acids are certainly important factors influencing the gut-brain axis.[32,39] Such studies cannot be performed in infants; hence, we rely on animal studies although application of those data to human physiology requires great care.[40] The current opinion, whether fucosylated or sialylated HMOs can directly be incorporated into the brain is controversial as addressed in the introduction. Our studies with ^{13}C-2´FL, ^{13}C-Fuc, ^{13}C-SL and ^{13}C-SA do not support a direct influence of HMOs on brain composition.[14,16] With regard to Fuc and fucosylated HMOs, there is so far no evidence that a direct transfer and uptake into brain cells occur in animals or humans.

Previous studies on the metabolic fate of Fuc support our results. In 1964, Coffey and coworkers addressed the metabolic question in rats using ^{14}C as a radioactive label of Fuc.[41] The authors observed a rapid elimination of Fuc in urine after intraperitoneal injection of ^{14}C-Fuc. Similar to this previous study, we also found urine as the major elimination route after oral and intravenous application of ^{13}C-labeled 2´FL or Fuc (**Figure** 4).

^{13}C-enrichment of brain tissue after oral Fuc application was relatively modest in scope and occurred only at the later time points indicating that Fuc was not readily enriched in the brain as an intact molecule. This conclusion is supported by earlier observations from Harsh et al. (1984) who found an uptake of L-Fuc in brain tumors but not in normal tissue.[42] The authors stated that their data imply a permissive blood-brain barrier in tumors rather than differences in Fuc metabolism. Wiese et al. (1994) observed that the uptake of L-Fuc into eukaryotic cells does not occur through a glucose transporter but potentially through facilitated diffusion.[43] To date, there is little information about an intracellular uptake of Fuc into the brain. GLUT-1 which is essential for the transport of glucose across the blood-brain barrier does not transport Fuc. The SLC database (http://www.bioparadigms.org/slc/intro.htm) lists transporters for metabolically activated carbohydrates such as GDP-Fuc (e.g., SLC35C1), but not for free L-Fuc. Therefore, we assume that if Fuc or 2´FL as such have an effect on brain function, it is more likely to be either through a direct effect of one or more bacterial metabolites transported to the brain or through an effect within the gut, e.g., via stimuli on the vagus nerve. As discussed in our previous publication,[14] the majority of ingested 2´FL reaches the colon where it can be used as a substrate for intestinal bacteria and catabolized into acetate and lactate, as has been reported for some *Bifidobacteria* strains.[26,28,44,45] Co-existing bacteria participate in cross-feeding relationships that influence HMO metabolization.[26] Whether

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HMO metabolites derived from various bacterial activities exert beneficial effects on the gut–brain axis is a highly relevant question to be addressed by future research.

3.3. Concluding Remarks

Our studies in mice receiving ^{13}C-labeled Fuc via oral gavage revealed an early rise of ^{13}C-enrichment in plasma (30 min after dosing) which had not been the case with ^{13}C-2´FL. However, ^{13}C-enrichment in the brain does not occur until 3 - 5 h after Fuc intake, when the ^{13}C-Fuc bolus has already reached the lower gut. These data are consistent with the notion that Fuc was absorbed in the upper small intestine, but could not cross the blood-brain barrier and that the later ^{13}C-enrichment in the brain may be derived from the uptake of Fuc metabolites resulting from bacterial fermentation as has been seen for 2´FL in the previous study. Metabolites, e.g. deriving from bacterial fermentation in the lower gut, however, can be enriched in tissues, including the brain. These HMO-derived metabolites (SCFA or other organic acids, such as lactic acids) may well affect brain function and composition, but most likely not by directly incorporating intact HMOs or their monosaccharides into brain structures. Thus, any benefit from dietary intake of 2'FL to an organ outside the gastrointestinal tract cannot be explained by absorption of intact fucose. The specific role of Fuc or 2´FL metabolites and to what degree they may be effective in the gut-brain communication needs to be investigated in further studies.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

S.R., E.V., R.B. and C.K. designed the study; S.R., C.B., M.R. and G.P.E. conducted research; S.K. performed statistical analysis; S.R. and C.K. drafted the manuscript; the authors read, revised and approved the final manuscript.

Data Availability Statement

Data available on request from the authors.

Keywords

^{13}C-labeled fucose, biological fluids, brain, metabolism, microbiota

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[1] C. G. Victora, R. Bahl, A. J. D. Barros, G. V. A. França, S. Horton, J. Krasevec, S. Murch, M. J. Sankar, N. Walker, N. C. Rollins, *Lancet* **2016**, *387*, 475.
[2] N. C. Rollins, N. Bhandari, N. Hajeebhoy, S. Horton, C. K. Lutter, J. C. Martines, E. G. Piwoz, L. M. Richter, C. G. Victora, *Lancet* **2016**, *387*, 491.
[3] M. B. Belfort, P. J. Anderson, V. A. Nowak, K. J. Lee, C. Molesworth, D. K. Thompson, L. W. Doyle, T. E. Inder, *J. Pediatr.* **2016**, *177*, 133.
[4] B. E. Lechner, B. R. Vohr, *Clinics Perinatol.* **2017**, *44*, 69.
[5] B. L. Horta, B. A. Sousa, C. L. de Mola, *Curr. Opin. Clin. Nutr. Metab. Care* **2018**, *21*, 174.
[6] W. W. Pang, P. T. Tan, S. Cai, D. Fok, M. C. Chua, S. B. Lim, L. P. Shek, S.-Y. Chan, K. H. Tan, F. Yap, P. D. Gluckman, K. M. Godfrey, M. J. Meaney, B. F. P. Broekman, M. S. Kramer, Y.-S. Chong, A. Rifkin-Graboi, *Eur. J. Nutr.* **2020**, *59*, 609.
[7] W. Witt, H. Nicolai von, F. Zilliken, *Nutr. Metab.* **1979**, *23*, 51.
[8] A. T. Mudd, S. A. Fleming, B. Labhart, M. Chichlowski, B. M. Berg, S. M. Donovan, R. N. Dilger, *Nutrients* **2017**, *9*, 1297
[9] A. J. Tarr, J. D. Galley, S. E. Fisher, M. Chichlowski, B. M. Berg, M. T. Bailey, *Brain Behav. Immun.* **2015**, *50*, 166.
[10] K. Obelitz-Ryom, S. B. Bering, S. H. Overgaard, S. F. Eskildsen, S. Ringgaard, J. L. Olesen, K. Skovgaard, S. Pankratova, B. Wang, A. Brunse, A. B. Heckmann, M. P. Rydal, P. T. Sangild, T. Thymann, *Nutrients* **2019**, *11*, 1335.
[11] S. Rudloff, C. Kunz, *Adv. Nutr.* **2012**, *3*, 398S.
[12] B. Wang, *Adv. Nutr.* **2012**, *3*, 465S.
[13] E. Oliveros, E. Vázquez, A. Barranco, M. Ramírez, A. Gruart, J. M. Delgado-García, R. Buck, R. Rueda, M. J. Martín, *Nutrients* **2018**, *10*, 1519.
[14] S. Kuntz, C. Kunz, C. Borsch, E. Vazquez, R. Buck, M. Reutzel, G. P. Eckert, S. Rudloff, *Mol. Nutr. Food Res.* **2019**, e1900035.
[15] M. Hobbs, M. Jahan, S. A. Ghorashi, B. Wang, *Foods* **2021**, *10*, 473.
[16] C. E. Galuska, S. Rudloff, S. Kuntz, C. Borsch, M. Reutzel, G. Eckert, S. P. Galuska, C. Kunz, *J. Funct. Foods* **2020**, *75*, 104268.
[17] M. Schneider, E. Al-Shareffi, R. S. Haltiwanger, *Glycobiology* **2017**, *27*, 601.
[18] H. E. Murrey, C. I. Gama, S. A. Kalovidouris, W.-I. Luo, E. M. Driggers, B. Porton, L. C. Hsieh-Wilson, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 21.
[19] H. Matthies, S. Staak, M. Krug, *Brain Res.* **1996**, *725*, 276.
[20] G. W. Hart, *Nat. Chem. Biol.* **2006**, *2*, 67.
[21] N. Tosh, S. Quadrelli, G. Galloway, C. Mountford, *Sci. Rep.* **2019**, *9*, 18806.
[22] T. V. Bliss, G. L. Collingridge, *Nature* **1993**, *361*, 31.
[23] W. Pohle, L. Acosta, H. Rüthrich, M. Krug, H. Matthies, *Brain Res.* **1987**, *410*, 245.
[24] E. Vazquez, A. Santos-Fandila, R. Buck, R. Rueda, M. Ramirez, *Brit. J. Nutr.* **2017**, *117*, 237.
[25] E. Vazquez, A. Barranco, M. Ramirez, A. Gruart, J. M. Delgado-Garcia, M. L. Jimenez, R. Buck, R. Rueda, *PLoS One* **2016**, *11*, e0166070.
[26] C. Milani, S. Duranti, F. Bottacini, E. Casey, F. Turroni, J. Mahony, C. Belzer, S. D. Palacio, S. A. Montes, L. Mancabelli, G. A. Lugli, J. M. Rodriguez, L. Bode, W. de Vos, M. Gueimonde, A. Margolles, D. van Sinderen, M. Ventura, *Microbiol. Mol. Biol. Rev.* **2017**, *81*, e00036-17.
[27] T. Katoh, M. N. Ojima, M. Sakanaka, H. Ashida, A. Gotoh, T. Katayama, *Microorganisms 8*, **2020**, 481.
[28] M. Sakanaka, A. Gotoh, K. Yoshida, T. Odamaki, H. Koguchi, J.-Z. Xiao, M. Kitaoka, T. Katayama, *Nutrients* **2019**, *12*, 71.
[29] P. Louis, G. L. Hold, H. J. Flint, *Nat. Rev. Microbiol.* **2014**, *12*, 661.
[30] K. C. Bauer, T. Rees, B. B. Finlay, *BioEssays* **2019**, *41*, 1800268.
[31] V. Osadchiy, C. R. Martin, E. A. Mayer, *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 322.
[32] B. Dalile, L. van Oudenhove, B. Vervliet, K. Verbeke, *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 461.

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[33] S. Rudloff, S. Obermeier, C. Borsch, G. Pohlentz, R. Hartmann, H. Brösicke, M. J. Lentze, C. Kunz, *Glycobiology* **2006**, *16*, 477.
[34] S. Rudloff, G. Pohlentz, C. Borsch, M. J. Lentze, C. Kunz, *Brit. J. Nutr.* **2012**, *107*, 957.
[35] V. Dotz, S. Rudloff, C. Meyer, G. Lochnit, C. Kunz, *Mol. Nutr. Food Res.* **2015**, *59*, 355.
[36] K. C. Goehring, A. D. Kennedy, P. A. Prieto, R. H. Buck, *PLoS One* **2014**, *9*, e101692.
[37] L. R. Ruhaak, C. Stroble, M. A. Underwood, C. B. Lebrilla, *Anal. Bioanal. Chem.* **2014**, *406*, 5775.
[38] S. Kuntz, C. Kunz, C. Borsch, E. Vazquez, R. Buck, M. Reutzel, G. P. Eckert, S. Rudloff, *Mol. Nutr. Food Res.* **2019**, *63*, 1900035.
[39] C. R. Martin, V. Osadchiy, A. Kalani, E. A. Mayer, *Cell. Mol. Gastroenterol. Hepatol.* **2018**, *6*, 133.
[40] E. A. Mayer, K. Tillisch, A. Gupta, *J. Clin. Invest.* **2015**, *125*, 926.
[41] J. W. Coffey, O. N. Miller, O. Z. Sellinger, *J. Biol. Chem.* **1964**, *239*, 4011.
[42] G. R. Harsh, R. N. Nishimura, B. E. Dwyer, V. A. Levin, *Exp. Neurol.* **1986**, *94*, 21.
[43] T. J. Wiese, J. A. Dunlap, M. A. Yorek, *J. Biol. Chem.* **1994**, *269*, 22705.
[44] C. Yamada, A. Gotoh, M. Sakanaka, M. Hattie, K. A. Stubbs, A. Katayama-Ikegami, J. Hirose, S. Kurihara, T. Arakawa, M. Kitaoka, S. Okuda, T. Katayama, S. Fushinobu, *Cell Chem. Biol.* **2017**, *24*, 515.e5.
[45] T. Katayama, *Biosci. Biotechnol. Biochem.* **2016**, *80*, 621.

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