Colonic Fermentation of Inulin Increases Whole-Body Acetate Turnover in Dogs

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ABSTRACT Metabolism of acetate from colonic fermentation was investigated in dogs. Beagle dogs (n = 9) were fed a control diet for 17 d followed by a 3% inulin–enriched diet (from chicory) for 4 and 21 d. On 3 occasions, the dogs were administered simultaneously infusions of \([1,2-\text{\textsuperscript{13}}\text{C}]\)acetate i.v. and \([1,2-\text{\textsuperscript{13}}\text{C}]\)acetate intrarectally. Peripheral acetate concentration and turnover did not change over time after consumption of an inulin-enriched diet for 4 d. After 21 d of consuming the inulin-enriched diet, the whole-body acetate turnover increased significantly by 31% from (mean ± SEM) 15.6 ± 2.2 to 20.4 ± 2.9 \(\mu\text{mol/(kg} \cdot \text{min)}\) without a change in concentration. The rate of colonic acetate production that reached the peripheral circulation was 4.8 ± 1.8 \(\mu\text{mol/(kg} \cdot \text{min)}\). However, no \([1,2-\text{\textsuperscript{13}}\text{C}]\)acetate tracer was recovered in the peripheral circulation. The fraction of oxidized tracer was higher in the gut (64 ± 3%) than in peripheral circulation (46 ± 3%) in dogs fed an inulin-enriched diet for 21 d. In conclusion, colonic fermentation of inulin occurred and indirectly stimulated whole-body acetate turnover in dogs fed an inulin-enriched diet for 21 d. J. Nutr. 135: 2845–2851, 2005.

KEY WORDS: • acetate • colonic fermentation • dogs • stable isotope

Dietary fiber plays an important role in the nutritive value of diets consumed by omnivorous species, including dogs. Fiber comprises that part of a vegetable diet that is indigestible by small intestine enzymes, and reaches the active microflora of the cecum and colon. This microflora degrades the fermentable part of these fiber substrates into gases and SCFAs, mainly acetate, propionate, and butyrate as major end-products. Although dogs have a smaller colon than many other species, SCFA likely are produced in sufficient amounts to induce beneficial effects in the whole body (1,2). These benefits include enhanced colonic motility, mucus production, and blood flow to the large intestine (3). Among SCFA, butyrate was found to minimize the development of cecocolonic carcinogenic tumors in a rat model (4). Ex and in vivo studies in rats suggested that propionate reduced cholesterol synthesis in hepatocytes probably by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase activity (5), whereas other authors had previously concluded that SCFA were not likely responsible for the hypercholesterolemic effect of water-soluble fiber (6). The major SCFA end-product is acetate, which is a directly available energy source. It is taken up and released by most tissues of nonruminants and especially by the intestine (7,8). A recent study in humans showed that oral intake of acetate modified the fibrinogen architecture network, thereby having a beneficial effect on blood fluidity (9). These SCFAs are considered to be relevant not only for gut health but also, especially acetate, for peripheral circulation metabolism (7–10).

Acetate is the main SCFA encountered in the peripheral circulation. It originates either from endogenous production or from colonic fermentation of dietary fiber. Most cells are capable of utilizing acetate via the acetyl-CoA synthetase enzyme and of releasing acetate via the acetyl-CoA hydrolase enzyme (11). Previous studies showed inconsistencies in acetate production rate that ranged from 3 to 24 \(\mu\text{mol/(kg} \cdot \text{min)}\) in the peripheral circulation of dogs (8,10). Acetate production in humans, as measured by the stable isotope dilution technique, was increased by 60% after oral intake of nondigestible lactulose (12). However, in dogs that were not adapted to fiber intake, the acetate turnover did not increase significantly after oral intake of lactulose (13). Although the dog’s colon is shorter than that of humans and their microflora is different (14), Zentek et al. (1) and Pouteau et al. (15) showed that there was hydrogen production after the intake of nondigestible fructans (a class of fermentable carbohydrates that includes inulin) had divergent effects (a decrease in fecal ammonia concentration, with no change in amines, indole, or phenols contents) on fermentative end-products in dogs. Still more recently, Spears and Fahey suggested that copious amounts of SCFA are produced in the large intestine of dogs that consumed products based on resistant starch (2). Few data exist on in vivo SCFA production in companion animals. The present work was performed first to determine acetate turnover and provide new data on the fate of acetate in the whole body of dogs before the fermentation state. Second, the study was extended to assess, via acetate turnover, whether the large intestine of dogs could degrade a nondigestible carbohydrate into SCFAs that were detectable in the peripheral blood circulation. A stable-isotope study was conducted to investigate the short- and long-term effects in...
dogs of an inulin-enriched diet on whole-body acetate turnover.

MATERIALS AND METHODS

Experimental design

Animals and diets. Healthy adult Beagle dogs (n = 9; 4 females, 5 males; 13.8–17.5 kg) were used in the present study. The dogs were supplied by the kennels of the National Veterinary School of Nantes (France) after approval from the ethical committee for clinical experimentation on animals of the National Veterinary School of Nantes. Dogs were adapted to a dry diet (control, 0% inulin) and then switched to a dry diet enriched with 6% chicory (3% inulin) (Table 1). Except for days on which kinetic studies were performed, dogs were fed once each day in the morning at a level of 532 kJ/kg0.75 (metabolizable energy content of the diet = 14.630 MJ/kg).

Study framework. The present study was a longitudinal clinical study. Each dog was food deprived for 22 h and then subjected to a kinetic study on 3 occasions. The first study was performed after the dogs had consumed the control diet for 17 d to normalize the status of their intestinal tract. A 2nd kinetic study was performed after consumption of a diet enriched with 6% chicory for 4 d as a transition period (short-term inulin). Finally, a 3rd kinetic study was performed after the dogs had consumed the inulin-enriched diet for 21 d, mimicking a chronic intake of inulin (long-term inulin). During the present study, home-living conditions were a prerequisite. Dogs were free in their kennels, except for the days of the kinetic studies when they were made as comfortable as possible.

Kinetic studies. During the kinetic studies, dogs were fed discrete meals (Fig. 1). For the control diet study, the dogs were fed their daily intake of the control diet in 16 equally portioned meals every 0.5 h from T = T0 to 8 h. During the short- and long-term inulin studies, the dogs were fed the same sized portions of the 6% chicory-enriched diet on the same schedule as above. The total food intake was 2.40 ± 0.02 g/(kg·h).

On the morning of the kinetic studies after 22 h of food deprivation, a polyethylene catheter (20 gauge, Vigon) was inserted into the cephalic vein of one forelimb for stable isotope tracer infusion. A second catheter was placed in the cephalic vein of the other forelimb for blood sampling. A venous blood sample (2 mL of heparinized blood) was collected at time zero (T0) and immediately in an Exetainer tube (20 mL) and 30 mL in a syringe. Immediately, the dogs were administered a primed i.v. infusion of [1-13C]acetate for 8 h (acetate = 125.8 ± 2.7 μmol/kg, infusion rate = 2.07 ± 0.05 μmol/kg·min) via the intracolonic (i.c.) tubing (Fig. 1).

Dogs were also placed in an air-regulated box linked to a breath gas monitor (DeltaTrac indirect calorimeter) from time T = 2 to 8 h corresponding to the i.v.-i.c. period steady state (i.v.-i.c. tracer only) and from time T = 6 to 8 h corresponding to the i.v.-i.c. period steady state (i.v. tracer). Venous blood (2 mL) and breath samples (aliquots of 10 mL in an Exetainer tube and of 30 mL in a syringe) were collected every 0.5 h throughout the study. Plasma and breath samples were kept at −80 °C and at +4 °C, respectively, until analysis. Feces were collected after the kinetic study on the same day or the next day if not available on d 1, and aliquots were kept at −80 °C until analysis. At the end of the kinetic studies, the catheters and intracolonic tubing were removed, and the dogs were moved out of the air-regulated box.

Analytical procedures

Plasma acetate isotopic enrichment and SCFA concentrations. Analysis of plasma acetate enrichments was based on a previously published method (17). The procedure was adapted to measure simultaneously plasma concentrations of acetate, propionate, and butyrate and isotopic enrichment of [1-13C]acetate (M + 1) and [1,2-13C2]acetate (M + 2) (18). Briefly, plasma (450 μL) was spiked with [D3]acetate (Isotec) as an internal standard, deproteinized, acidified, and extracted with diethyl-ether (99.8%, Fluka). SCFAs were derivatized with tert-butyldimethylsilyl-imidazole (TBDS, Fluka Chemika) and analyzed by GC–MS (model 5970A, Hewlett Packard; SSQ700, Finnigan Mat). Calibration curves for plasma concentrations showed excellent linearity from 1 to 1000 μmol/L for acetate, propionate, and butyrate. Calibration curves for isotopic enrichments (M + 1) and (M + 2) ranged from 0.5 to 15 mol % excess (MPE) for

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**TABLE 1**

Composition by product categories of control and inulin-enriched diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
<th>Inulin-enriched diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g</td>
<td>g/100 g</td>
<td></td>
</tr>
<tr>
<td>Cereals (wheat and maize)</td>
<td>57.456</td>
<td>51.243</td>
</tr>
<tr>
<td>Meat and animal by-products (poultry)</td>
<td>19.794</td>
<td>19.781</td>
</tr>
<tr>
<td>Plant by-products</td>
<td>10.705</td>
<td>10.697</td>
</tr>
<tr>
<td>Oils and fats</td>
<td>6.469</td>
<td>6.469</td>
</tr>
<tr>
<td>Soybean protein extracts</td>
<td>6.176</td>
<td>6.172</td>
</tr>
<tr>
<td>Extruded chicory roots</td>
<td>1.552</td>
<td>1.551</td>
</tr>
<tr>
<td>Minerals</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>Vitamins</td>
<td>0.263</td>
<td>0.263</td>
</tr>
<tr>
<td>Preservatives (including antioxidants)</td>
<td>0.263</td>
<td>0.263</td>
</tr>
</tbody>
</table>

1 In the inulin-enriched diet, extruded chicory roots replaced an equal amount of cereal.

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2 Abbreviations used: GC/IRMS, GC/isotope ratio MS; i.c., intracolonic; MPE, mol % excess; PDB, Pee Dee Belemnite; Ra, rate of appearance, TBDSM, tert-butyldimethylsilyl-imidazole; TEE, total energy expenditure.
INULIN INCREASES PLASMA ACETATE IN DOGS

[1-13C]acetate and [1,2-13C2]acetate and showed excellent linearity (18).

**Fecal SCFA concentrations.** SCFA concentrations in feces were measured using an adaptation of the procedure for plasma described above. Aliquots of feces (250 mg) were diluted in 1 mL of aqueous solution (0.1% HgCl2, 0.1% H3PO4), and 50 µL of [1-13C]acetate, as internal standard, was added. The aqueous solution was homogenized by mixing on a vortex (20 min). After centrifugation (5 min, 12000 x g) 20 µL of HCl (32%) was added. Part of the upper liquid layer (450 µL) was then extracted with diethyl ether (3 mL). Thereafter, sample preparation was identical to that described above. Fecal SCFA concentrations were expressed in µmol/g whole feces. [1,2-13C2]acetate enrichment in feces was determined simultaneously and expressed in MPE.

**Indirect calorimetry.** Breath gas exchanges (oxygen and carbon dioxide) were measured by indirect calorimetry (Datex-Ohmeda, Deltrac II) using a controlled-ventilation box adapted to Beagle dogs. Oxygen consumption (V\textsubscript{O2}) and carbon dioxide production (V\textsubscript{CO2}) were expressed in mL/min. A laboratory standard gas (Quick Cal calibration gas, Datex-Engstrom) was used for calibration.

**Carbon dioxide isotopic enrichment.** Isotopic enrichment of breath carbon dioxide was measured using an automatic GC linked to an isotopic ratio MS (GC/IRMS, BreathMAT Plus™, Finnigan Mat). A laboratory carbon dioxide standard gas calibrated against the international Pee Dee Belemnite carbonate (PDB) was used as a reference. The data were obtained in δ13C (‰).

**Hydrogen breath test.** Hydrogen and methane concentrations in breath samples were analyzed the day of the kinetic studies using GC (Quintron Microlyser™, Model DP, Quintron Instruments). Results were expressed as parts per million (1 ppm = 0.05 µmol/L for both gases). The minimal detectable breath-hydrogen and -methane were 1 ppm for both gases. The chromatograph was calibrated with a hydrogen and methane reference mixture.

**Calculations**

**Acetate metabolism model.** Acetate metabolism was investigated using a dual tracer approach, [1-13C]acetate was used as the tracer for determination of the peripheral acetate turnover and [1,2-13C2]acetate was used for evaluation of central acetate production. [1-13C]acetate in plasma was determined from13C acetate isotopic enrichment expressed in MPE.

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From the steady state (no significant change in acetate concentration) and isotopic enrichment and the assumption that the tracer was administered into one whole-body compartment, the following equations can be used for assessing kinetic parameters. The acetate compartment was regarded as a uniform pool in which the production rates (rate of appearance) included supplies mainly from exogenous colonic fermentation, liver, and peripheral tissues, and in which the global utilization rates (rate of disappearance) included uptakes from intestine, liver, and peripheral tissue.

**Peripheral rate of appearance of acetate.** The peripheral rate of appearance of acetate (Ra in µmol/(kg·min)) was calculated according to the equation for steady state during the last 2 h of the i.v.-period (Ra\textsubscript{i.v.-period}) and of the i.v.-i.c.-period (Ra\textsubscript{i.v.-i.c.-period}) of kinetic studies:

\[
Ra_{i.v.-i.c.} = i_{i.v.} \cdot (E_{Mt+2}/E_{PaM+1} - 1)
\]

where \(i_{i.v.}\) is the i.v. infusion rate [µmol/(kg·min)], \(E_{Mt+2}\) and \(E_{PaM+1}\) are the isotopic enrichment of the tracer solution ([1-13C]acetate) and of arterial plasma, respectively, given in MPE. \(E_{PaM+1}\) is either the isotopic enrichment during the i.v.- or i.v.-i.c.-periods for determining Ra\textsubscript{i.v.-i.c.}.

According to published data on acetate metabolism using radio-labeled or stable isotope tracers (7,20,21), calculations of Ra were carried out from arterial isotopic enrichments. For this reason, plasma venous isotopic enrichment (MPE) was corrected to estimate the arterial enrichment (Epa\textsubscript{a}). The arterial enrichment was determined from venous enrichment (MPE) divided by a correction factor (0.34) that was obtained from a published study performed on the same breed of dog from the same kennels under identical conditions at a steady postprandial state (8). The correction factor was the mean ratio of venous to arterial plasma acetate isotopic enrichment. Use of estimated arterial isotopic enrichment allowed the rate of appearance of acetate to be calculated.

**Colonial acetate entering the peripheral circulation.** The exogenous acetate supply (colonic fermentation of chicory inulin) reaching the peripheral circulation [Ra\textsubscript{p}\textsubscript{e}, in µmol/(kg·min)] was estimated from the difference in rates of appearance of peripheral acetate between the i.v. period (Ra\textsubscript{i.v.-i.c.}) and the i.v.-i.c. period (Ra\textsubscript{i.v.-i.c.-period}) calculated using [1-13C]acetate as tracer. We assumed a constant endogenous production rate of acetate for this approach equal to Ra\textsubscript{i.v.}.

\[
Ra_{i.v.-i.c.} = Ra_{i.v.} - Ra_{i.v.}
\]

This exogenous acetate supply represents acetate that is not catabolized by the large intestine and the liver and that is available to the peripheral circulation.

**Colonial and whole-body acetate production rate.** Colonial and whole-body production rates (Ra\textsubscript{i.v.-i.c.-period}) of peripheral (colonic fermentation of chicory inulin) and of arterial plasma, respectively, given in MPE.

**Arterial isotopic enrichment (Epa\textsubscript{a}) was estimated from venous plasma isotopic enrichment (MPE) divided by the 0.34 correction factor noted above.**

**Splanchnic first-pass retention.** Splanchnic (intestinal and liver) first-pass removal (Rs, in %) was calculated as follows:

\[
Rs = [1 - (Ra_{i.v.-i.c.}/Ra_{i.v.})] \times 100
\]

where Ra\textsubscript{i.v.-i.c.} is the rate of appearance of acetate during the i.v.-i.c.-period estimated from [1-13C]acetate intravenous infusion and Ra\textsubscript{i.v.} is the rate of appearance of acetate during the i.v.-period estimated from [1,2-13C2]acetate intracolonic infusion (22). Ra\textsubscript{i.v.-i.c.} and Ra\textsubscript{i.v.} were calculated from time T = 6 to 8 h at steady state.

**Fraction of oxidized acetate.** To measure the fraction of oxidized acetate, values of carbon dioxide isotopic enrichment (δ13C in ‰) were transferred to mol % (MP) (23):

\[
MP = 100 \cdot R \cdot ([0.001 \cdot \delta^{13}C + 1]/[1 + R \cdot (0.001 \cdot \delta^{13}C + 1)]
\]

where R is the ratio 13C/12C of the international PDB standard (R
0.0112372) and δ13C is the 13C enrichment of the samples expressed from IRMS measurements (in ‰). The isotopic enrichment of expired CO2 was then expressed in terms of MPE calculated as below:

\[
MPE = MP_{0} - MP_{1}
\]

where MP, and MP\textsubscript{0} are the MP of the sample at time T and at time 0, respectively.

The fraction of intravenous [1-13C]acetate tracer oxidized (fox\textsubscript{a}) was determined from the flux of exhaled 13CO\textsubscript{2} (F\textsubscript{13CO\textsubscript{2}} in µmol/(kg·min)) during the i.v. period:

\[
F_{13CO_{2}} = fox_{a} \cdot c \cdot i_{v}
\]
where $F_{\text{i.v.-i.c.}}$ is initially calculated from the measured isotopic enrichment of exhaled CO$_2$ ($E_{\text{CO}_2}$ in MPE) at plateau during the i.v.-period multiplied by the rate of expired CO$_2$ [$V_{\text{CO}_2}$ in mmol/(kg·min)] obtained from indirect calorimetry. Coefficient $c$ is the recovery coefficient equal to 0.78 for the [1-13C]acetate tracer (24), taking into account the dilution and loss of carbon dioxide in the body bicarbonate pool. Therefore,

$$foxi = F_{\text{i.v.-i.c.}}/(c \cdot i.v.)$$

The fraction of intracolonic [1,2-13C]acetate tracer oxidized ($foxi$) was determined from the flux of exhaled 13CO$_2$ ($F_{\text{13CO}_2}$; in mmol/(kg·min)) during the i.v.-i.c. period. $F_{\text{13CO}_2}$ was initially calculated from the measured isotopic enrichment of exhaled CO$_2$ ($E_{\text{CO}_2}$ in MPE) at plateau during the i.v.-i.c. period multiplied by the rate of expired CO$_2$ [$V_{\text{CO}_2}$ in mmol/(kg·min)] obtained from indirect calorimetry. Because the tracer was doubly labeled, the $i.v.$ infusion was equivalent to 2 infusions at the same rate, one of [1-13C]acetate and another of [2-13C]acetate. Therefore, the coefficient $c$ was applied to the equivalent [1-13C]acetate infusion and the coefficient $c'$ to the equivalent [2-13C]acetate infusion. The coefficient $c'$ was 0.41 to take into account the slower time to recovery in breath because this carbon undergoes futile cycles via the citric acid cycle before entering the bicarbonate pool (8, 24).

Hence, if one assumes that $F_{\text{i.v.-i.c.}}$ is constant during the observed period, then:

$$foxi = [F_{\text{i.v.-i.c.}} - F_{\text{13CO}_2}]/(c \cdot i.v.)$$

The oxidation of acetate ($Oxi$, in mmol/(kg·min)) was determined during the i.v.-period, as follows (25):

$$Oxi = (E_{\text{CO}_2} \cdot V_{\text{CO}_2})/(c \cdot E_{\text{pao}} \cdot t)$$

The percentage of $V_{\text{CO}_2}$ from oxidation of acetate (%$V_{\text{CO}_2}$) was calculated during the i.v.-period:

$$%V_{\text{CO}_2} = 100 \cdot E_{\text{CO}_2} / (E_{\text{pao}} \cdot 1 + c)$$

The energy ($E_{\text{ace}}$) derived from acetate was calculated during the i.v.-period assuming a production of 879 kJ/oxidized mol (26). The acetate energy contribution ($E_{\text{ace}}$ in %) was calculated as follows:

$$E_{\text{ace}} = 100 \cdot (Oxi \cdot 0.879) / TEE$$

where oxidation of acetate (Oxi) was transposed in mol/d and TEE was the total energy expenditure (in kJ/d) calculated as follows from indirect calorimetry (27):

$$\text{TEE} = 4.184 \cdot [3.941 \cdot V_{\text{CO}_2}/RQ + 1.106 \cdot V_{\text{CO}_2}]$$

where $RQ$ is the respiratory quotient obtained from the ratio of $V_{\text{CO}_2}$ (carbon dioxide production, L/d) to $V_{\text{O}_2}$ (oxygen consumption, L/d).

Statistics. Differences between hydrogen breath gas excretions, plasma acetate, propionate, and butyrate concentrations, plateau values of 13C acetate isotopic enrichments, whole-body peripheral rates of appearance of acetate, and the energy acetate contributions when dogs were fed control and inulin-enriched diets were evaluated by paired t test because the study had a longitudinal design. We assumed that no carry-over effect between diets occurred and that all effects were due to dietary chicory addition. Paired t tests were also used to compare data at plateau between the i.v. period and the i.v.-i.c. period of kinetic studies. Differences with $P < 0.05$ were considered significant. Data are presented as means ± SEM.

RESULTS

Kinetic studies. Both the control diet and the 6% chicory-enriched diet were well liked by the dogs, and no secondary effects such as diarrhea occurred during the 38-d of experiment. The dogs’ body weights did not differ between the control and long-term inulin treatments (15.0 ± 0.4 and 14.8 ± 0.4 kg, respectively).

Breath test. No methane was detected in breath tests in the kinetic studies. Hydrogen concentrations were ~1–2 ppm (0.05–0.10 μmol/L) in all kinetic studies from time 0 to 4 h. From time 4 to 8 h, hydrogen concentrations increased to 4 ppm in the control study and to 7 ppm in the short- and long-term inulin kinetic studies. The cumulative hydrogen monitored during the 8 h of study was greater when dogs consumed the long-term inulin diet than when they consumed the control diet ($P < 0.05$, Fig. 2). Similarly, the area under the curve of breath hydrogen (in ppm/min) was greater when dogs consumed the long-term inulin diet (903 ± 417) than when they consumed the control diet (1220 ± 231, $P < 0.05$); the value when dogs consumed the short-term inulin diet was 1374 ± 251, which was not different from that of dogs consuming the control diet ($P > 0.05$). Orocecal transit time was 4.2 ± 0.3 and 4.2 ± 0.5 h in dogs administered the short- and long-term inulin treatments, respectively, and did not differ from controls (4.5 ± 0.3 h, both $P > 0.05$).

SCFA concentrations. Fecal SCFA concentrations did not differ when dogs consumed the 3 diets. Acetate, propionate, and butyrate concentrations did not differ in venous plasma and were 156.2 ± 3.5, 11.2 ± 0.4 and 25.1 ± 0.1 μmol/L, respectively.

Isotopic enrichment in plasma. There was a rapid increase in plasma [1,13C]acetate M + 1 reaching a steady state, i.e., within 1 h for control and short-term inulin treatments and within 1.5 h for the long-term inulin treatment. Plateau isotopic enrichments in [1,13C]acetate were evaluated during the last 2 h of each i.v.- and i.v.-i.c. period. Enrichments did not differ, although absolute enrichment was lower in dogs administered the long-term inulin treatment during the colonic fermentation i.v.-i.c. period compared with the basal i.v. period (Table 2). Enrichment [1,2-13C]acetate M + 2 from intracolonic infusion was detected below 2 MPE in plasma from only 3 dogs. Enrichment of [1,2-13C]acetate in feces was 2.7 ± 1.4, 2.1 ± 0.7 and 9.9 ± 3.5 MPE for control, short-term inulin, and long-term inulin treatments, respectively. A loss of [1,2-13C]acetate tracer of 4.2 ± 2.0 and 3.2 ± 1.0% was estimated.

**FIGURE 2** Cumulative breath hydrogen in dogs that consumed discrete meals every 0.5 h during the 8-h kinetic studies after consumption of control (17 d), short-term inulin (4 d), and long-term inulin (21 d) diets. Values are means ± SEM, $n = 9$. *Different from control, $P < 0.05$. 

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TABLE 2
Whole-body acetate production. Isotopic M + 1 and M + 2 enrichments of acetate were assessed at the plateau level of the i.v. and i.v.-i.c. periods. The whole-body rates of appearance of acetate Ra_i.v. and Ra_i.v.-i.c. were calculated during the control, short-term, and long-term inulin kinetics. The rates of appearance of acetate from colon and whole body (Ra_i.c.) and the splanchnic retention (Rs) were evaluated during the i.v.-i.c. periods.

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>Plateau isotopic M + 1 enrichment</th>
<th>Rate of appearance of acetate in peripheral circulation</th>
<th>Plateau isotopic M + 2 enrichment</th>
<th>Rate of appearance from colon and whole body</th>
<th>Splanchnic bed retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v. period</td>
<td>i.v.-i.c. period</td>
<td>Ra_i.v.</td>
<td>Ra_i.v.-i.c.</td>
<td>i.v.-i.c. period</td>
</tr>
<tr>
<td>Control Inulin</td>
<td>4.4 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>15.5 ± 2.3</td>
<td>16.0 ± 2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Short term</td>
<td>4.3 ± 0.7</td>
<td>4.4 ± 0.4</td>
<td>16.2 ± 2.0</td>
<td>14.0 ± 1.3</td>
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<tr>
<td>Long term</td>
<td>4.2 ± 0.6</td>
<td>3.2 ± 0.4</td>
<td>15.6 ± 2.2</td>
<td>20.4 ± 2.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1 Values are mean ± SEM, n = 9. * Different from the i.v. period, P < 0.05.
2 Values are mean (each value from n = 2 kinetic studies from 3 different dogs); no statistical analysis was performed.

in feces for control and short-term inulin treatments, respectively. A loss of 10.9 ± 3.3% was estimated during the long-term inulin treatment.

Rates of appearance and colonic supply of acetate. The rate of appearance of acetate in the whole-body peripheral circulation from T = 0 to 4 h (Ra_i.v.) during control, short-term inulin, and long-term inulin treatments was 15–16 μmol/(kg·min), with no differences among the treatments (Table 2). The rate of appearance Ra_i.v.-i.c. from T = 4 to 8 h did not differ between the control and short-term inulin treatments (Table 2). However, there was a significant increase of 31% in rate of appearance of acetate (Ra_i.v.-i.c.) after the colonic fermentation state (i.v.-i.c. period) in the long-term inulin treatment compared with the baseline rate of appearance of acetate (Ra_i.v.), Ra_i.v.-i.c. = 20.4 ± 2.9 μmol/(kg·min) (P < 0.05). Subtracting Ra_i.v.-i.c. from Ra_i.v. after long-term inulin treatment revealed that the supply of acetate that reached peripheral circulation from the colonic bacterial fermentation of nondigested inulin was 4.8 ± 1.8 μmol/(kg·min).

M + 2 acetate was detected in plasma of only 3 dogs. Colonic and whole-body rate of appearance of acetate, Ra_i.c., was evaluated [63 ± 4 μmol/(kg·min), n = 3], but large variations were found. However, the mean splanchnic bed retention was evaluated in these 3 dogs to be 64 ± 9% within all kinetics studies. In dogs showing no appearance of M + 2 acetate in peripheral circulation, 100% splanchnic bed retention was assumed. Therefore, the overall mean splanchnic bed retention was 89 ± 21, 92 ± 13, and 94 ± 8% for control, short-term inulin, and long-term inulin treatments, respectively.

Acetate oxidation, contribution to TEE (i.v. period), and fraction of tracer acetate oxidized (i.v. and i.v.-i.c. periods). Total energy expenditure (TEE) was measured for all treatments at both the i.v. and i.v.-i.c. periods (Table 3). During the i.v.-i.c. period, TEE was 206 ± 10, 213 ± 11, and 228 ± 6 kJ/(kg·d) for control, short-term inulin, and long-term inulin treatments, respectively (P > 0.05). Indirect calorimetry indicated respiratory quotients of 0.88–0.92. Breath V_CO2 var-

TABLE 3
Carbon dioxide and acetate oxidation. The isotopic enrichments of breath CO₂ and breath CO₂ (V_CO2) and fraction of acetate oxidized fox_i.v. (from i.v.-infusion) and fox_i.c. (from i.c. infusion) were assessed at steady state before and during colonic fermentation (i.v. period and i.v.-i.c. period, respectively). Acetate oxidation rate, the percentage of rate of appearance (% Ra), TEE, and acetate energy contribution to TEE were assessed at steady state during the i.v.-period of kinetics

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>CO₂ before colonic fermentation (i.v. period)</th>
<th>CO₂ during colonic fermentation (i.v.-i.c. period)</th>
<th>Acetate oxidation before colonic fermentation (i.v. period)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂ isotopic enrichment</td>
<td>V_CO2</td>
<td>fox_i.v.</td>
</tr>
<tr>
<td></td>
<td>MPE</td>
<td>mL/min</td>
<td>MPE</td>
</tr>
<tr>
<td>Control Inulin</td>
<td>0.33 ± 0.02</td>
<td>90.2 ± 6.5</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Short term</td>
<td>0.36 ± 0.01</td>
<td>88.9 ± 6.4</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>Long term</td>
<td>0.31 ± 0.02</td>
<td>103.3 ± 4.8</td>
<td>0.64 ± 0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Symbols indicate different from the i.v. period, * P < 0.05; † P < 0.01. * Short term different from control, P < 0.05.
** Long term different from control, P < 0.05.
Whole-body acetate production rate appears from either an endogenous source, such as peripheral tissues and the liver, or from exogenous cecocolonic production (7,28). To evaluate the exogenous production of acetate in dogs fed a diet rich in inulin, 9 Beagle dogs participated individually in 3 kinetic studies. The first kinetic study was performed after a washout period (control diet), and the second and third after the feeding of an inulin-enriched diet for 4 and 21 d, respectively. The kinetic studies consisted of an i.v. infusion of [1-13C]acetate and of an i.c. infusion of [1,2-13C2]acetate. The whole-body appearance rate of acetate was measured at a steady postprandial state before the diet reached the colon. The whole-body appearance rate of acetate increased by 31% up to 20 μmol/(kg·min) 4–6 h after the start of the inulin-enriched diet feeding in dogs adapted for 21 d to this diet. This increase occurred during colonic fermentation, which was indicated by an increase in breath hydrogen. These data show that the colonic microflora of dogs adapted for 21 d to an inulin-enriched diet produced hydrogen and a large amount of acetate. However, not all of the labeled acetate was recovered in plasma, especially the intestinal [1,2-13C2]acetate tracer, indicating that much of the colonic acetate escaped detection.

Kinetic studies were designed in 2 periods, with the first as an i.v. period related to a steady baseline before the colonic fermentation of inulin and the second as an i.v.-i.c. period (T = 4–8 h) related to the colonic fermentation of chicory inulin. The hydrogen breath test corroborated our initial hypothesis that during the i.v. period, negligible colonic acetate would form, whereas during the i.v.-i.c. period, a noticeable amount of acetate produced by colonic fermentation would reach the peripheral circulation in dogs fed an inulin-enriched diet for 21 d. This exogenous colonic acetate increased whole-body acetate turnover.

Whole-body acetate turnover was reported recently to be ~3 μmol/(kg·min) in dogs undergoing [13C]acetate infusion when glucose was infused in parallel (10). Using radiolabeled [14C]acetate, Bleiberg et al. (7) estimated peripheral acetate turnover in dogs to be 8 μmol/(kg·min). A recent study in Beagle dogs using an infusion of [1,13C]acetate showed an acetate turnover of 24 μmol/(kg·min) (8). Discrepancies occurred among studies and probably originate from the methodology and from the various physiologic states of the dogs. The turnover of acetate in Beagle dogs before the fermentation state in this study was ~16 μmol/(kg·min).

After a short period of adaptation to chicory (of 4 d), there was no change in acetate turnover in this study. Similarly, a previous study did not show an increase in acetate turnover in Beagle dogs after acute lactulose ingestion (13). An acetate turnover increase [5 μmol/(kg·min)] was observed in humans after an oral intake of 20 g of lactulose without any adaptation period to the nondigestible disaccharide (12). In dogs, an increase in acetate turnover was observed only after 21 d feeding an inulin-enriched diet. Dogs have a shorter colon with a microflora that is probably less adapted and responsive to carbohydrate degradation compared with humans. This could explain the observation in the acute study. Nevertheless, efficient activity of the colonic microflora was demonstrated by an increase in both acetate turnover and breath hydrogen after 21 d of adaptation. In our study, the dogs consumed ~10 g of inulin/d and each 8-h kinetic study. Accounting for the stoichiometric equation established in vitro by Roberfroid et al. (29), the theoretical acetate production rate in the dog’s colon could be estimated to be 10.8 μmol/(kg·min). If one applies to dogs, a model that was proposed in humans and takes into account a splanchic bed of 60%, ~4.4 μmol/(kg·min) of acetate would reach the peripheral circulation in the present study (12). This predicted increase closely matched the exogenous acetate supply that was determined to reach the peripheral circulation (4.8 μmol/(kg·min). The present observation therefore confirmed the validity, in dogs, of the model of acetate metabolism previously described in humans (12).

Nevertheless, the most striking result was the lack of detection of colonic [1,2-13C2]acetate in the peripheral circulation. Although infusion of [1,2-13C2]acetate into the large intestine was confirmed in all individuals by carbon dioxide isotopic enrichment in breath, plasma [1,2-13C2]acetate enrichment was detected in only 3 dogs. About 40–50% of the doubly labeled acetate that is administrated into the large intestine was catabolized into carbon dioxide. In contrast to Propst et al. (30) who observed an increase in fecal SCFA after ingestion of oligofructose or inulin, there was no change in acetate and other SCFA concentrations in feces. The acetate remaining in the colon was then either metabolized by bacteria or utilized by enterocytes and further by liver cells. The liver was indeed described to synthesize fatty acids from acetate depending on acetate portal concentrations (31). The liver would play a role in the homeostasis of...
plasma acetate (32,33). This suggests that the increase in acetate turnover in the peripheral circulation might not originate directly from the colon. An explanation would be that portal acetate enters a nonhomogenous acetyl-CoA pool in the extramitochondrial area of hepatocytes (11,34,35). With acetate and acetyl-CoA molecule exchanges in equilibrium, colonic acetate would thereby enter the cytosolic area of hepatocytes and would not mix with endogenous production of acetyl-CoA and subsequent acetate. Because the cellular pool of acetate could be overloaded by the supply of colonic acetate, the colonic acetate would likely be diverted from the peripheral route into mitochondrial oxidation or cellular restoration (lipid synthesis) (31). Assessment of lipid 13C-enrichment in liver during i.v. vs. i.c. tracer infusions would clarify the nonhomogenous pools. Colonic acetate would not reach the peripheral circulation; this was confirmed by a recent observation that the liver of dogs under surgery retains ~100% of labeled acetate (10). It is possible that the arrival of colonic acetate would indirectly stimulate a release of hepatic acetate into the peripheral circulation matching the model discussed above. The net increase in peripheral acetate turnover, which was demonstrated in the present study, would originate de novo from the liver, after indirect stimulation from the colonic fermentation of inulin. The present work show in vivo that the fate of acetate in the whole body, especially in the splanchnic bed, is far from being clearly understood and warrants further investigation and clarification.

Finally, acetate metabolism in dogs originates mainly from an endogenous regulation and production. Although direct colonic acetate could not be detected in the peripheral circulation, dogs adapted for 21 d to inulin from chicory showed 1) a remarkable increase in peripheral acetate turnover simultaneous with an increase in breath hydrogen and 2) a lower fraction of acetate oxidized in the colon than in the peripheral circulation. More colonic acetate was available in the splanchnic bed, not for oxidation, but likely for anabolism. The feeding of inulin to dogs for 21 d had an indirect effect on the whole-body metabolism of acetate, probably by stimulating the liver acetate-acetyl-CoA cycle. Complementary kinetic studies are required to elucidate the fate of all of the acetate in the whole body. It is clear that acetate plays an important role in the splanchnic bed and whole-body metabolism of dogs.

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