Chicory increases acetate turnover, but not propionate and butyrate peripheral turnovers in rats

Etienne Pouteau*, Florence Rochat, Alfred Jann, Isabelle Meirim, Jose-Luis Sanchez-Garcia, Kurt Ornstein, Bruce German and Olivier Ballèvre

Nestlé Research Centre, Nutrition and Health Department, Vers-Chez-Les-Blanc, PO Box 44, 1000 Lausanne 26, Switzerland

(Received 8 December 2006 – Revised 19 June 2007 – Accepted 29 June 2007)

Chicory roots are rich in inulin that is degraded into SCFA in the caecum and colon. Whole-body SCFA metabolism was investigated in rats during food deprivation and postprandial states. After 22 h of food deprivation, sixteen rats received an IV injection of radioactive $^{13}$C-labelled SCFA. The volume of distribution and the fractional clearance rate of SCFA were 0.25–0.27 litres/kg and 5.4–5.9 %/min, respectively. The half-life in the first extracellular rapidly decaying compartment was between 0.9 and 1.4 min. After 22 h of food deprivation, another seventeen rats received a primed continuous IV infusion of $^{13}$C-labelled SCFA for 2 h. Isotope enrichment ($^{13}$C) of SCFA was determined in peripheral arterial blood by MS. Peripheral acetate, propionate and butyrate turnover rates were 29, 4 and 0.3 μmol/kg per min respectively. Following 4 weeks of treatment with chicory root or control diets, eighteen fed rats received a primed continuous IV infusion of $^{13}$C-labelled SCFA for 2 h. Intestinal degradation of dietary chicory lowered caecal pH, enhanced caecal and colonic weights, caecal SCFA concentrations and breath H2. The diet with chicory supplementation enhanced peripheral acetate turnover by 25 % ($P < 0.017$) concomitant with an increase in plasma acetate concentration. There were no changes in propionate or butyrate turnovers. In conclusion, by setting up a multi-tracer approach to simultaneously assess the turnovers of acetate, propionate and butyrate it was demonstrated that a chronic chicory-rich diet significantly increases peripheral acetate turnover but not that of propionate or butyrate in rats.

Short-chain fatty acids: Chicory: Kinetics: Rats

Dietary fibres are fermented in the large intestine of man by anaerobic bacteria to produce SCFA, such as acetate, propionate and butyrate, and some gases. SCFA are found in large quantities in portal vein blood after intestinal absorption and to a lesser extent in peripheral blood. Acetate is the most prominent SCFA in peripheral blood, it is favoured by most cells to a lesser extent in peripheral blood. Acetate is the most prominent SCFA in peripheral blood, it is favoured by most cells.

Abbreviations: FCR, fractional clearance rate; IV, intravenous; MPE, mole percent excess; MRT, mean residence time; MS, mass spectrometry; Ra, rate of appearance; T1/2, half-life of first phase (rapid decay); T2/3, half-life of second phase (elimination decay); VD, volume of distribution.

* Corresponding author: Dr Etienne Pouteau, fax +41 21 785 85 44, email etienne.pouteau@rdls.nestle.com
Service Vétérinaire, Etat de Vaud, Lausanne, Switzerland). Wistar rats were obtained from the Charles River Company (L’Arbresle, France). [1-14C]acetate, Na salt (50 mCi), [1-14C]propionate, Na salt (250 mCi) and [1-14C]butyrate, Na salt (50 mCi) were purchased from NEN Life Science Products, Inc. (Boston, MA, USA). [1,2-13C2]acetate, [1-13C]acetate, [1-13C]propionate and [1-13C]butyrate Na salt tracers were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). A conventional diet was used in experiments 1 and 2, which contained 23·5 % protein, 5·5 % fat, 52 % carbohydrate and 3 % fibre (Kliba 3310; Promivi Kliba SA, Kaiseraugst, Switzerland). The diets in experiment 3 were the same conventional diets enriched or not with 10 % chicory (chicory dry root; Friskies SA, Amiens, France). The chicory was dry chicory roots composed of 50–60 % inulin, a non-digestible fructo-oligosaccharide. The chicory diet contained 8 % fibre. The metabolisable energy of the diet was 14·0 kJ/g. The animals were adapted to the diet for at least 1 week before all experiments.

Rat model. A rat model was developed for intravenous (IV) tracer infusion and intra-arterial blood sample collection. The rat was anaesthetised with isoflurane (Baxter AG, Volketswil, Switzerland) for the duration of catheter insertion and tracer kinetics. Particular attention was taken to gently isolate the femoral vein and/or artery without injuring them. For all experiments, a catheter (24G Insyte; Becton Dickinson AG, Bale, Switzerland) was inserted into the femoral artery and a first blood sample was taken at time zero (100 μl). Each rat was injected with either [1-14C]acetate, Na salt (5·497 (SEM 0·088) mCi, 2·7483 μmol), [1-14C]propionate (6·407 (SEM 0·057) mCi, 0·1140 μmol) or [1-14C]butyrate (6·990 (SEM 0·007) mCi, 0·3548 μmol) in a randomised order. Arterial blood samples (100 μl) were collected every 30 s until 5 min, every 60 s until 10 min and at 12, 14, 16, 18, 20, 25 and 30 min. The rats were then killed. The blood samples were analysed the same day.

Experiments

Experiment 1. The experiment had a parallel design. Sixteen rats (eight males and eight females; weight 322·7 ± 13·3 g) were divided into three groups; one received the acetate tracer injection (n 5), another the propionate injection (n 6) and the other the butyrate injection (n 5). Rats that were deprived of food for 22 h were submitted to kinetic studies in which either radiolabelled acetate, propionate or butyrate tracers were used (Fig. 1). The rats were anaesthetised, a catheter was inserted into the femoral artery and a first blood sample was taken at time zero (100 μl). Each rat was injected with either [1-14C]acetate (5·497 (SEM 0·088) mCi, 2·7483 μmol), [1-14C]propionate (6·407 (SEM 0·057) mCi, 0·1140 μmol) or [1-14C]butyrate (6·990 (SEM 0·007) mCi, 0·3548 μmol) in a randomised order. Arterial blood samples (100 μl) were collected every 30 s until 5 min, every 60 s until 10 min and at 12, 14, 16, 18, 20, 25 and 30 min. The rats were then killed. The blood samples were analysed the same day.

Fig. 1. Principle of using isotope-labelled SCFA intravenous infusions to investigate intestinal fermentation from peripheral metabolism of SCFA. Bacterial fermentation in the intestine produces SCFA that are transported through the liver to the peripheral blood circulation. The liver and whole body produce endogenous SCFA. From the isotope-dilution principle, the isotope enrichments in arterial blood reflect the whole-body SCFA turnovers that were estimated in food-deprived rats (Expts 1 and 2) and in the postprandial state (Expt 3 with control and chicory diets).
Chicory and short-chain fatty acid turnover

Stable isotope tracers. Measurements of [1,2-¹³C₂]acetate, [1-¹³C]acetate, [1-¹³C]propionate and [1-¹³C]butyrate enrichments and SCFA concentrations in plasma were performed as previously described. Briefly, plasma samples (450 μl) were spiked with 10 μl [¹³C₆] acetate internal standard. Samples were deproteinised with sulfosalicylic acid solution (10 μl, 1 g/litre; Fluka, Buchs, Switzerland) and centrifuged at 2200 g for 10 min. The supernatant fraction was transferred into a vial containing 10 μl hydrochloric acid (10 M) (Panreac, Barcelona, Spain), to which 3 ml diethyl ether (99-8 %; Fluka, Buchs, Switzerland) were added. The vials were vortexed for 15 min and centrifuged for 10 min at 1200 g, then 8 μl tert-butyl-dimethyl-silyl-imidazole (Fluka Chemika, Buchs, Switzerland) were added to the separated organic phase. The sealed vials were heated to 60°C for 30 min after which they were cooled and evaporated to 200-300 μl. Then 2 μl were injected into a gas chromatograph–mass spectrometer (SSQ7000; Finnigan Mat, Bremen, Germany). Chromatographic separations were carried out using a 30 m × 0.32 mm capillary column (DB5: J&W Scientific, Folsom, CA, USA) using the following temperature programme: initial temperature at 40°C for 1 min, then 70°C/min up to 60°C, 10°C/min up to 110°C and 70°C/min up to 250°C for 1 min. Ammonia chemical ionisation and selected ion monitoring were used on ions m/z 192, 193 and 194 for acetate, m/z 206 and 207 for propionate and m/z 220 and 221 for butyrate for isotope enrichment determination. Selected ion monitoring at m/z 195 was used for internal standard and concentration determination. Plasma concentrations were expressed in μmol/l and isotope enrichments M₀+2 and M₀+1 in mol percent excess (MPE). Calibration curves for plasma concentrations showed excellent linearity from 1 to 1000 μmol/l for acetate, propionate and butyrate. Calibration curves for isotope enrichment (M₀+2 and M₀+1) were linear from 0.5 to 99 MPE for [1,2-¹³C₂]acetate, [1-¹³C]acetate, [1-¹³C]propionate and [1-¹³C]butyrate. 

Caecal and colonic short-chain fatty acid analysis. SCFA concentrations in caecum and colon contents were measured using a method similar to that described earlier for plasma. Samples of faeces (250 mg) were diluted in 1 ml aqueous solution (0.1 % HgCl₂, 0.1 % H₃PO₄). The aqueous solution was homogenised by vortexing (20 min) and centrifuging (5 min; 1200 g). Then 20 μl hydrochloric acid (32 %) were added. Part of the upper liquid layer (450 μl) was then extracted with diethyl ether (3 ml). Thereafter, the sample preparation was identical to that described for plasma earlier. Analysis was performed using the same chromatographic conditions on another gas chromatograph–mass spectrometer (6890 Series; Hewlett Packard, Palo Alto, CA, USA) in electronic ionisation mode with selected ion monitoring at m/z 117, 118, 119, 120 and 131, 132, 133, 134 and 145, 146, 147, 148 and 149 for labelled acetate, propionate and butyrate, respectively. SCFA concentrations in caecal contents were expressed in μmol/g (g of whole caecal content). 

Calculations 

Radiolabelled tracers. Radiolabelled [¹⁴C]SCFA tracers were injected and distributed into a first pool from which tracers were rapidly eliminated into a second pool. A two-compartment

Experiment 2. The experiment had a parallel design. Seventeen rats (adult males; 389±1 (SEM 8.4) g) were divided into three groups; one received the acetate tracer infusion (n = 6), another the propionate infusion (n = 5) and the other the butyrate infusion (n = 6). Stable isotopes of acetate, propionate or butyrate tracers were used to determine their individual turnover rates in rats deprived of food for 22 h (Fig. 1). The catheters were inserted under anaesthesia and a first arterial blood sample was collected at time zero (800 μl). Each rat received, intravenously, a primed continuous infusion of either [1-¹³C]acetate (52.3 (SEM 2.1) μmol/kg and 0.98 (SEM 0.04) μmol/kg per min), [1-¹³C]propionate (48.0 (SEM 1.1) μmol/kg and 0.97 (SEM 0.02) μmol/kg per min) or [1-¹³C]butyrate (49.7 (SEM 1.9) μmol/kg and 1.04 (SEM 0.05) μmol/kg per min) solubilised in 0.9 % NaCl sterile solution. Arterial blood samples (800 μl) were collected at 60, 75, 90, 105 and 120 min. A final blood sample was collected from the portal vein just before the rats were killed. All blood samples were centrifuged (10 min at 1200 g) and plasma stored at −80°C until analysis.

Experiment 3. The experiment had a parallel randomised and controlled design. Eighteen rats (adult males; 378±2 (SEM 2.5) g) were randomly divided into two groups; one received the chicory diet (chicory group; n = 10) and the other the conventional diet (control group; n = 8) for 4 weeks and stable-isotope kinetics were performed on the last day (Fig. 1). Food was not restricted the night before the kinetic studies. Each rat was fed by oral administration 1/12 of its daily intake of either the conventional or the chicory diet (according to their group assignment) every 30 min for 6 h in order to mimic continuous feeding. In the remaining time, each rat was placed in a 10 litre airtight transparent box for 12 min per h during the experiment to collect partially respired air. After the last feeding by oral administration, the catheters were inserted under anaesthesia and a first arterial blood sample was collected (800 μl). Each rat was then administered, intravenously, a primed continuous infusion of [1,2-¹³C₂]acetate, [1-¹³C]propionate and [1-¹³C]butyrate simultaneously (all tracers solubilised in 0.9 % NaCl sterile solution; 287 (SEM 5), 97 (SEM 2) and 49 (SEM 1) μmol/kg priming doses and 2.84 (SEM 0.04), 0.96 (SEM 0.01) and 0.48 (SEM 0.01) μmol/kg per min infusion rates, respectively). Arterial blood samples (800 μl) were collected at 60, 75, 90, 105 and 120 min. A fast blood sample was collected from the portal vein before killing. Mucosa samples from the caecum and the colon were obtained by scraping with a glass slide. Caecal and colonic contents and respective mucosal and non-mucosal intestinal tissues were collected and weighed. Intestinal contents and plasma were stored at −80°C until analysis.

Analysis

Radiolabelled tracers. Blood samples were prepared for β + radioactivity counting. Blood samples (100 μl) were centrifuged and 40 μl plasma were pipetted into a scintillation flask containing 20 ml scintillant (Ultima Gold, Packard Bioscience Comp., the Netherlands). A scintillation counter (PerkinElmerTM, Regford, Switzerland) measured and recorded β + detection in counts per min. Data in counts per min were transformed into dpm accounting for quenching.
modelling approach was used. The decay of [14C]SCFA tracers (in dpm) as a function of time, $y(t)$, during kinetics was fitted by a bi-exponential curve using SigmaPlot (version 4.00) software.12 The equation of the fitting curve was:

$$ y(t) = A_1 \times e^{-k_1 t} + A_2 \times e^{-k_2 t} $$

where $A_1$ and $A_2$ are constants (in dpm), and $k_1$ and $k_2$ are decay constants (in per min).

The rapid decay from the first pool is defined by:

$$ y_1(t) = A_1 \times e^{-k_1 t}, $$

The elimination decay from the second pool is defined by:

$$ y_2(t) = A_2 \times e^{-k_2 t}. $$

The values $A_1$, $A_2$, $k_1$ and $k_2$ were determined using a non-linear least-squares estimation. This procedure determines the values that minimise the value for the residual sum of squares (RSS), defined as:

$$ RSS = \sum \left( z(t) - y(t) \right)^2 $$

where $z(t)$ are measured data at time $t$, $y(t)$ is the value at time $t$ derived from the equation to fit the data from $n$ number of samples per kinetic.

Constants $A_1$ and $A_2$ were thereafter first transformed into $\mu Ci$, since it is known that $1 \mu Ci$ equals $2.22 \times 10^6$ dpm, and further into $\mu Ci/l$, taking into account that $40 \mu l$ plasma were analysed. From this curve fitting, the following parameters were calculated:

The area under the curve (AUC in $\mu Ci \times \text{min/l}$) of the different [14C]SCFA tracer decays:

$$ AUC^\infty = \frac{A_1}{k_1} + \frac{A_2}{k_2} $$

where $A_1$ and $A_2$ are expressed in $\mu Ci/l$, and $k_1$ and $k_2$ in per min.

The volume of distribution (VD in litres/kg) of the different SCFA, where SCFA are considered to be instantaneously and homogeneously distributed:

$$ VD = \frac{\text{dose}}{A_1 + A_2} \times \frac{1}{W} $$

where dose is the radiolabelled tracer dose injected (in $\mu Ci$), and $W$ is the rat weight in kg.

The fractional clearance rate (FCR in %/min) of the different SCFA, the fractional rates at which SCFA are removed from VD:

$$ FCR = 100 \times \frac{A_1 + A_2}{AUC^\infty} $$

The total clearance (C in ml/min) of the different SCFA from VD:

$$ \text{Total} \ C = FCR \times VD \times W \times 1000; $$

The mean residence time (MRT in min) of the different SCFA in VD:

$$ MRT = \frac{\int_0^\infty t \times y(t) dt}{\int_0^\infty y(t) dt} = \frac{A_1 + A_2}{A_1 + A_2} \times \frac{1}{k_1} + \frac{1}{k_2} $$

The half-life of the first phase of the different SCFA, rapid decay from the first compartment ($T_1/2$) (in min):

$$ T_1/2 = \ln(2) \times k_1 $$

The half-life of the second phase of the different SCFA, elimination decay from the second compartment ($T_2/2$) (in min):

$$ T_2/2 = \ln(2) \times k_2 $$

Stable-isotope tracers. The [13C]SCFA tracers were continuously IV infused until an equilibrium was reached between the tracers and the whole-body SCFA production. The plateau values of isotope enrichment at steady state were equalled to the mean isotope enrichments during the last 30 min tracer infusion. Using the isotope-dilution principle13, the rates of appearance or turnover ($Ra$ in $\mu mol/kg$ per min) of acetate, propionate and butyrate were calculated using the steady-state equation, as follows:

$$ Ra = i \times \left( \frac{IE_a}{IE_a} - \frac{IE_{pv}}{IE_{pv}} \right) $$

where $i$ is the IV infusion rate ($\mu mol/kg$ per min) of [13C]SCFA tracers, $IE_a$ and $IE_{pv}$ are the [13C]SCFA isotope enrichments of the tracer solution ([1,2-13C2]acetate, [1-13C]acetate, [1,13C]propionate or [1-13C]butyrate) and of arterial plasma at plateau, respectively, given in MPE.

The fractional extraction of the different SCFA by the intestine ($%\text{extrac}$ in %) was as follows:

$$ %\text{extrac} = 100 \times \left( \frac{IE_a \times C_a}{IE_{pv} \times C_{pv}} - \frac{IE_{pv} \times C_{pv}}{IE_a \times C_a} \right) $$

where $C_a$ and $C_{pv}$ are concentrations of SCFA, and $IE_a$ and $IE_{pv}$ are isotope enrichments of [13C]SCFA at plateau in the femoral artery (a) and in the portal vein (pv), respectively. The utilisation or uptake of acetate, propionate and butyrate from intestinal tissues was evaluated assuming a blood flow in the portal vein of 15 ml/min (in 300 g rats) according to data from Jaeschke et al.14. Plasma flow was estimated (assuming 45 % packed cell volume) to be 6-75 ml/min15, calculated as follows:

$$ \text{Uptake} = %\text{extrac} \times C_a \times \text{plasma flow} $$

where ‘plasma flow’ is expressed in litres/kg per min and was estimated from blood flow, packed cell volumes and rat body weight. ‘Uptake’ is expressed in $\mu mol/kg$ per min.

The production or release of acetate, propionate and butyrate from intestinal tissues was evaluated assuming a plasma flow in the portal vein as above. The following calculation...
was performed:

\[ \text{Release} = \text{uptake} + (C_{pu} - C_a) \times \text{plasma flow} \]

where plasma flow is expressed in litres/kg per min and Release in \( \mu \)mol/kg per min.

Statistics

Statistics were performed using Statistica (version 5.1 G software; StatSoft Ltd, London, UK). One-way ANOVA were performed in experiment 1 between kinetic parameters of acetate, propionate and butyrate groups. Kinetic parameters were \( A_1, A_2, k_1, k_2, VD, FCR, \) total clearance, \( MRT, T_{1/2} \) and \( T_{2/1/2} \). One-way ANOVA was used in experiment 2 to compare parameters of the acetate, propionate and butyrate groups followed by a Tukey honest significant difference (HSD) test for unequal \( n \) per group. Paired \( t \) tests were performed in experiment 3 to compare outcomes after the chicory diet treatment with those after the control diet. All data are presented as mean values with their standard errors. Differences were considered to be statistically significant at \( P < 0.05 \).

Results

Experiment 1: Kinetic parameters of plasma short-chain fatty acids

The radiolabelled SCFA decays (\([1-14C]\)acetate, \([1-14C]\)propionate and \([1-14C]\)butyrate tracers) are shown in Fig. 2. Mean parameters of bi-exponential decays \( (A_1, A_2, k_1, k_2) \) and kinetic parameters are presented in Table 1; no significant differences were observed between acetate, propionate and butyrate. Distribution volumes were not significantly different; similarly no differences were observed for \( FCR, \) total clearance, \( MRT \) and \( T_{2/1/2} \) between the SCFA. Rapid decay half-life \( (T_{1/2}) \) was not significantly shorter for acetate than for butyrate.

Experiment 2: Turnover of plasma short-chain fatty acids

The plasma isotope enrichments of \([1-13C]\)acetate, \([1-13C]\)propionate and \([1-13C]\)butyrate reached a plateau during the last 30 min of each IV infusion (Fig. 3). Equal infusion rates of \([1-13C]\)acetate, \([1-13C]\)propionate and \([1-13C]\)butyrate showed different plateau values of isotope enrichments at 3.5 (SEM 0.6), 22.0 (SEM 3.7) and 76.6 (SEM 3.6) MPE \((P < 0.005)\), respectively. At the end of the IV infusions, no labelled acetate enrichment was detected in the portal vein, as opposed to labelled propionate (1.8 (SEM 0.6) MPE) and butyrate (7.3 (SEM 5.6) MPE). The turnover rates of acetate, propionate and butyrate in the whole-body peripheral circulation were 29 (SEM 4), 4 (SEM 2) and 0.3 (SEM 0.1) \( \mu \)mol/kg per min, respectively in 22 h food-deprived rats (with \( R_{acetate} > R_{propionate} > R_{butyrate}; P < 0.0001 \)).

Experiment 3: Intestinal production of short-chain fatty acids

The body weights of the rats increased during both 4-week diet treatments to 498 (SEM 8) g \((+32\%)\) and 495 (SEM 9) g \((+31\%)\), with no difference between the control and chicory diets, respectively. Food intake did not differ between the diet treatments (24 (SEM 1) g/d). The arterial concentrations of acetate were higher after the chicory than the control diet treatment \((P = 0.026)\), but no difference was observed for propionate and butyrate (Table 2). The isotope enrichments of \([1,2-13C]\)acetate, \([1-13C]\)propionate and \([1-13C]\)butyrate reached a plateau during the multi-tracer infusion (Fig. 4). The plateau isotope enrichments in arterial blood of \([1,2-13C]\)acetate were lower after chicory than after the control treatment \((P = 0.025); \) Table 2). No difference was observed between treatments in \([1-13C]\)propionate isotope enrichment
in arterial and portal vein blood (Table 2). No difference was observed in [1- 13C]butyrate isotope enrichment in arterial blood. Portal vein [1- 13C]butyrate enrichment was lower after chicory than after control diet treatment ($P = 0.035$; Table 2). The $R_{1/2}$ of acetate in the whole body was significantly higher after the chicory-enriched than after the control diet treatment ($P = 0.017$; Table 2). No difference was observed in the $R_{1/2}$ of propionate and butyrate between diet treatments (Table 2). Intestinal extraction and rates of uptake and release of acetate, propionate and butyrate were not different between diet treatments (Table 3). The mean pH in the caecal lumen was lower in the chicory group (5.7 (SEM 0.1)) compared with the control group (6.0 (SEM 0.1); $P = 0.017$). Rats submitted to the chicory-enriched diet demonstrated a significant increase in whole caecal and whole colonic weights, as well as in empty caecal and empty colonic weights compared with those on the control diet ($P = 0.01$; Fig. 5). The caecal content was also significantly higher after the chicory diet compared with control diet treatments (Table 2). The concentration of each SCFA evolved differently in the colon compared with the more proximal caecum; the colonic concentration of butyrate was decreased after chicory treatment compared with control treatment (Table 4). No methane was detected in expired breath in any of the rats. The area under the curve (0–6 h) of breath $H_2$ was significantly higher in rats that consumed the chicory diet (176 (SEM 34) parts per million £h) compared with the controls (66 (SEM 6) parts per million £h; $P = 0.002$).

### Discussion

Dry chicory roots contain about 50–60% inulin, which is a non-digestible fructo-oligosaccharide. The intestinal fermentation of inulin produces exogenous SCFA that mix with those produced endogenously. We investigated the plasma kinetics of bi-exponential decay curves of radiolabelled short-chain fatty acid tracers (Expt 1) (Table 1).

#### Table 1. Kinetic parameters of bi-exponential decay curves of radiolabelled short-chain fatty acid tracers (Expt 1)*

<table>
<thead>
<tr>
<th></th>
<th>$A_1$ (µCi/l)</th>
<th>$k_1$ (per min)</th>
<th>$A_2$ (µCi/l)</th>
<th>$k_2$ (per min)</th>
<th>$V_D$ (litres/kg)</th>
<th>$F_C$ (%)</th>
<th>Total clearance (ml/min)</th>
<th>$M_R$ (min)</th>
<th>$T_{1/2}$ (min)</th>
<th>$T_{2/2}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]acetate†</td>
<td>46.8 (6.3)</td>
<td>0.033 (0.067)</td>
<td>16.9 (2.5)</td>
<td>0.016 (0.004)</td>
<td>0.26 (0.03)</td>
<td>5.4 (1.1)</td>
<td>4.7 (0.9)</td>
<td>89.5 (30.6)</td>
<td>0.9 (0.1)</td>
<td>64.4 (21.4)</td>
</tr>
<tr>
<td>[14C]propionate‡</td>
<td>49.9 (6.0)</td>
<td>0.073 (0.176)</td>
<td>29.0 (4.7)</td>
<td>0.020 (0.003)</td>
<td>0.27 (0.02)</td>
<td>5.5 (0.7)</td>
<td>4.4 (0.4)</td>
<td>55.9 (11.2)</td>
<td>1.2 (0.2)</td>
<td>40.8 (8.2)</td>
</tr>
<tr>
<td>[14C]butyrate†</td>
<td>69.7 (4.9)</td>
<td>0.596 (0.140)</td>
<td>24.0 (3.5)</td>
<td>0.017 (0.004)</td>
<td>0.25 (0.01)</td>
<td>5.9 (1.2)</td>
<td>4.5 (1.1)</td>
<td>78.4 (27.2)</td>
<td>1.4 (0.2)</td>
<td>58.0 (19.3)</td>
</tr>
</tbody>
</table>

* $A_1$ and $A_2$ are constants; $k_1$ and $k_2$ are decay constants; kinetic parameters are: volume of distribution ($V_D$); fractional clearance rate ($F_C$); total clearance; mean residence time ($M_R$); half-life of rapid decay ($T_{1/2}$); half-life of elimination decay ($T_{2/2}$). No significant differences were observed between the SCFA.

† For acetate and butyrate tracer groups, $n = 5$.
‡ For propionate tracer group, $n = 5$.

* MPE, mole percent excess.
Table 2. Concentrations, isotope enrichments and rates of appearance of plasma acetate, propionate and butyrate in arterial and portal vein blood in rats submitted to 4-week chicory or control diets (Expt 3) (Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Control (n 8)</th>
<th>Chicory (n 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentrations (μmol/l)</td>
<td>Isotope enrichments (MPE)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>287</td>
<td>22</td>
</tr>
<tr>
<td>Portal vein</td>
<td>756</td>
<td>105</td>
</tr>
<tr>
<td>Propionate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>Portal vein</td>
<td>148</td>
<td>37</td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Portal vein</td>
<td>237</td>
<td>72</td>
</tr>
</tbody>
</table>

MPE, mole percent excess.
*Mean value was significantly different from that of the control group (P<0·05).

and turnover rates of SCFA in food-deprived and fed rats (Fig. 1). Interestingly the turnover rates of propionate and butyrate were not negligible; however, a 4-week chicory diet did not enhance their turnovers. We observed that a 4-week chicory diet increased intestinal fermentation and was accompanied by a 25% increase in peripheral acetate turnover in rats.

Non-ruminant models have been used to study SCFA production and metabolism. Kien et al. evaluated butyrate turnover in piglets that received a [13C]butyrate infusion into the caecum. While the rat model has been limited to the determination of SCFA concentrations in blood and intestinal contents, in the present study, an isotope approach was used to take into account the dynamics of SCFA metabolism. The radioisotope-tracer experiment, in the present study, did not reveal significant differences between SCFA kinetics in rats. As a consequence, we used the same 2 h infusion rate of different SCFA tracers to determine SCFA turnover rates in experiment 2. It appeared, however, that the SCFA had significantly different turnover rates when measured in the peripheral circulation, since the stable-isotope dilution method in experiment 2 is more accurate for turnover rate determinations. Consequently the last experiment, experiment 3, was conducted using adapted priming doses and infusion rates of the different [13C]SCFA tracers, by proportionally doubling the labelled acetate and lowering the labelled butyrate priming doses and infusion rates. A concomitant multi-tracer infusion technique was additionally developed in experiment 3, which could be applicable in human subjects.

The study of SCFA plasma decays in experiment 1 revealed unique data on the plasma kinetics of the different SCFA. SCFA were rapidly cleared from the first pool (half-life T1/2 < 2 min), which represents the whole-body extracellular volume, compared with the second, intracellular pool, which cleared much more slowly (T1/2 = 40–65 min). No significant differences in any of the plasma kinetic parameters of SCFA were observed, probably because their extracellular and intracellular distribution volumes are similar. The distribution volumes of the SCFA are very similar to those of glycerol and acetate in dogs (0·27 (SEM 0·16) litres/kg)23. The turnover rate of acetate in the peripheral circulation of 22 h food-deprived rats was three times higher than that of human subjects in the post-absorptive state (8·4 (SEM 0·6) μmol/kg per min)3 and similar to that of 24 h food-deprived dogs (24·4 (SEM 2·4) μmol/kg per min)24. The turnover of propionate in 22 h food-deprived rats was higher than that (0·6 μmol/kg per min) reported by Sbaï et al. in metronidazole-treated human patients with propionic acidemia and that (0·3 μmol/kg per min) reported by Walter et al. in four healthy human subjects. The use of different tracers and different species can probably explain these discrepancies. When the three experiments were compared, the following logical ranking was observed, Aacetate < Apropionate < A1butyrate (NS) and kacetate > kpropionate > k1butyrate (NS) in experiment 1 (Table 1), in parallel with Raacetate > Raproionate > R1butyrate (P<0·05) in experiments 2 and 3. The ranking of the different SCFA turnover rates was identical, but not proportional, to their plasma concentrations and is due to the fact that an increase in turnover rate is not necessarily accomplished by an increase in plasma concentration, especially when the Ra and rate of disappearance of the SCFA stays in equilibrium. This reinforces the relevance of the turnover determination in our experiments.

In experiment 3, we observed a clear caeco-colonic fermentation of the non-digestible inulin component of the chicory diet. The rats consuming the 4-week chicory diet demonstrated, as expected24,25, a higher caecal and colonic weight, increased intestinal hypertrophy, higher SCFA concentrations in the caecum, a fall in pH and increased H2 concentrations in breath compared with those who consumed the control diet. Interestingly, in experiment 3 (Table 2) we observed that the acetate concentration in the portal vein was positively correlated with that in arterial blood (r 0·62) and Ra of acetate (r 0·56) and inversely correlated with acetate isotope enrichment in arterial blood (r −0·62). Non-labelled acetate, probably originating from the caecum, diluted the tracer and lowered the isotope enrichment after the chicory compared with the control diet. The Ra of acetate was also correlated with the acetate concentration in the caecum (r 0·63). The estimated release of acetate from the intestine (Table 3) was correlated with the Ra and with the concentration of acetate in the
In the present study we showed that acetate turnover increased by 25% in peripheral blood circulation, while propionate and butyrate turnover rates were unchanged in chicory-treated rats. In a previous study, dogs adapted to 3-week chicory intake showed a similar 31% increase in acetate turnover\(^26\). In a previous clinical trial\(^27\), healthy adults that were submitted to an acute lactulose oral intake showed a sharp 63% increase in acetate turnover rate. No comparisons are available for propionate and butyrate turnovers to our knowledge. The present approach provides information about the net contribution of intestinal fermentation to peripheral metabolism, in terms of carbon flux available for either energy or for anabolic processes. Previous approaches using arterio-venous differences combined with a measure of blood flow provide similar information, however, with less accuracy. The latter, and tracer methods, have been thoroughly studied in ruminants\(^28-29\). Measurements of SCFA concentrations in the intestinal lumen or portal vein only provide static information about the production and absorption of SCFA at a particular moment in time\(^30\) and neglect the dynamic nature and physiological meaning of SCFA production rates. The present approach could have been improved by catheterising the portal vein to assess true SCFA production rates from the intestine in rats. Nevertheless, the present approach could be used in clinical research to measure SCFA turnover rates. SCFA turnover rates may differ in man and we cannot exclude, unlike acetate, that peripheral propionate and butyrate turnover rates can be beneficially modulated by consuming dietary fibres.

From the present, and previous studies, we have attempted to describe SCFA metabolism (Fig. 1). After absorption through the intestinal endothelial cells, SCFA are activated by enzyme acyl-CoA synthetase to serve as fuel for cellular energy or as an intermediate for intestine metabolism\(^24,31-33\). Following absorption, butyrate is activated to produce butyryl-CoA. Butyrate is mainly metabolised in the colonocytes and is known to serve as a fuel for intestinal cells\(^34\). Acetate and propionate are absorbed and partially escape the filtering capacity of the intestine. Intestinal acetate release increased 30% into the portal vein, propionate release increased, but not significantly, while intestinal butyrate release did not increase after chicory ingestion in the present study. Independent of the diet, the liver retains most of the portal propionate and butyrate\(^35\), where propionyl-CoA and butyryl-CoA are probably involved in gluconeogenesis and lipogenesis in hepatocytes\(^36,37\). The non-negligible concentrations and turnover rates of propionate and butyrate were unchanged and constant in the post-hepatic circulation; these originate either from an endogenous production or from discrete amount of SCFA escaping the splanchnic filter. To a lesser proportion, acetate was retained and probably metabolised by the liver\(^35,38\). Considering that approximately 0.2 g inulin/h were reaching the rat hindgut, during the last trial, and taking into account the stoichiometric macrobiota degradation of inulin into SCFA after Roberfroid et al.\(^39\), it is possible to theoretically estimate that approximately 50 µmol acetate/kg per min was produced in the gut. By comparing the chicory and control diet groups, the difference in acetate turnovers assessed in the peripheral circulation was about 5 µmol/kg per min; that is, only 10% of the theoretical caeco-colonic production. This suggests that a large amount of acetate is taken up and used by the splanchnic tissues in rats. The role(s) of such
low-molecular-weight SCFA metabolites is still not fully understood. More investigations using stable isotopes would contribute to the understanding of the function of acetate, propionate and butyrate in whole-body energy metabolism.

In the present study, simultaneous IV infusions of labelled acetate, propionate and butyrate were used to determine the concurrent SCFA turnover rates in the peripheral circulation of rats. As much as 590 μmol acetate/h was turned over in peripheral blood circulation, with turnover rates of 115 and 40 μmol/h for propionate and butyrate in food-deprived rats, respectively. Propionate and butyrate turnover rates appeared to be non-negligible and their role in systemic metabolism remains to be elucidated. The intestine and the liver probably play a role in the peripheral homeostasis of propionate and butyrate independently of non-digestible carbohydrate fermentation in the gut. Rats consuming a chicory diet for 4 weeks significantly increased peripheral acetate turnover but turnover rates of propionate and butyrate did not change when compared with rats on a control diet. Acetate constitutes an ideal marker of intestinal fermentation in rats. The present quantitative determination of SCFA turnover rates after dietary fibre ingestion is applicable in human subjects and remains to be investigated.

Acknowledgements

The authors thank M. Gyger, S. N. Mafuala-Muana and M. Marchesini for their fruitful advice and assistance to perform the animal protocol. We thank Kevin Acheson for improving the writing style of the manuscript. Nestlé Research Centre financially supported the present study.

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