

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

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(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 ^{14}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 $\mu\text{mol/kg}$ 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 H_2 . 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^{1,2}. 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 of the body as an energetic substrate and its oxidation contributes to about 7% whole-body resting energy expenditure in man³. All SCFA contribute to gut health and maintenance^{2,4} and may provide further benefits for peripheral metabolism¹. The intestinal production of SCFA has been assessed *in vitro*⁵, in *in vivo* animal models (pigs, dogs, rats)¹ and also in human subjects⁶. Most studies have been limited to the determination of SCFA concentrations in biological fluids in animals and human subjects and only a few have investigated the peripheral turnover rate of acetate or of propionate^{3,7–9}. To our knowledge no studies have investigated the peripheral turnover rates of propionate and butyrate after dietary fibre ingestion in either animals or human subjects.

The production of SCFA in the intestine occurs at a rate that depends upon the rate of entry of unabsorbed nutrients into the intestine and bacterial activity within the gut. It varies from a minimum during fasting, to a maximum several hours after dietary fibre ingestion and is dose dependent. After

their production in, and absorption from, the gut SCFA are transported to the liver, where they combine with those produced endogenously during substrate metabolism. To our knowledge the endogenous production of propionate and butyrate is limited, but has not yet been evaluated. If it is possible to differentiate intestinal SCFA from those that are endogenously produced using labelling, one can estimate the intestinal production rate of SCFA (Fig. 1)¹⁰. The primary objective of the present study was to develop an isotope-dilution technique to determine whole-body SCFA turnover in a rat model. In a first experiment we investigated the kinetics of acetate, propionate and butyrate in whole-body peripheral circulation and in a second experiment we assessed their individual peripheral turnover rates in rats deprived of food for 22 h. The second objective was to quantify in a third experiment the effect of 4-week chicory treatment on whole-body peripheral SCFA turnover in rats.

Materials and methods

Animals and diets

The animal experiments were approved by the Cantonal Committee for Ethics in Animal Experimentation (no. 1272;

Abbreviations: FCR, fractional clearance rate; IV, intravenous; MPE, mole percent excess; MRT, mean residence time; MS, mass spectrometry; *R_a*, rate of appearance; *T*_{1/2}, half-life of first phase (rapid decay); *T*_{2/2}, half-life of second phase (elimination decay); *VD*, volume of distribution.

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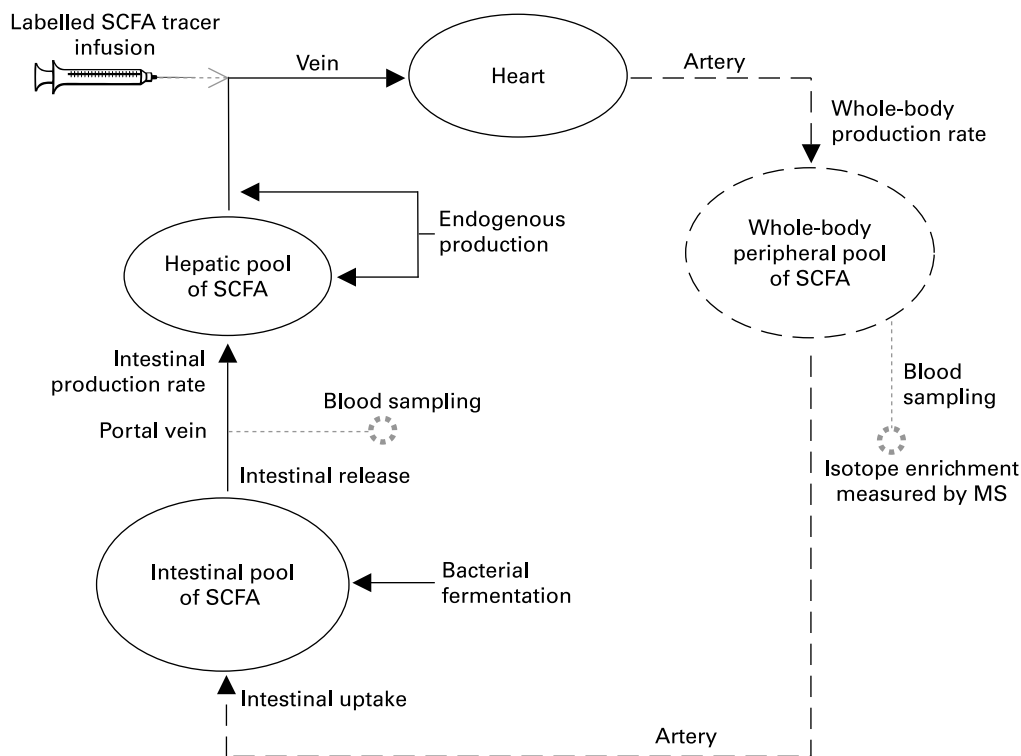


Fig. 1. Principle of using isotope-labelled SCFA intravenous infusions to investigate intestinal fermentation from peripheral metabolism of SCFA. Bacterial fermentation in the intestine produces SCFA that are transported through the liver to the peripheral blood circulation. The liver and whole body 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).

Service Vétérinaire, Etat de Vaud, Lausanne, Switzerland). Wistar rats were obtained from the Charles River Company (L'Arbresle, France). $[1-^{14}\text{C}]$ acetate, Na salt ($50\ \mu\text{Ci}$), $[1-^{14}\text{C}]$ propionate, Na salt ($250\ \mu\text{Ci}$) and $[1-^{14}\text{C}]$ butyrate, Na salt ($50\ \mu\text{Ci}$) were purchased from NENTM Life Science Products, Inc. (Boston, MA, USA). $[1,2-^{13}\text{C}_2]$ acetate, $[1-^{13}\text{C}]$ acetate, $[1-^{13}\text{C}]$ propionate and $[1-^{13}\text{C}]$ 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 for blood sampling. For experiments 2 and 3, a catheter (24G Insyte) was also inserted into the femoral vein for tracer infusions. A first blood sample (100–800 μl) was collected at time zero, in a heparinised tube, and was replaced

by injecting the same volume of sterile saline solution (0.9% NaCl). Regular blood sampling (800 μl) was possible as the sampling catheter was flushed with diluted heparin (10% in saline solution) after each sample collection. Particular attention was taken to maintain body temperature constant under isoflurane anaesthesia. This rat model could be kept operational for 5 h. At the end of the kinetic measurements, portal blood was eventually collected just before the rat was killed by exsanguination.

Experiments

Experiment 1. The experiment had a parallel design. Sixteen rats (eight males and eight females; weight $322.7 \pm 13.3\ \text{g}$) 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 $[1-^{14}\text{C}]$ acetate ($5.497\ (\text{SEM}\ 0.088)\ \mu\text{Ci}$, $2.7483\ \mu\text{mol}$), $[1-^{14}\text{C}]$ propionate ($6.407\ (\text{SEM}\ 0.057)\ \mu\text{Ci}$, $0.1140\ \mu\text{mol}$) or $[1-^{14}\text{C}]$ butyrate ($6.990\ (\text{SEM}\ 0.007)\ \mu\text{Ci}$, $0.3548\ \mu\text{mol}$) 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.

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 μ l). Each rat received, intravenously, a primed continuous infusion of either [$1\text{-}^{13}\text{C}$]acetate (52.3 (SEM 2.1) μ mol/kg and 0.98 (SEM 0.04) μ mol/kg per min), [$1\text{-}^{13}\text{C}$]propionate (48.0 (SEM 1.1) μ mol/kg and 0.97 (SEM 0.02) μ mol/kg per min) or [$1\text{-}^{13}\text{C}$]butyrate (49.7 (SEM 1.9) μ mol/kg and 1.04 (SEM 0.05) μ mol/kg per min) solubilised in 0.9% NaCl sterile solution. Arterial blood samples (800 μ l) 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 1200g) 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 μ l). Each rat was then administered, intravenously, a primed continuous infusion of [$1,2\text{-}^{13}\text{C}_2$]acetate, [$1\text{-}^{13}\text{C}$]propionate and [$1\text{-}^{13}\text{C}$]butyrate simultaneously (all tracers solubilised in 0.9% NaCl sterile solution; 287 (SEM 5), 97 (SEM 2) and 49 (SEM 1) μ mol/kg priming doses and 2.84 (SEM 0.04), 0.96 (SEM 0.01) and 0.48 (SEM 0.01) μ mol/kg per min infusion rates, respectively). Arterial blood samples (800 μ l) were collected at 60, 75, 90, 105 and 120 min. A last 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 μ l) were centrifuged and 40 μ l plasma were pipetted into a scintillation flask containing 20 ml scintillant (Ultima Gold, Packard Bioscience Comp., the Netherlands). A scintillation counter (PerkinElmerTM, Regendorf, Switzerland) measured and recorded β + detection in counts per min. Data in counts per min were transformed into dpm accounting for quenching.

Stable isotope tracers. Measurements of [$1,2\text{-}^{13}\text{C}_2$]acetate, [$1\text{-}^{13}\text{C}$]acetate, [$1\text{-}^{13}\text{C}$]propionate and [$1\text{-}^{13}\text{C}$]butyrate enrichments and SCFA concentrations in plasma were performed as previously described¹¹. Briefly, plasma samples (450 μ l) were spiked with 10 μ l [$^2\text{H}_3$] acetate internal standard. Samples were deproteinised with sulfosalicylic acid solution (10 μ l, 1 g/litre; Fluka, Buchs, Switzerland) and centrifuged at 2200g for 10 min. The supernatant fraction was transferred into a vial containing 10 μ l 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 1200g, then 8 μ l 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 μ l. Then 2 μ l were injected into a gas chromatograph (model 5890A; Hewlett Packard, Palo Alto, CA, USA) connected to a quadrupole mass spectrometer (SSQ7000; Finnigan Mat, Bremen, Germany). Chromatographic separations were carried out with a 30 m \times 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 $70^{\circ}\text{C}/\text{min}$ up to 60°C , $10^{\circ}\text{C}/\text{min}$ up to 110°C and $70^{\circ}\text{C}/\text{min}$ 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 μ mol/l and isotope enrichments $M_0 + 2$ and $M_0 + 1$ in mole percent excess (MPE). Calibration curves for plasma concentrations showed excellent linearity from 1 to 1000 μ mol/l for acetate, propionate and butyrate. Calibration curves for isotope enrichment ($M_0 + 2$ and $M_0 + 1$) were linear from 0.5 to 99 MPE for [$1,2\text{-}^{13}\text{C}_2$]acetate, [$1\text{-}^{13}\text{C}$]acetate, [$1\text{-}^{13}\text{C}$]propionate and [$1\text{-}^{13}\text{C}$]butyrate¹¹.

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% HgCl_2 , 0.1% H_3PO_4). The aqueous solution was homogenised by vortexing (20 min) and centrifuging (5 min; 1200g). Then 20 μ l hydrochloric acid (32%) were added. Part of the upper liquid layer (450 μ l) 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, respectively¹¹. SCFA concentrations in caecal contents were expressed in μ mol/g (g of whole caecal content).

Calculations

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

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) software¹². The equation of the fitting curve was:

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

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:

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

The elimination decay from the second pool is defined by:

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

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:

$$RSS = \sum_{i=1}^n (z(t) - y(t))^2$$

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 μCi , since it is known that $1 \mu\text{Ci}$ equals 2.22×10^6 dpm, and further into $\mu\text{Ci/l}$ taking into account that $40 \mu\text{l}$ plasma were analysed. From this curve fitting, the following parameters were calculated¹²:

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

$$AUC_0^\infty = \frac{A_1}{k_1} + \frac{A_2}{k_2}$$

where A_1 and A_2 are expressed in $\mu\text{Ci/l}$, 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 μCi), 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:

$$FCR = 100 \times \frac{A_1 + A_2}{AUC_0^\infty}$$

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:

$$MRT = \frac{\int_0^\infty t \times y(t) dt}{\int_0^\infty y(t) dt}$$

$$MRT = \frac{\frac{A_1}{k_1^2} + \frac{A_2}{k_2^2}}{\frac{A_1}{k_1} + \frac{A_2}{k_2}}$$

The half-life of the first phase of the different SCFA, rapid decay from the first compartment ($T_{1/2}$) (in min):

$$T_{1/2} = \frac{\ln(2)}{k_1}$$

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

$$T_{2/2} = \frac{\ln(2)}{k_2}$$

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 (R_a in $\mu\text{mol/kg}$ per min) of acetate, propionate and butyrate were calculated using the steady-state equation, as follows:

$$R_a = i \times \left(\frac{IE_t}{IE_a} - 1 \right)$$

where i is the IV infusion rate ($\mu\text{mol/kg}$ per min) of [¹³C]SCFA tracers, IE_t and IE_a 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 (%*extrac* in %) was as follows:

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

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:

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

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 $\mu\text{mol/kg}$ 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:

$$\text{Release} = \text{uptake} + (C_{pv} - C_a) \times \text{plasma_flow}$$

where *plasma_flow* is expressed in litres/kg per min and *Release* in $\mu\text{mol/kg}$ 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 $T_{2/2}$. 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 SCFA concentrations between artery and portal vein and also to compare uptake and release from intestinal tissues. Unpaired 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 $P \leq 0.05$.

Results

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

The radiolabelled SCFA decays ($[^{14}\text{C}]$ acetate, $[^{14}\text{C}]$ propionate and $[^{14}\text{C}]$ butyrate tracers) are shown in Fig. 2. Mean parameters of bi-exponential decays (A_1 , A_2 , k_1 and 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 $T_{2/2}$ 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 $[1-^{13}\text{C}]$ acetate, $[1-^{13}\text{C}]$ propionate and $[1-^{13}\text{C}]$ butyrate reached a plateau during the last 30 min of each IV infusion (Fig. 3). Equal infusion rates of $[1-^{13}\text{C}]$ acetate, $[1-^{13}\text{C}]$ propionate and $[1-^{13}\text{C}]$ 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) $\mu\text{mol/kg}$ per min, respectively in 22 h food-deprived rats (with $Ra_{\text{acetate}} > Ra_{\text{propionate}} > Ra_{\text{butyrate}}$; $P < 0.0001$).

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)

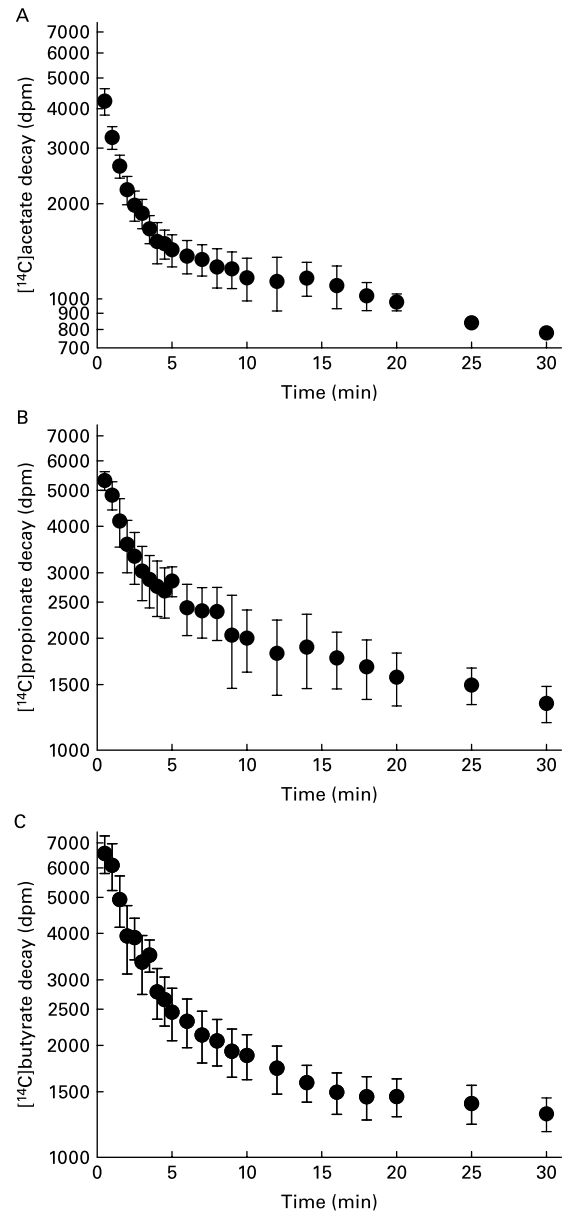


Fig. 2. Decays of the tracer plasma level (common log scale) used for bi-exponential curve fitting and kinetic parameter determination in adult rats that received (A) a $[1-^{14}\text{C}]$ acetate, (B) a $[1-^{14}\text{C}]$ propionate or (C) a $[1-^{14}\text{C}]$ butyrate intravenous injection (Expt 1). Data are means, with their standard errors represented by vertical bars (n 5 for acetate and butyrate tracer groups; n 6 for propionate tracer group).

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 $[1,2-^{13}\text{C}_2]$ acetate, $[1-^{13}\text{C}]$ propionate and $[1-^{13}\text{C}]$ butyrate reached a plateau during the multi-tracer infusion (Fig. 4). The plateau isotope enrichments in arterial blood of $[1,2-^{13}\text{C}_2]$ acetate were lower after chicory than after the control treatment ($P = 0.025$; Table 2). No difference was observed between treatments in $[1-^{13}\text{C}]$ propionate isotope enrichment

Table 1. Kinetic parameters of bi-exponential decay curves of radiolabelled short-chain fatty acid tracers (Expt 1)* (Mean values with their standard errors)

	A_1 ($\mu\text{Ci/l}$)		k_1 (per min)		A_2 ($\mu\text{Ci/l}$)		k_2 (per min)		VD (litres/kg)		FCR (%/min)		Total clearance (ml/min)		MRT (min)		T_1 1/2 (min)		T_2 1/2 (min)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
[^{14}C]acetate†	46.8	6.3	0.833	0.067	16.9	2.5	0.016	0.004	0.26	0.03	5.4	1.1	4.7	0.9	89.5	30.6	0.9	0.1	64.4	21.4
[^{14}C]propionate‡	49.9	6.0	0.733	0.176	29.0	4.7	0.020	0.003	0.27	0.02	5.5	0.7	4.4	0.4	55.9	11.2	1.2	0.2	40.8	8.2
[^{14}C]butyrate‡	69.7	4.9	0.596	0.140	24.0	3.5	0.017	0.004	0.25	0.01	5.9	1.2	4.5	1.1	78.4	27.2	1.4	0.2	58.0	19.3

* A_1 and A_2 are constants; k_1 and k_2 are decay constants; kinetic parameters are: volume of distribution (VD); fractional clearance rate (FCR); total clearance; mean residence time (MRT); half-life of rapid decay (T_1 1/2); half-life of elimination decay (T_2 1/2). No significant differences were observed between the SCFA.

† For acetate and butyrate tracer groups, n 5.

‡ For propionate tracer group, n 6.

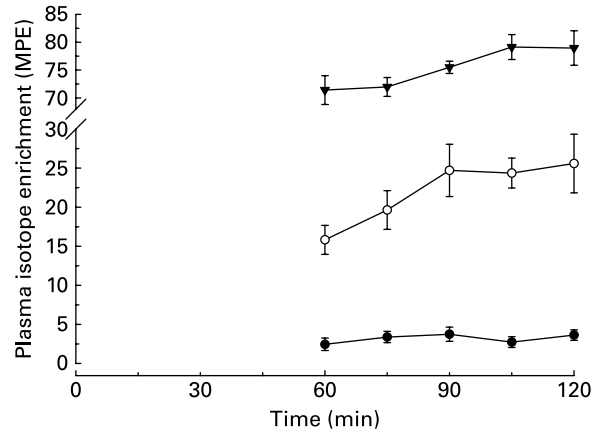


Fig. 3. [^{13}C]isotope enrichments of acetate (●), propionate (○) and butyrate (▼) in arterial plasma during separate [^{13}C]SCFA primed continuous IV infusions in rats (Expt 2). Data are means, with their standard errors represented by vertical bars (n 6 for acetate and butyrate tracer groups; n 5 for propionate tracer group). MPE, mole percent excess.

in arterial and portal vein blood (Table 2). No difference was observed in [^{13}C]butyrate isotope enrichment in arterial blood. Portal vein [^{13}C]butyrate enrichment was lower after chicory than after control diet treatment ($P=0.035$; Table 2). The Ra 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 Ra 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, as well as non-mucosal colonic tissue weight ($P<0.05$; Fig. 5). Concentrations of acetate, propionate, iso-butyrate and iso-valerate were significantly increased in the caecal content after 4-week-chicory treatment compared with control treatment (Table 4), while concentrations of butyrate and valerate were not. 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 \times h) compared with the controls (66 (SEM 6) parts per million \times 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 endogenously¹⁶. We investigated the plasma kinetics

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)

Diet group...	Concentrations ($\mu\text{mol/l}$)				Isotope enrichments (MPE)				Rate of appearance ($\mu\text{mol/kg per min}$)			
	Control (<i>n</i> 8)		Chicory (<i>n</i> 10)		Control (<i>n</i> 8)		Chicory (<i>n</i> 10)		Control (<i>n</i> 8)		Chicory (<i>n</i> 10)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetate												
Artery	287	22	352*	22	13.0	0.9	10.6*	0.7	19.7	1.6	24.7*	1.5
Portal vein	756	105	953	79	2.4	0.7	1.5	0.3	–	–	–	–
Propionate												
Artery	20	3	21	3	22.7	2.8	22.6	2.3	3.8	0.6	3.7	0.5
Portal vein	148	37	172	31	2.0	0.6	1.8	0.2	–	–	–	–
Butyrate												
Artery	9	2	9	1	28.8	3.6	26.5	3.4	1.3	0.2	1.6	0.3
Portal vein	237	72	238	58	1.6	0.6	0.6*	0.2	–	–	–	–

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 peripheral 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^{1,17}. Kien *et al.* evaluated butyrate turnover in piglets that received a [¹³C]butyrate infusion into the caecum¹⁷. While the rat model has been limited to the determination of SCFA concentrations in blood and intestinal contents^{19–21}, 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 2h 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 $T_{1/2} < 2$ min), which represents the whole-body extracellular volume, compared with the second, intracellular pool, which cleared much more slowly ($T_{2/2} = 40–65$ 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 glycerol²² 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) $\mu\text{mol/kg per min}$)³ and similar to that of 24 h food-deprived dogs (24.4 (SEM 2.4) $\mu\text{mol/kg per min}$)²³. The turnover of propionate in 22 h food-deprived rats was higher than that (0.6 $\mu\text{mol/kg per min}$) reported by Sbaï *et al.*¹⁸ in metronidazole-treated human patients with propionic acidemia and that (0.3 $\mu\text{mol/kg per min}$) reported by Walter *et al.*⁹ 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, $A_{\text{acetate}} < A_{\text{propionate}} < A_{\text{butyrate}}$ (NS) and $k_{\text{acetate}} > k_{\text{propionate}} > k_{\text{butyrate}}$ (NS) in experiment 1 (Table 1), in parallel with $Ra_{\text{acetate}} > Ra_{\text{propionate}} > Ra_{\text{butyrate}}$ ($P < 0.05$) 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 accompanied 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 expected^{24,25}, a higher caecal and colonic weight, increased intestinal hypertrophy, higher SCFA concentrations in the caecum, a fall in pH and increased H_2 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

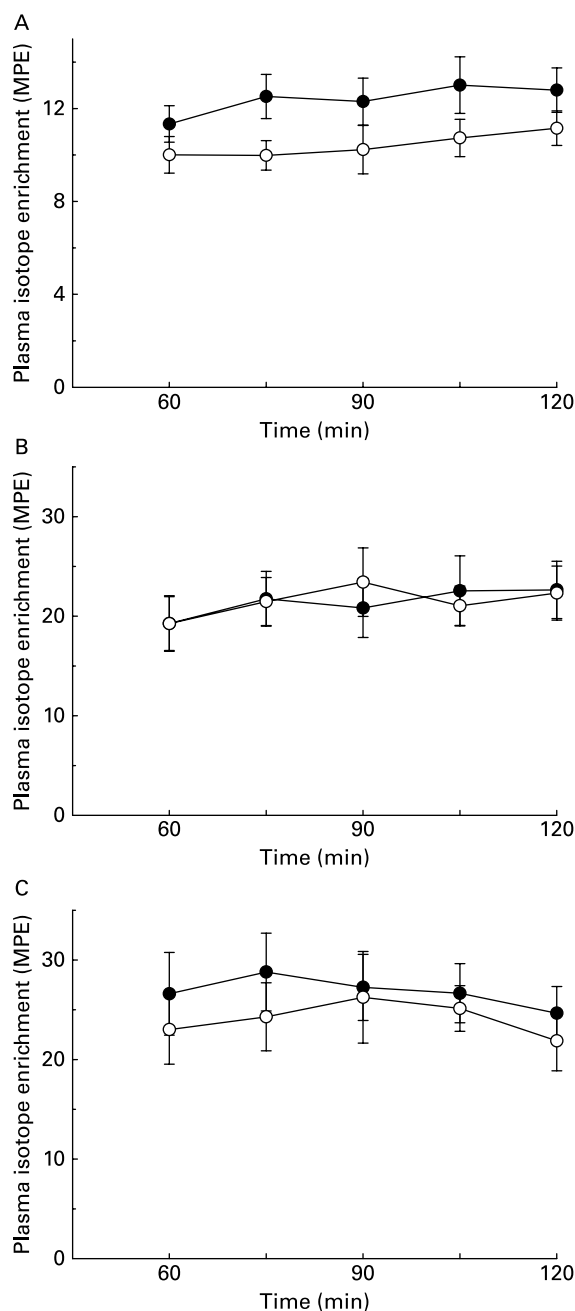


Fig. 4. Time-course of isotope enrichments of (A) [1,2-¹³C₂]acetate, (B) [1-¹³C]propionate and (C) [1-¹³C]butyrate in arterial plasma during simultaneous IV infusions of labelled acetate, propionate and butyrate in rats previously submitted to a 4-week chicory diet (O) or a 4-week control diet (●) (Expt 3). Data are mean values, with their standard errors represented by vertical bars.

peripheral circulation (r 0.50 and 0.58, respectively; Table 2). No such correlations could be observed for propionate or butyrate. However, a lower isotope enrichment of butyrate in the portal vein after chicory (Table 2) suggests that some non-labelled butyrate escapes mucosal uptake after its production in the intestine. A lower concentration of butyrate was observed in the colon after the chicory than after the control diet and was possibly due to a more efficient transport of SCFA through the gut barrier during fermentation.

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 turnover²⁶. In a previous clinical trial²⁷, 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 time³⁰, 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 cells³⁴. 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 butyrate³⁵, 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 microbiota degradation of inulin into SCFA after Roberfroid *et al.*³⁹, it is possible to theoretically estimate that approximately 50 μ mol 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 μ mol/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

Table 3. Extraction, uptake and release of acetate, propionate and butyrate by intestine in rats submitted to 4-week chicory or control diets (Expt 3)*

(Mean values with their standard errors)

Diet group...	Extraction (%)				Uptake ($\mu\text{mol/kg}$ per min)				Release ($\mu\text{mol/kg}$ per min)			
	Control (n 8)		Chicory (n 10)		Control (n 8)		Chicory (n 10)		Control (n 8)		Chicory (n 10)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetate	62	12	63	7	2.5	0.6	3.2	0.4	8.6	1.4	11.3	1.1
Propionate	54	7	51	9	0.14	0.03	0.4	0.3	1.7	0.5	2.0	0.5
Butyrate	39	14	38	14	0.08	0.04	0.04	0.01	2.8	0.9	2.4	0.9

* No significant differences were observed between the diets.

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 $590 \mu\text{mol}$ acetate/h was turned over in peripheral blood circulation, with turnover rates of 115 and $40 \mu\text{mol/h}$ 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

Table 4. Concentrations ($\mu\text{mol/g}$ whole content) of acetate, propionate and butyrate in the caecal and colonic contents of rats submitted to 4-week chicory or control diets (Expt 3)

(Mean values with their standard errors)

Diet group...	Caecum				Colon			
	Control (n 8)		Chicory (n 10)		Control (n 8)		Chicory (n 10)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acetate	47	6	85**	10	39	5	44	5
Propionate	14	2	22*	3	11	2	10	2
Iso-butyrate	0.8	0.2	0.2**	0.1	0.5	0.1	0.1**	0.1
Butyrate	46	6	56	6	25	5	16*	3
Iso-valerate	0.9	0.3	0.2*	0.1	0.5	0.1	0.3**	0.1
Valerate	1.6	0.2	1.4	0.2	0.9	0.1	0.4**	0.1

Mean value was significantly different from that of the control group: * $P < 0.05$, ** $P < 0.01$.

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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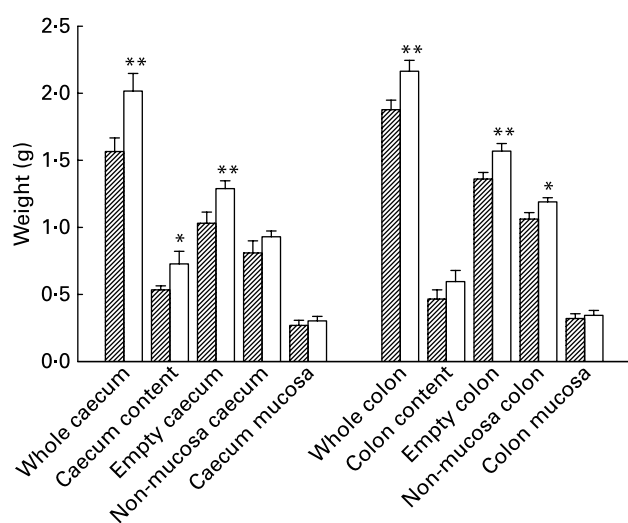


Fig. 5. Weights of whole organs, contents, empty organs, mucosa and non-mucosa tissues of caecum and of colon, respectively, of rats submitted to a 4-week chicory diet (□) or a 4-week control diet (▨) in Expt 3. Data are mean values, with their standard errors represented by vertical bars. Mean value was significantly different from that of the control group: * $P < 0.05$, ** $P < 0.01$ (unpaired t test).

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