Absorption and metabolism of octanoate by the rat colon in vivo: concentration dependency and influence of alternative fuels

J R Jørgensen, M D Fitch, P B Mortensen, S E Fleming

Background: Compared with short and long chain fatty acids, medium chain fatty acids (MCFAs) have been shown to provide the highest colonic absorption of substrate carbon. Moreover, colonic epithelial cells fulfil their basic energy requirements as easily from MCFAs as from short chain fatty acids.

Aims: To further characterise octanoate as a colonic luminal substrate, we determined in vivo the influence of (i) substrate concentration and (ii) alternative luminal fuels, on rat colonic transport and metabolism.

Methods: Segments of rat proximal colon (8 cm) were cannulated and perfused for 100 min with 14C labelled octanoate. The right colic vein was also cannulated and venous blood analysed for total 14C, 14CO2, and metabolites by scintillation counting and high performance liquid chromatography.

Results: Tracer appearance in mesenteric blood stabilised after 20–40 minutes of perfusion. Increasing luminal octanoate concentrations from 2 to 40 mM resulted in linear increases in total carbon absorption. Maximum CO2 production was reached near 10 mM. A substantial proportion of octanoate was absorbed without being metabolised (59–94%). The luminal presence of a mixture of alternative fuels had no influence on either octanoate transport or metabolism.

Conclusions: This study demonstrated substantial concentration dependent colonic absorption of octanoate, rendering this MCFCA a potential and much needed high energy substrate for patients with compromised small bowel function. Moreover, octanoate meets the basic energy requirements of colonic epithelial cells in vivo as well as butyrate. This study also demonstrates the divergence of in vitro and in vivo data regarding fatty acid absorption and metabolism in the colonic epithelium.

Materials and Methods

In this in vivo model, rats were surgically operated on to cannulate a segment of proximal colon, isolate the vasculature, and cannulate the right colic vein draining this segment. The lumen was then perfused with 14C labelled octanoate solutions, and all blood draining the segment was collected from the right colic vein and analysed for total 14C, 14CO2, and metabolites. The experimental protocols are based on procedures that have been used previously.

Chemicals

[1-14C] labelled octanoate (sodium salt) was obtained from American Radiolabelled Chemicals, Inc. (St Louis, Missouri, USA). Unlabelled substrates (sodium salts), acetylcysteine, and antibiotics were obtained from Sigma Chemical Co., Inc.

Abbreviations: acyl-CoA, acyl-Coenzyme A; HPLC, high pressure liquid chromatography; K_m, the Michaelis-Menten constant; MCFAs, medium chain fatty acids; LCT, long chain triacylglycerol; MCT, medium chain triacylglycerol; SCFAs, short chain fatty acids; V_max, maximum reaction rate.
(St Louis, Missouri, USA). Sodium pentobarbital was obtained from Abbott Laboratories, Inc. (North Chicago, Illinois, USA). Sodium heparin was obtained from Elkins-Sinn, Inc. (Cherry Hill, New Jersey, USA).

Animals
Male Sprague-Dawley rats, six months of age, weighing 496 (8) g (mean (SEM)), were obtained from Simonsen Laboratories, Inc., (Gilroy, California, USA). All procedures involving animals were reviewed and approved by the Animal Care and Use Committee, University of California, Berkeley, California, USA.

Substrates and solutions
All substrates were prepared in Krebs-Henseleit buffer without calcium and with antibiotics and acetylcysteine, as previously described. Unlabelled octanoate was dissolved in this solution to produce final concentrations of 2, 5, 10, or 40 mM octanoate. Ethanol solutions of [1-14C] labelled octanoate were evaporated to near dryness and then added in trace amounts to the solutions to produce an average specific activity of 0.08 (0.02) µCi/µmol. Octanoate at 10 mM was also tested in a mixture of 10 mM each of unlabelled acetate, propionate, butyrate, glucose, and glutamine at a specific activity of 0.04 (0.00) µCi/µmol octanoate.

Surgical procedure and lumen perfusion
One rat was anaesthetised and placed on an operating pad with a rectal temperature probe. A nose cone ventilator provided oxygen while the respired gases were exhausted with a rectal temperature probe. A nose cone ventilator was inserted into the lumen of the proximal colon at the caecocolonic junction and also secured tightly. A second cannula for the exit of effluent into the caecum was exteriorised onto a 37°C platform. The middle colic artery and vein were tied off and then cut to allow repositioning of the middle region of the colon within the body cavity. The right colic vein was then exposed. A cannula for infusion of substrate solution was inserted into the lumen of the colon segment at the position of the middle colic vein and secured tightly in place. A second cannula for the exit of effluent was inserted into the lumen of the proximal colon at the caecocolonic junction and also secured tightly. The segment was then flushed to clear it of excess mucus and any remaining digesta. The cannula supplying donor blood was installed into the left saphenous vein and another cannula was inserted into the aorta to measure blood pressure. Donor blood infusion rate was adjusted to maintain a systolic pressure of 160–180 mm Hg. Finally, a cannula was inserted into the right colic vein for blood collection.

The lumen was perfused at 1.0 ml/min with labelled substrate solution. Blood from the right colic vein was quantitatively collected over 10 minute intervals throughout the 100 minute perfusion period. Whole blood was analysed immediately after collection for 14C and 14CO2. Expired 14CO2 caught in the liquid trap was also quantitated.

Blood metabolite analysis
Metabolites for high pressure liquid chromatography (HPLC) analysis were extracted from frozen whole blood aliquots using a modified Bligh and Dyer methanol:chloroform procedure. Equal volumes of blood taken at time points 70, 80, and 90 minutes were pooled and spiked with a solution of unlabelled standards. The procedure has been previously described. All scintillation counting was performed in a 1600TR liquid scintillation counter (Packard Instr., Co.).

Calculations
Calculations were based on the specific activities of octanoate in solutions and the appearance of radioactivity in mesenteric blood, as described previously. Data are reported as nmol octanoate carbon per gram wet weight per minute, where wet weight refers to the fresh weight of the blotted colonic segment exposed to the substrate.

Experimental design and statistical analyses
Fifteen rats were randomly assigned to five different substrate groups so that a total of three rats were exposed to each substrate solution. Because of unequal variance among substrates, statistical differences among group means were determined on log transformed data. When comparing total absorption of substrates and CO2 production, group means were based on the marginal mean value of three consecutive 10 minute intervals (70, 80, and 90 minute time points) under steady state conditions (see results section), and a two way ANOVA on repeated measures was used. When comparing data from HPLC analysis of pooled blood, one way ANOVA was used. Differences were considered to be statistically significant at p<0.05. The Tukey-HSD procedure was used as a follow up test for multiple comparisons. The statistical procedures in SPS 8 were used to perform statistical analyses.

RESULTS

Validity of the perfusion technique
The cannulated colonic segments had a mean length of 7.7 (SEM 0.3) cm and a mean wet weight of 1.06 (0.05) g, and consisted of the proximal third of the entire colon length (excluding the caecum). Sufficient substrate was perfused through the cannulated segment to prevent substantial changes in substrate concentration of the luminal perfusate and thereby maintain a constant availability of substrate to the epithelium during the experiments. On average, <3% of the substrate was transported into mesenteric blood during the 100 minute perfusion period (octanoate in 2 mM, 3.2 (0.6)%; in 5 mM, 2.5 (0.8)%; in 10 mM, 3.5 (0.1)%; in 40 mM, 2.3 (0.2)%; and in 10 mM in a mix, 3.0 (0.2)%).

Breath CO2 was quantitatively collected throughout the experiments. Blood samples were also taken from the aorta at the end of the experiments. The sum of radioactivity in breath CO2, and whole body blood taken from all experiments was 2.6 (0.4)% and 0.3 (0.1)%, respectively, of the radioactivity transported into the mesenteric blood. Furthermore, significant differences were not seen for any of these two measures between substrate solutions, indicating that the procedure effectively prevented transport of luminal perfusate into tissues other than the cannulated intestinal segment, regardless of substrate concentration. Thus by restricting metabolism by other tissues, this method enabled us to accurately assess the fate of luminal octanoate in the perfused colonic segment. Of the total radioactivity that was perfused in substrate solutions, 101.7 (1.2)% was recovered.

Pooling of blood collected for HPLC analysis during steady state absorption
Total octanoate carbon absorption from each substrate solution appeared to stabilise and reach steady state conditions after 20–40 minutes of perfusion (fig 1). Metabolism of octanoate to CO2 required approximately 40 minutes of perfusion to stabilise (fig 1). Thus data from three consecutive 10 minute intervals, corresponding to the 60–90 minutes of perfusion, were chosen for statistical analysis. For these 10 minute intervals, no substrate by time interaction was seen (p=0.64), allowing statistical comparison among the corresponding mean values for the five substrates (two way ANOVA). Correspondingly, no time effect within substrate was noted (p=0.25), showing that steady state absorption of substrates occurred during the 60–90 minute perfusion period. This allowed volumetric pooling of blood from this period for later HPLC analysis of metabolites. Steady state conditions for the 60–90 minute perfusion period were also achieved for the rate of substrate oxidised to CO2 (time interaction not statistically significant, p=0.20) (fig 2). In addition, no substrate
by time interaction was seen for this period (p=0.50), allowing statistical analysis using a single mean steady state value.

Influence of octanoate concentration on transport and metabolism

Total octanoate carbon absorption increased linearly (r=0.990, p<0.01) with increases in the octanoate concentration of the luminal perfusate (fig 3A). Also, the rate at which carbon was transported into mesenteric blood as octanoate increased linearly with luminal octanoate concentration (r=0.991, p<0.01) (fig 3B). Increasing the luminal octanoate concentration did not significantly increase metabolism of octanoate to CO₂ or ketone bodies (p=0.14; fig 3C) or to ketone bodies (p=0.31; fig 3D).

Influence of alternative luminal fuels on the transport and metabolism of octanoate

The rate at which total octanoate carbon was absorbed from a mix of 10 mM each of octanoate, acetate, propionate, butyrate, glucose, and glutamine was not significantly different from the rate of octanoate carbon absorption from 10 mM octanoate alone (fig 4A). Similarly, the presence of alternative fuels in the luminal perfusate did not significantly influence the appearance in mesenteric blood of octanoate carbon absorbed as octanoate, CO₂, or ketone bodies (fig 4B–D).

Relative absorption of unmetabolised substrate and metabolites

The relative proportion of substrate transported into mesenteric blood without being metabolised was substantial, at 59% for 2 mM octanoate (table 1). Relative proportions increased significantly with increasing concentrations of up to 94% for 40 mM octanoate. The corresponding proportion of substrate oxidised to CO₂ was 18% for 2 mM octanoate, and decreased significantly with increasing concentrations to 2% for 40 mM octanoate. Similarly, ketone bodies comprised 13% of total octanoate carbon when octanoate was perfused at 2 mM, and this proportion decreased significantly with increasing concentrations to 1% at 40 mM octanoate. Small proportions of lactate and other metabolites accounted for the remaining substrate found in mesenteric blood. The presence of five other fuels in the luminal perfusate did not significantly influence the relative proportions of metabolites of octanoate in mesenteric blood.

DISCUSSION

The data reported here clearly demonstrate significant absorption of octanoate by the colon. More importantly, this study describes the concentration dependency of both octanoate transport into the blood and its metabolism, primarily to CO₂ and ketone bodies, by epithelial tissues. The Michalis-Menten constant for oxidation of octanoate to CO₂ in vivo is more than 16-fold higher than similar measurements using isolated colonic epithelial cells and is comparable with the in vivo value for butyrate. Also, contrary to previous in vitro studies, octanoate absorption in vivo was shown not to be affected by the simultaneous presence of other energy providing substrates. From these observations, it is likely that colonic absorption of MCFAs may be responsible for a large part of the benefit seen with oral medium chain triacylglycerol (MCT) therapy to patients with compromised small bowel function.

The ability of the colon to absorb octanoate was directly demonstrated by measuring octanoate appearance in the mesenteric venous blood, along with its metabolites, over a wide concentration range (fig 3A). This luminal substrate was available to the epithelial cells for oxidation to CO₂ and ketone bodies, and for other metabolic processes within the cell (table 1). Even at the lowest concentration however the majority was transported unmetabolised into venous blood for distribution throughout the body (fig 3B, table 1).

The maximum total absorption of octanoate carbon when provided at 40 mM was 2.5-fold higher than that of equimolar butyrate, measured previously using identical methodology. The in vivo value for butyrate. Also, contrary to previous in vivo is more than 16-fold higher than similar measurements using isolated colonic epithelial cells and is comparable with the in vivo value for butyrate. Thus the potential for octanoate to provide energy to the whole body in patients with compromised small bowel function can be exploited using dietary modifications. MCTs are in fact already used in a range of malabsorptive disorders, as substitution of medium chain for long chain triacylglycerols (LCT) has been shown to reduce fat excretion. Although this reduction has been explained by a more rapid absorption of MCT than of LCT in the small bowel, colonic absorption of MCFAs may have been a contributing factor. This view is supported by a study in short bowel patients where substitution of MCT for 50% of existing dietary LCT was done. This dietary change resulted in luminal output concentrations of between 36 and 40 mM of octanoate and decanoate, respectively, in patients without a colon in continuity, whereas concentrations were much lower for patients with a colon in continuity (4 and 10 mM, respectively). Hence in some instances appreciable MCFAs may be available to the colon, resulting in a significant gain in fat and energy absorption. As colonic absorption of MCFAs is also manifold greater than that of LCFAs, the potential energetic gain to the patient achieved by replacing LCT with MCT becomes increasingly important with concurrent decreasing small bowel function.

Oxidation of octanoate to CO₂ by colonic epithelial cells in vivo reached maximum velocity (Vₘ₉₅) at a concentration of
approximately 10 mM. Although a precise value for the apparent Michaelis-Menten constant ($K_m$) cannot be calculated from the few data points, it is evident by extrapolation of the curve in fig 3C that $K_m$ is approximately 2.5 mM. These estimated $K_m$ and $V_{max}$ values are similar to those reported previously for butyrate oxidation by the colonic epithelium in vivo and demonstrate that octanoate can easily be used to meet the energy needs of epithelial cells. Thus a fundamental benefit of dietary MCT treatment may lie in the ability of the colon epithelium itself to utilise MCFAs as a primary substrate for maintenance of healthy tissue or for repair of damaged tissue.

Although the oxidation kinetics determined in this study agree well with previous values for butyrate in vivo, the $K_m$ differed dramatically from that determined using isolated colonic epithelial cells. In vitro, octanoate was found to be an excellent substrate with a $K_m$ equal to that of butyrate (0.16 mM and 0.13 mM, respectively, for octanoate and butyrate) and a maximum energy production from oxidation even exceeding that of butyrate. The current work showed that the concentration needed to achieve half maximum oxidation of octanoate was 16-fold higher in vivo than when using isolated cells. This agrees with the result that the $K_m$ for butyrate

**Figure 3** Influence of octanoate concentration on absorption and transport into mesenteric blood of total octanoate (A), unmetabolised octanoate (B), carbon dioxide (C), and ketone bodies (D).

**Figure 4** Influence of the simultaneous presence of other fuels on absorption and transport into mesenteric blood of total octanoate (A), unmetabolised octanoate (B), carbon dioxide (C), and ketone bodies (D). All substrates were present at 10 mM. The mix was composed of octanoate plus acetate, propionate, butyrate, glucose, and glutamine.
oxidation was also manifold higher in vivo than in isolated colonicocytes. The similarity in absorption and oxidation kinetics between octanoate and butyrate reinforces the finding that MCFAs can provide energy to the colon at levels comparable with or exceeding those of butyrate. The higher \( K_e \) seen in vivo for both butyrate and octanoate may be due to a smaller surface area exposed to the substrate solution in the intact perfused colon. In vivo, only the epithelial surface is in contact with luminal contents, and this may be further influenced by a mucus layer. Absorbed substrate is easily transported out of the cell and into the bloodstream, keeping intracellular substrate concentrations below luminal concentrations. Isolated cells, exposed to substrate on all surfaces, are likely in near equilibrium with the surrounding substrate solution, and saturating conditions for oxidation can be reached at a lower solution concentration. Thus in vitro substrate absorption and oxidation rates measured using isolated cells do not always reflect in vivo conditions.

Another significant finding of the current work is that production of \( \text{CO}_2 \) from oxidation of octanoate in vivo was not affected by the presence of other key nutrients. Absorption of total octanoate, transport of unmetabolised octanoate into the blood, and metabolism of octanoate to \( \text{CO}_2 \) and ketone bodies were not affected when equimolar (10 mM) amounts of acetate, propionate, butyrate, glucose, and glutamine were simultaneously present in the luminal perfusate (fig 4). This is in contrast with studies using isolated colonic epithelial cells which showed that butyrate inhibits the oxidation of octanoate and vice versa. The lower apparent \( K_e \) for SCFAs and MCFAs in isolated cells allows saturating conditions to be achieved at much lower substrate concentrations and may accentuate the effects of competition. In vivo however comitant influx of five other primary fuels resulted in no measured decreases in transport or metabolism, even when production of \( \text{CO}_2 \) and ketone bodies from octanoate was at or near maximum. The ability to transport octanoate across the basolateral membrane into the blood may help keep substrate concentrations within the cell below saturation. Thus in vivo it appears that octanoate does not compete with SCFAs for activation to acyl-Coenzyme A (acyl-CoA). Neither does the acetyl-CoA derived from octanoate compete with other acetyl-CoA molecules for oxidation in the tricarboxylic acid cycle or for conversion to ketone bodies. This reinforces the idea that two distinct activating enzymes must be present in colonic epithelial cells: one which activates acetate and butyrate mitochondrialy and another which activates octanoate.

In summary, in vivo octanoate absorption in the proximal rat colon was concentration dependent. Octanoate was transported into venous blood predominantly unchanged while a portion was metabolised by the colonic epithelium, primarily to \( \text{CO}_2 \) and to ketone bodies. Absorption, transport, and metabolism of octanoate were not affected by the simultaneous presence of other luminal substrates, including SCFAs, glucose, and glutamine. These results demonstrate that the colon can effectively use octanoate as oxidative fuel to meet its own energy demands and that it can absorb significant energy which can then be made available throughout the body. Colonic absorption of MCFAs is likely a major contributing factor to the success of oral MCT therapy in small bowel compromised patients.

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REFERENCES

Table 1 Relative proportions of octanoate carbon absorbed and transported into mesenteric blood during the 60–90 minute perfusion period of proximal rat colon

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Total</th>
<th>Unmetabolised substrate</th>
<th>( \text{CO}_2 )</th>
<th>Ketone bodies*</th>
<th>Lactate</th>
<th>Other metabolites†</th>
<th>Known</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>100</td>
<td>58.9 (1.8)*</td>
<td>18.3 (1.1)*</td>
<td>13.3 (2.1)*</td>
<td>3.2 (0.3)*</td>
<td>2.1 (0.2)*</td>
<td>4.2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>75.0 (2.7)*</td>
<td>10.1 (0.6)</td>
<td>6.9 (1.4)*</td>
<td>1.4 (0.1)*</td>
<td>2.0 (0.3)*</td>
<td>4.6 (1.2)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>88.5 (0.8)*</td>
<td>5.0 (0.6)*</td>
<td>3.5 (0.1)*</td>
<td>0.6 (0.0)*</td>
<td>0.8 (0.3)*</td>
<td>1.8 (0.1)</td>
<td></td>
</tr>
<tr>
<td>10 in mix‡</td>
<td>100</td>
<td>86.5 (0.5)</td>
<td>5.4 (0.4)*</td>
<td>3.5 (0.2)*</td>
<td>0.6 (0.1)*</td>
<td>1.7 (0.3)*</td>
<td>2.3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>93.6 (1.2)</td>
<td>2.2 (0.3)*</td>
<td>0.8 (0.1)*</td>
<td>0.2 (0.1)*</td>
<td>1.1 (0.2)*</td>
<td>2.1 (0.7)</td>
<td></td>
</tr>
</tbody>
</table>

p Value

Values are per cent (mean [SEM]), n=3 for each substrate concentration. Values within columns with different superscripts are significantly different at p<0.05.

* Acetoacetate + 3-OH butyrate.
† The known fraction of other metabolites consists of acetate, propionate, butyrate, decanoate, and laurate. The unknown fractions were not further characterised.
‡ The substrate “10 in mix” consists of 10 mM of octanoate in a mixture of 10 mM each of acetate, propionate, butyrate, glucose, and glutamine.
§ Despite an overall p value of 0.04, none of the pairwise comparisons (Tukey) were significantly different.
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