Effect of acetate and propionate on fasting hepatic glucose production in humans

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Objective: Short chain fatty acids (SCFA, e.g. acetate and propionate) produced from bacterial colonic fermentation may be involved in the improvement of fasting glucose concentration observed with high dietary fibre diets. Because fasting blood glucose is related to hepatic glucose production, we have tested the effect of propionate and acetate on hepatic glucose production.
Setting: The study was carried out in the Clinical Research Center for Human Nutrition.
Subjects: Six healthy young volunteers.
Interventions: The subjects received, in a random order: acetate (12 mmol/h), or propionate (4 mmol/h), or acetate + propionate (12 mmol/h + 4 mmol/h), or an isotonic sodium salt solution (saline) in 3 h gastric infusions. Blood glucose and plasma insulin was monitored. Hepatic glucose production was measured with an isotopic method using [6,6-$^2H_2$] glucose.
Results: No changes were observed in blood glucose, plasma insulin concentrations or hepatic glucose production with any of the infused solutions. An increase in free fatty acid (FFA) plasma concentration related to the fasting state was observed with the saline solution, but not with the SCFA infusions ($P < 0.05$). There was also an increase in β-hydroxybutyrate concentration with the saline and the acetate solutions, but not with the propionate or acetate + propionate solutions.
Conclusions: SCFA, administered at a rate calculated on the basis of a continuous daily fermentation of 30 g dietary fibres, do not change hepatic glucose production or fasting blood glucose. Propionate and acetate decrease plasma FFA, and further studies are needed to explore this effect on glucose tolerance and insulin sensitivity.
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Descriptors: acetate, glucose tolerance, hepatic glucose production, human, propionate, short chain fatty acids

Introduction

High dietary fibre diets and soluble fibre supplements may offer some improvement in carbohydrate metabolism (Vinik & Jenkins, 1988). The lowered blood glucose concentrations observed after fibre consumption is usually attributed to the slowed rate of intestinal transit and an attenuated stimulus (Vinik & Jenkins, 1988). However, chronic ingestion of fibre could be associated with low fasting blood glucose levels related to a better insulin sensi-

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tivity as estimated from plasma insulin measurements, urinary excretion of C-peptide and euglycaemic hyperinsulinic clamp (Anderson, Herman & Zakim, 1973; Fukagawa *et al.*, 1990; Anderson *et al.*, 1991). The potential mechanisms involved in these effects of dietary fibres are still unclear. One of these mechanisms (Vinik & Jenkins, 1988; Bergman, 1990) is related to the bacterial fermentation of dietary fibre in the colon that generates short chain fatty acids (SCFA, e.g. acetate and propionate). Indeed hepatic glucose production is the main source of fasting blood glucose (De Fronzo, 1988), and could be, in part, controlled by SCFA (Anderson & Bridges, 1984). *In vitro* data from isolated rat hepatocytes (Anderson & Bridges, 1984) indicate that acetate increases gluconeogenesis from lactate and decreases glycolysis. On the other hand, although propionate was partly converted to glucose, its net effect was to decrease gluconeogenesis and to increase glycolysis (Anderson & Bridges, 1984).

Human data on the effect of SCFA in hepatic glucose production is less consistent. Scheppach *et al.*'s study, in fasting and intravenously fed glucose volunteers, showed no change in hepatic glucose production with oral doses of 15 mmol sodium acetate given every 15 min for 2 h. In this study, there was also no effect of acetate on insulin, glucagon or gastric inhibitor polypeptide levels, but it did temporarily stop the rise in plasma free fatty acids (FFA) observed during the fasted state and it did increase β-hydroxybutyrate concentrations. In another study, Iki Jarvinen, Koivisto & Ilikahri (1988) showed no effect in hepatic glucose production from an intravenous infusion of acetate. Wolever *et al.* (1989) gave a large dose of sodium acetate and sodium propionate alone and in association with a 30 min rectal infusion of glucose and found no change in blood glucose levels when compared to controls who were given an isotonic saline solution. Venter, Vorster & Cummings (1990), on the other hand, reported a decrease in fasting blood glucose and a maximal rise in insulin during glucose tolerance test in 10 healthy female volunteers supplemented with 7.5 g sodium propionate for 7 weeks. Therefore, acetate does not affect hepatic glucose production in humans, but the effect of propionate alone or in combination with acetate is still unclear. The present study was designed to test the acute effect of propionate and propionate + acetate on hepatic glucose production in healthy subjects and to compare the observed findings to the effect of acetate alone. The tested hypothesis was that propionate would decrease glucose hepatic production even in the presence of acetate. The rate of administration of SCFA was chosen in order to match a daily fermentation of a 30 g supplement of dietary fibres (Cummings, 1981).

Materials and methods

Subjects

Six healthy volunteers (three male, three female, mean age 22 ± 1.5 years) participated in the study. All volunteers were of normal weight (BMI: 21.2 ± 0.8 kg/m^2) and had normal physical examination. There was no medical history of diabetes mellitus in any of the volunteers and they did not take any medications during the experimental protocol. The design of the study and possible risks were carefully explained to each subject before written consent was obtained. The experimental protocol was approved by the ethics committee of the University Hospital.

Experimental protocol

Each subject was tested four times, with an interval of ≥1 week between sessions. For 3 days before each test, subjects consumed a weight maintenance diet in our metabolic unit to supply their usual energy-intake (based on a nutritional inquiry). Protein, fat and carbohydrate provided, respectively, 15%, 35%, and 55% of total energy consumed. Foods containing dietary fibres (e.g. non-digestible starch) were avoided. After a 12 h overnight fast all tests were begun at 8 a.m. A line was inserted for blood sampling in a heated hand vein to provide arterialised blood. In the other arm, a second catheter was inserted in an antecubital vein for infusion of the isotope solution. A tungsten-weighted feeding tube (Sherwood Medical, Tullamare, Ireland) was placed into the stomach for the infusion of SCFA solutions. After baseline blood samples were obtained, a priming dose of 22 μmol/kg [6,6-$^2H_2$]glucose 99 atom % enrichment, Tracer Technologies Inc, Somerville, MA) was given as a bolus injection. Immediately

following the priming dose, a 5 h continuous infusion of [6,6-$^2H_2$]glucose tracer was started, at a rate of 20 μmol.kg^{-1}.h^{-1}. Sterile isotope solutions, tested for sterility and pyrogenicity prior to use, were prepared in normal saline solutions and infused through a 0.22 μm Millipore filter. A 3 h continuous infusion of SCFA solution was started, through the feeding tube, 2 h after the start of each experiment. Subjects received in a random order: (1) sodium acetate (Aldrich, France); (2) sodium propionate (Aldrich, France); (3) a mixture of sodium acetate and sodium propionate; (4) saline solution (9% NaCl solution, Agettan, France). SCFA were prepared in saline solution, the rate of infusion being 12 mmol/h for acetate (concentration: 40 mmol/l) and 4 mmol/h for propionate (concentration: 13.5 mmol/l). Osmolarity of the solutions was adjusted with sodium salt at 280 mOsm/l. The rate of delivery of the SCFA was chosen in order to match the mean theoretical production in 24 h of 30 g dietary fibres (Cummings, 1981). Blood samples were taken at times: 0, 60, 75, 90, 105, 120, 150, 180, 210, 240, 255, 270, 285 and 300 min for blood glucose, acetate, FFA, hydroxybutyrate and insulin concentrations, and for isotope enrichment measurements. Plasma samples were stored at –80°C until the time of assay. Breath hydrogen levels were measured before each test in order to indicate any colonic fermentation process.

Analytical methods

Plasma samples were analysed for glucose using the glucose oxidase method, for insulin using a radioimmunoassay (ORIS, Gif sur Yvette, France) and for acetate, FFA, β-hydroxybutyrate, cholesterol and triglycerides, using enzymatic methods (Boehringer Mannheim, Meilland, France). Breath hydrogen and methane concentrations were determined simultaneously on a Microlyser DP gas chromatograph (Quintron Instruments, Milwaukee, WI).

Plasma isotope enrichment of [6,6-$^2H_2$]glucose was measured by Electron ionization (70eV) gas chromatography–mass spectrometry (Hewlett–Packard Model 5971A) of the glucose penta acetate derivatives (Wolfe, 1992). Single ion monitoring was carried out on the frament ion m/z 242 and its isotopes; m/z 243, 244, 245, 246.

Calculations and statistical analysis

Mole fraction of the [6,6-$^2H_2$]glucose tracer was calculated, using peak areas. Mole fraction was defined as the ratio of the enriched ion (m/z 244) relative to all ions monitored, and was corrected using daily calibration curves as previously described (Biolo, Tessari & Inchiostro, 1992).

During the fasting state there is no glucose coming from the gut which is produced only by the liver from both glycolysis and gluconeogenesis. Therefore the appearance rate of glucose in the plasma is equivalent to the total hepatic glucose production (De Fronzo, 1988). Hepatic glucose production (HGP) at steady state was determined from the rate of appearance as previously reported (Wolfe, 1992): HPG = iMFt(1/MFp – 1), where MFt and MFp are the mole fraction enrichments of the tracer and of the plasma and the rate of tracer infusion, respectively.

Data are shown as mean ± s.e.m. Comparisons were made with an analysis of variance and a paired Student's *t*-test when appropriate. Area under the curve was calculated using the trapezoide rule, and all the statistical calculations were made with the Statgraphic software package (Statistical Grafic Corporation, Rockville, MD).

Results

Blood glucose concentrations tended to decrease (non-significantly) from the start to the end of all experiments (Figure 1). There were no differences due to any of the tested products. Insulin concentrations (Figure 1) did not change and there was no statistical difference between the products tested. A slight increase in plasma FFA concentrations was observed before the start of the gastric infusion (≈0.1 mmol/l), which was not different for any of the products tested. From the beginning of the gastric infusion, FFA continued to increase (from 0.4 ± 0.02 to 0.44 ± 0.04 mmol/l) in the control experiment with saline. On the other hand, no change was observed with the infusions of the SCFA. During the gastric infusion, the area under the curve of delta FFA concentrations (Figure 2) was significantly higher with saline as compared to the SCFA (saline *vs* acetate, propionate, or acetate + propionate; $P < 0.05$).

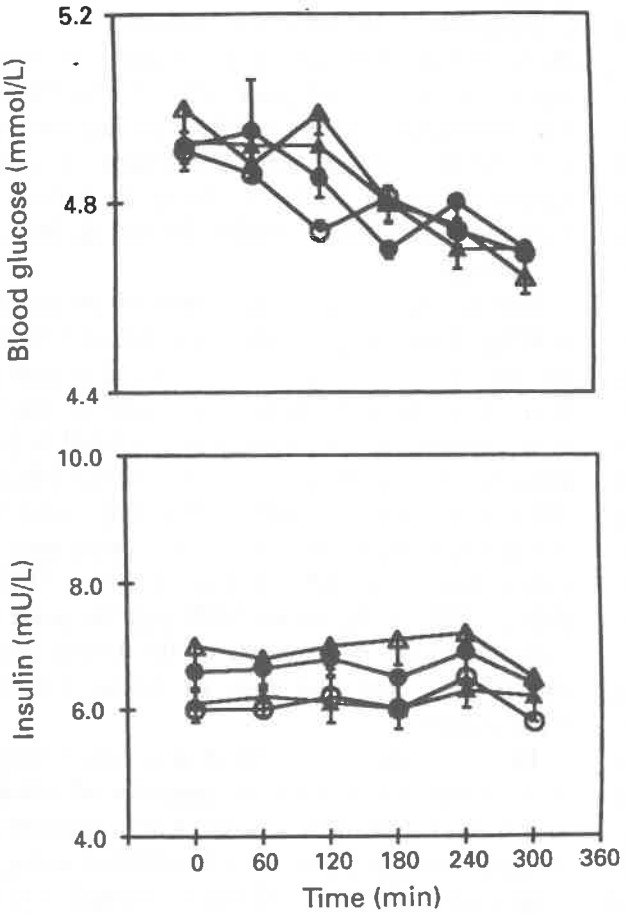

Figure 1 Blood glucose (mmol/l) and plasma insulin (mUI/l) concentrations observed in six volunteers receiving in a random order from 120 to 300 min of the experiments: ○ saline solution; ● acetate (12 mmol/h); △ propionate (4 mmol/h) and ▲ propionate + acetate (4 mmol/h + 12 mmol/h). No significant differences were observed between the four experiments.

Acetate had no effect on the rise in β-hydroxybutyrate concentrations seen in control experiment (Figure 3). Propionate alone or together with acetate, however, inhibited this rise. The differences of the areas under the curve (Figure 3) between saline or acetate as compared to propionate or acetate ± propionate were statistically significant ($P < 0.05$).

Blood cholesterol and triglycerides were not statistically different for any of the products tested.

Initial acetate concentrations were 80 ± 5.6 µmol/l for all experiments and were almost doubled after the start of acetate or acetate + propionate gastric infusion (157 ± 6.2 µmol/l). No change was observed with the saline or the propionate infusion.

Breath hydrogen concentrations were low at the start of all experiments (2.5 ± 0.2 ppm)

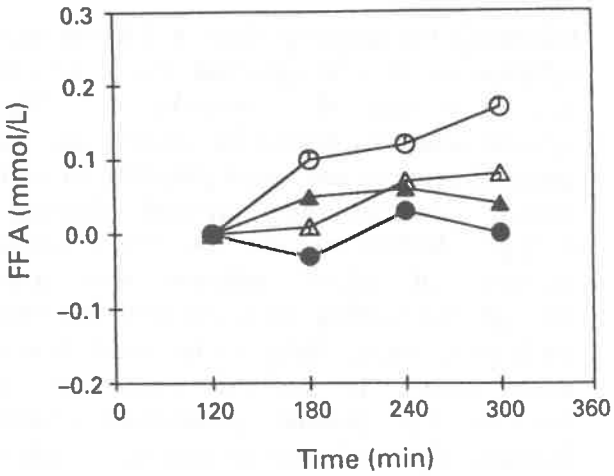

Figure 2 Delta free fatty acids observed in six volunteers receiving in a random order from 120 to 300 min of the experiment: ○ saline solution; ● acetate (12 mmol/h); △ propionate (4 mmol/h) and ▲ propionate + acetate (4mmol/h + 12 mmol/h). Area under the curve was significantly higher ($P < 0.05$) for the saline compared to SCFA solutions.

suggesting no detectable colonic bacterial fermentation and therefore no or little SCFA production from the large bowel during the studies.

Hepatic glucose production is shown in Table 1. No significant difference was observed in hepatic glucose production between any of the SCFA infusions as compared to the control experiment (saline) but there was a non-significant decrease by the end of the experiments related to the length of the fasting state.

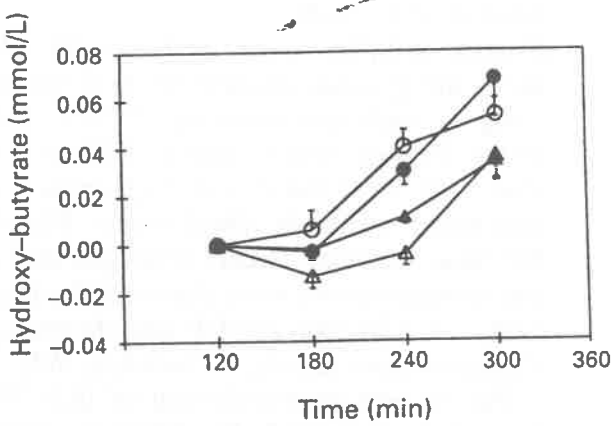

Figure 3 Delta β-hydroxybutyrate observed in six volunteers receiving in a random order from 120 to 300 min of the experiments: ○ saline solution; ● acetate (12 mmol/h); ▲ propionate + acetate (4 mmol/h + 12 mmol/h). Area under the curve was significantly higher ($P < 0.05$) for saline and acetate compared to propionate or propionate + acetate solutions.

Table 1 Hepatic glucose production (μmol/kg.min) measured in 6 healthy volunteers at baseline and after three hours gastric infusion of acetate (12 mmol/h), propionate (4 mmol/h), acetate + propionate (12 mmol/h and 4 mmol/h respectively) and NaCl. No significant differences were observed within and between experiments. Data are reported as mean (s.e.m.)

	Acetate	*Propionate*	*Acetate + propionate*	*NaCl*
Basal	11.61 (0.43)	1.33 (0.61)	11.97 (0.38)	11.38 (0.35)
Gastric infusion	9.46 (0.41)	9.24 (0.33)	9.33 (0.34)	9.22 (0.30)

Discussion

Improvement in glucose tolerance observed with soluble dietary fibres may be related to SCFA produced from bacterial colonic fermentation (Vinik & Jenkins, 1988). In this study, we have tested the hypothesis that this effect of SCFA on fasting glucose metabolism is related to a change in hepatic glucose production. Acetate or propionate alone or in combination were given by continuous gastric infusion over 3 h in healthy volunteers at a rate equivalent to the mean daily SCFA production from 30 g of dietary fibres. We did not observe any difference on hepatic glucose production with the tested SCFA gastric infusions. We therefore reject our original hypothesis. However, all SCFA infusions inhibited the normal rise in plasma FFA concentration related to the fasting state. The acetate had no effect on the rise of ketones observed in control experiments. This rise was inhibited by propionate. Anderson noted a few years ago the opposite effect of high concentrations of acetate (5 mmol/l) and propionate (5 mmol/l) on glucose production in isolated rat hepatocytes (Anderson & Bridges, 1984). Acetate as butyrate and long chain fatty acids increase glucose production from lactate. This effect was probably mediated largely by activating pyruvate carboxylase via acetyl CoA generation (Pilkis, Park & Claus, 1978; Brocks, Siess & Wieland, 1980). In sharp contrast, propionate administration decreased gluconeogenesis in rat hepatocytes because it appeared to lower acetyl CoA concentrations and inhibited pyruvate carboxylase activity further via the production of two specific inhibitors of this enzyme: methyl malonyl CoA and succinyl CoA (Blair, Cook & Lardy, 1973). Also in Anderson & Bridges' study (1984), glycolysis was modified by SCFA. Glycolysis decreased with acetate via an inhibition of phosphofructokinase, a key enzyme of this pathway, due to an enhanced citrate production within the cell (Rennie & Holloszy, 1977). Propionate increased glycolysis because its metabolism is accompanied by a reduction in hepatic citrate concentration (Blair *et al.*, 1973). Moreover, propionate has been shown to inhibit hepatic acetate metabolism (Anderson & Bridges, 1984). In summary, propionate could decrease hepatic glucose production via an inhibition of neoglucogenesis and enhanced glycolysis, whereas acetate shows the opposite effect that does not occur in the presence of propionate.

We observed no changes in human hepatic glucose production as estimated by using the isotopic tracer kinetic method with a continuous gastric administration of acetate that doubled plasma acetate level. The same finding was reported in two previous human studies using the same measurement approach (Scheppach *et al.*, 1988; Iki-Järvinen *et al.*, 1988). In one study, sodium acetate was given by venous infusion, but the hepatic concentration of this substrate was probably low due to the dilution in the total body acetate pool, and due to extensive loss of acetate to muscle tissue (Iki-Järvinen *et al.*, 1988; Bergman, 1990). In the other study, this pitfall was overcome by giving a large amount of acetate by mouth (135 mmol over 2 h). During 1 h, a 7-fold increase of plasma acetate was observed and hepatic glucose production was not changed at $\approx 11\ \mu mol.kg^{-1}.min^{-1}$ compared to hepatic glucose production in fasting individuals (Scheppach *et al.*, 1988).

In a human controlled study, Venter observed in 10 healthy young females a decrease in fasting serum glucose and maximum insulin increments during glucose tolerance tests after 7 weeks of supplementation with a daily oral dose

of 7.5 g sodium propionate (Venter *et al.*, 1990). Changes in hepatic glucose production could explain the drop in fasting blood glucose, but we observed no changes in our study, although sodium propionate was administered at the same rate (4 mmol/h) over a shorter period of time.

Neither did we observe changes in hepatic glucose production when sodium acetate and propionate were given together. This result is not surprising because there was no effect from these substrates when given alone. Moreover, this result is in agreement with a study previously reported by Wolever who observed no decrease in blood glucose when a large bolus of acetate and propionate was given per rectum (Wolever *et al.*, 1989).

No change in insulin concentration was observed, although it was previously shown that propionate could stimulate insulin secretion at very high concentrations in ruminant animals, but this does not seem to occur in non-ruminant animals (Manns, Boda & Willes, 1967; Horino *et al.*, 1968; de Jong, 1982). Cholesterol and triglyceride plasma levels did not change either, although opposite results have been reported for these variables after a short- and a long-term administration of SCFA (Scheppach *et al.*, 1988; Wolever *et al.*, 1989; Venter *et al.*, 1990). This particular aspect of lipid metabolism clearly needs further controlled human experiments.

The significant inhibition of the rise in plasma FFA concentration in the fasted state is also in agreement with previous studies (Crouse *et al.*, 1968; Scheppach *et al.*, 1988; Akanji, Bruce & Frayn, 1989). Indeed, the short-term effect of acetate on FFA plasma concentrations was reported in animals and humans and was related to an inhibitor effect of acetate on lipolysis possibly analogous to the antilipolytic function of ketone bodies (Nilsson & Belfrage, 1978; Balasse, 1986). The same decrease was observed when acetate and propionate were given together per rectum and when propionate was given orally for 7 weeks (Wolever *et al.*, 1989; Venter *et al.*, 1990). This effect of SCFA on FFA could explain the improvement on glucose tolerance observed with dietary fibres. Indeed a better insulin sensitivity and subsequently glucose tolerance in humans is related to reverse changes in FFA metabolism (Reaven, 1988; Kleiber *et al.*, 1992). This relationship, called the glucose–fatty-acid cycle, was described by Randle a few years ago and appears to be one of the major factors controlling glucose utilisation in humans (Randle *et al.*, 1963).

Although acetate may be used in ketone body synthesis (Bush & Milligan, 1971) we did not observe a significant increase in hydroxybutyrate as compared to the saline infusion; a slight but non-significant increase was observed at the end of each experiment. The same result in blood hydroxybutyrate was observed when acetate was given by an intravenous infusion with an accompanying decrease in plasma FFA (Balasse, 1986). From *in vitro* data on rat liver it has been suggested that FFA concentration in the perfusate was a key factor in determining the rate of ketogenesis (Krebs & Hems, 1970). The fact that, with acetate, no decrease in blood ketone bodies was observed, but a decrease in FFA was, suggests that acetate may contribute to hydroxybutyrate synthesis. On the contrary, propionate inhibited ketone body synthesis in our study and in previous studies (Bush & Milligan, 1971). Hydroxybutyrate concentration was similar in the mixed or propionate experiment and the acetate contribution to ketone body synthesis, as suggested above, probably did not occur when propionate was also infused. Therefore, the reported inhibitor effect of propionate on acetate utilisation in rat hepatocytes (Anderson & Bridges, 1984) probably occurs *in vivo* as well.

One can argue that the administered quantity of SCFA was too small. The rate of SCFA administration was calculated on the basis of a total colonic fermentation of 30 g of dietary fibres over 24 h (Cummings, 1981). From theoretical calculations, this amount of fibre should produce 200 mmol of acetate and 90 mmol of propionate. Moreover, this rate of SCFA administration represents the fasting turnover of acetate in human adults (Kien *et al.*, 1992) and 2–3 times that of propionate (Thompson *et al.*, 1990). Finally, the plasma concentration of acetate was doubled with the gastric infusion and was in a range usually reported in nutritional studies of supplementation with dietary fibre (Pomare, Branch & Cummings, 1985; Bridges *et al.*, 1992). We did not measure propionate concentration. Because of its hepatic metabolism, no change in propionate concentrations is usually reported after its ingestion (Bergman, 1990).

Finally, we have observed in this study significant changes in FFA and ketone body plasma concentration as previously reported, which suggests that the administered amount was high enough to induce metabolic changes. It should also be noted that we measured total fasting hepatic glucose production, but we did not estimate the contribution from gluconeogenesis or glycogenolysis. Special approaches are necessary to discern the effect of SCFA on these two hepatic pathways (Wolfe, 1992). However, total hepatic glucose production is the main factor controlling glucose tolerance in the fasted state and is mainly related to gluconeogenesis due to the subjects having fasted for more than 12 h (De Fronzo, 1988). Finally, the time of our study might be considered too short and the next step should be long-term studies with an initial adaptation phase. It should be noted, however, that the response time for many gene transcriptions involved in glucose metabolism, such as for phosphoenolpyruvate carboxykinase, a key enzyme of gluconeogenesis, is usually short in response to hormones or fatty acids and is in the time range of our study (Clarke & Abraham, 1992; Magnuson, Quinn & Granner, 1987).

In conclusion, a gastric infusion of SCFA at a rate within nutritional recommendations for dietary fibre consumption does not change hepatic glucose production. The main effect of acetate or propionate is on FFA plasma concentration and this needs further investigation in order to explain the beneficial role of dietary fibre in glucose tolerance in humans.

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