Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-α

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In animals, colonic infusion of SCFA does not affect glucagon-like peptide-1 (GLP-1) release whereas intravenous infusion does and SCFA may directly stimulate peptide YY (PYY) release. It is unknown whether SCFA and their route of administration affect human blood concentrations of GLP-1 and PYY. Our aim was to conduct a pilot study to determine the effects of intravenous and rectal acetate on blood concentrations of GLP-1, PYY, ghrelin, adiponectin and TNF-α in hyperinsulinaemic human subjects. Six hyperinsulinaemic female subjects were given 20 mmol sodium acetate intravenously, 60 mmol acetate rectally, or normal saline rectally or intravenously on four separate occasions in randomised order, with blood samples collected at 0, 10, 15, 30, 45 and 60 min. Change in plasma PYY was significantly higher after acetate and rectal infusions (9.69 and 13.78 pg/ml) compared with saline and intravenous (0.60 and 3.1 pg/ml; P < 0.01), respectively. Change in plasma GLP-1 was increased by rectal and acetate infusions (0.25 and 0.23 mmol/l) v. intravenous and saline (−0.26 and −0.19 mmol/l; P < 0.01). Acetate decreased TNF-α v. saline (−0.8 and 0.15 pg/ml; P < 0.05). Rectal infusions increased TNF-α and ghrelin (0.2 and 98.27 pg/ml) v. intravenous (−0.9 and −40 pg/ml; P < 0.01). There was no effect of treatment on plasma adiponectin. These preliminary results suggest that acetate raises plasma PYY and GLP-1, and suppresses TNF-α. Also, distending the rectum increases PYY, GLP-1, TNF-α and ghrelin in hyperinsulinaemic females. Increasing colonic fermentation products, particularly acetate, could yield a new mechanism for modifying weight gain.

Acetate: Infusion: Hormones

High intake of dietary fibre is associated with reduced weight gain1) and reduced risk for CVD and type 2 diabetes2–5). These effects may be due partly to the effects of increased colonic fermentation on gut- and adipose-derived hormones. Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are gut hormones involved in the regulation of appetite6–8). Animal and cell-culture studies suggest that colonic distension or the SCFA products of fermentation may up-regulate PYY and proglucagon gene expression9) and stimulate PYY and GLP-1 release10). In addition to its effects on appetite regulation, GLP-1 may also protect against type 2 diabetes by increasing insulin secretion and sensitivity and preserving the mass of insulin-secreting β-cells in the pancreas11,12). Adiponectin and TNF-α are hormones produced by adipose tissue which may influence the risk of type 2 diabetes and CVD via effects on insulin sensitivity and endothelial function13,14).

The effects of SCFA on gut and adipose hormones may differ in different animal species and may depend on whether SCFA are infused intravenously (IV) or rectally9,10). The effects in human subjects are not known. Since acetate is the major SCFA produced during colonic fermentation, our objectives were to conduct a pilot study to compare the effects of IV v. rectal infusion of sodium acetate v. sodium chloride (normal saline) on blood concentrations of GLP-1, PYY, ghrelin, adiponectin and TNF-α in hyperinsulinaemic human subjects. We studied hyperinsulinaemic subjects because, being at increased risk for obesity, type 2 diabetes and CVD, they could stand to benefit from any protective effects of increased fibre intake.

Subjects and methods

Subjects

Six females (age 44 (SEM 4) years; BMI 31.0 (SEM 1.0) kg/m²) with high fasting plasma insulin (105 (SEM 19) pmol/l) were recruited from Toronto and the surrounding area. Subjects were excluded from the study if they were pregnant, had a history of endocrine or gastrointestinal problems, diabetes, recent surgery, or had taken antibiotics within the previous 3 months. All but one of the subjects were participants in a previous study. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Subjects Review Committee, Office of Research Services, St Michael’s Hospital and University of Toronto. Written informed consent was obtained from all subjects.

Abbreviations: CGRP, calcitonin gene-related peptide; GLP-1, glucagon-like peptide-1; IV, intravenous; PYY, peptide YY.

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Study design

The study followed a single-blind, randomised cross-over design where subjects were infused IV and rectally with sodium acetate or saline on four different occasions. The four treatments consisted of (a) 20 mmol sodium acetate (1·66 g) dissolved in 100 ml sterile water given IV; (b) 100 ml saline given IV; (c) 60 mmol sodium acetate (4·92 g) dissolved in 300 ml water given rectally; (d) 300 ml water given rectally. The IV infusions were performed manually by a physician at a rate of 12·5 ml per min. The rectal infusions were also performed manually by the research coordinator at a rate of 37·5 ml per min using five 60 ml syringes. For the rectal infusions approximately 2 cm of the end of a piece of Tygon tubing (outer diameter 4 mm; Norton Performance Plastics, Akron, OH, USA) was inserted in subjects’ rectums. The other end of tubing was connected to a three-way tap and the dispensing syringe. The treatments were prepared by the Pharmacy at St Michael’s Hospital on the morning they were required.

Subjects came to the Nutrition Centre at St Michael’s Hospital at about 08.00 hours after a 12 h overnight fast on four separate occasions approximately 1 week apart. Upon arrival, an IV catheter was inserted into an arm vein for blood taking and a fasting blood sample obtained. The IV infusions were given via the same catheter at a rate of 12·5 ml per min over 8 min; after the test solution had been infused the catheter was flushed with 20 ml normal saline. For the rectal infusions, subjects inserted the plastic tubing into the rectum and the treatments were administered using a syringe at a rate of 37·5 ml per min for 8 min. Blood samples for glucose, insulin, hormones and acetate were collected fasting and at 10, 15, 30, 45 and 60 min after the start of the infusion.

Blood samples for plasma acetate concentrations

Blood was collected into tubes with potassium oxalate and centrifuged at 600 g for 10 min at 4°C. The plasma was removed and stored at −70°C until sample preparation and analysis by GC as previously described (18).

Blood samples for plasma glucose and insulin

Blood was collected into Vacutainer™ SST™ (BD Canada, Mississauga, ON, Canada) tubes. Insulin inhibitor (50 μl; Banting and Best Diabetes Centre, Mount Sinai Hospital, Toronto, ON, Canada) was added immediately. The samples were mixed and centrifuged at 600 g for 10 min at 4°C and the plasma removed and stored at −70°C until analysis of glucose using a enzymic reference method with hexokinase (16) and insulin by electrochemiluminescence immunoassay (17).

Blood samples for plasma glucagon-like peptide-1 and peptide YY

Blood was collected into ice-cooled EDTA tubes. Then 50 μl each of dipeptidyl peptidase-IV inhibitor (Millipore, Billerica, MA, USA) and aprotinin (Trasylol®; Bayer Inc., Toronto, ON, Canada) were added and mixed within 30 s of collection. The samples were centrifuged at 300 g for 15 min at 4°C and the plasma removed and stored at −70°C until analysis. GLP-1 was analysed by ELISA (Millipore) at the Banting and Best Diabetes Centre. This is a procedure that captures active GLP-1 (γ, α-amide and γ, γ'7) by a monoclonal antibody with specific binding to the N-terminal region of the molecule. PYY was measured in quadruplicate by a commercially available RIA (Millipore), using 125I-labelled bioactive PYY as a tracer and PYY3-36 antibody raised in guinea-pigs. This procedure captures human PYY3-36 with an intra- and inter-assay CV of 6·5 and 7·5%.

Blood samples for plasma adiponectin and TNF-α

Blood was collected into ice-cooled EDTA tubes. The samples were centrifuged at 300 g for 15 min at 4°C and the plasma was removed and stored at −70°C until analysis. Adiponectin was analysed in duplicate by RIA (Millipore), using 125I-labelled murine adiponectin as a tracer and a multispecies adiponectin rabbit antibody. Intra- and inter-assay CV were 4·5 and 8·1%. TNF-α was analysed by the sandwich enzyme immunoassay technique (18) at the Banting and Best Diabetes Centre.

Blood samples for plasma ghrelin

Blood was collected into ice-cooled EDTA tubes and 50 μl aprotinin (Trasylol®; Bayer Inc.) was added immediately. The samples were then mixed and centrifuged at 300 g for 15 min at 4°C, and 5 ml HCl (200 μl) was added to the plasma for a final concentration of 0·05 M. The plasma was removed and stored at −70°C until analysis. Active ghrelin was analysed in quadruplicate by a commercially available RIA (Millipore), using 125I-labelled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the C-terminal end of human ghrelin. Intra- and inter-assay CV were 8·5 and 13·2%.

Statistical analysis

The elimination rate constant for acetate (Kac) was the regression slope of the exponential fitted to the disappearance curve of acetate after the end of the IV infusion (10–60 min). The plasma half-time was calculated as 0·693/Kac. The change in plasma concentrations between the four treatments were compared using repeated-measures ANOVA for the main effects of acetate (acetate x route) and time and their interactions. Differences in plasma concentrations of acetate, glucose, insulin and hormones were assessed by repeated-measures ANOVA for the main effects of acetate (acetate x route) and time and their interactions. If there was no significant interaction between acetate, route and time, the data were analysed as a two-factor ANOVA with repeated measures on route and acetate. Differences in plasma concentrations between the four treatments were assessed as a secondary analysis by two-factor ANOVA with repeated measures on time and treatment; if there was a significant time x treatment interaction ANOVA was performed at each time point. For all analyses, individual means were compared using Tukey’s test to correct for multiple comparisons with the criterion for significance being (two-tailed) P<0·05. The analyses were not adjusted for multiple endpoints. All statistical analyses were performed.
by version 9.1 of SAS (SAS Institute Inc., Cary, NC, USA). Results are expressed as mean values with their standard errors.

**Results**

All subjects completed the four treatments. Fasting plasma acetate, glucose and hormone concentrations did not differ significantly between treatments (Table 1).

### Acetate, glucose and insulin

There were significant main effects of route \((P<0.01)\) and acetate \((P<0.0001)\) and significant route \(\times\) acetate \(\times\) time \((P<0.0001)\) and route \(\times\) acetate \((P<0.025)\) interactions, for plasma acetate (Table 2). Plasma acetate was significantly greater after IV acetate than all other treatments at 10 min and significantly greater after IV acetate than IV saline and rectal acetate at 15 min. From 30 to 60 min plasma acetate was significantly greater after rectal acetate than after all other treatments (Fig. 1). After IV acetate the elimination rate constant was 0.33 (SEM 0.03) per min with a serum half-life of 2.2 (SEM 0.2) min. There were no significant effects for either plasma glucose or insulin (Table 2, Fig. 2).

### Glucagon-like peptide-1

Plasma GLP-1 was significantly higher after rectal than IV infusions and significantly higher after acetate than saline infusions (significant main effects of route and acetate; Table 2). When comparing the IV and rectal acetate infusions, IV infusions increased plasma GLP-1 to a significantly greater extent than rectal infusions (route \(\times\) acetate interaction; \(P=0.01\)), but no significant differences were found at the individual time points (Fig. 3(a)).

### Peptide YY

For PYY, there were significant main effects of time \((P<0.025)\), route \((P<0.001)\) and acetate \((P<0.001)\), and a significant time \(\times\) acetate interaction \((P<0.05)\) (Table 2). Plasma PYY was significantly higher after rectal than IV infusions and significantly higher after acetate than saline. At 10 min, plasma PYY was significantly greater after rectal saline than IV saline. After rectal acetate, plasma PYY continued to increase throughout the experiment, being significantly higher than IV saline at 15 and 30 min, higher than IV acetate and IV saline at 45 min, and higher than all other treatments at 60 min (time \(\times\) treatment interaction; \(P<0.001\); Fig. 3(b)).

### Ghrelin

There was no effect of treatment on plasma ghrelin concentrations (Fig. 3(c)). Plasma ghrelin was significantly higher after rectal than IV infusions (main effect of route; \(P<0.01\) but the main effect of acetate was not significant \((P=0.07)\); Table 2). Plasma ghrelin tended to be highest after rectal saline infusion (Fig. 3(c)), but the route \(\times\) acetate interaction was not significant.

### TNF-\(\alpha\) and adiponectin

There were significant main effects of route \((P<0.05)\) and acetate \((P<0.05)\), such that plasma TNF-\(\alpha\) was higher after rectal than IV infusions and after saline and acetate (Table 2); there was no significant route \(\times\) acetate interaction (Fig. 3(d)). Plasma adiponectin concentrations were not affected by treatment, route or acetate (Table 2).

### Discussion

Increased colonic fermentation may influence gut and adipose hormones by distending the colon or by increasing serum SCFA concentrations. Our preliminary results suggest that both these effects may acutely stimulate PYY and GLP-1 secretion in human subjects. Furthermore, raising serum acetate acutely suppressed TNF-\(\alpha\) while distending the rectum raised plasma TNF-\(\alpha\) and ghrelin concentrations.

It has been estimated that 220–760 mmol SCFA are produced daily from the colonic fermentation of malabsorbed starch and dietary fibre, depending on the types of carbohydrate foods in the diet\(^{(19)}\). Since acetate, propionate and butyrate exist in human colonic contents in molar ratios of

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**Table 1.** Fasting values for metabolic parameters by treatment

(Mean values with their standard errors)

<table>
<thead>
<tr>
<th>Variable</th>
<th>I-S Mean</th>
<th>I-S SEM</th>
<th>I-A Mean</th>
<th>I-A SEM</th>
<th>R-S Mean</th>
<th>R-S SEM</th>
<th>R-A Mean</th>
<th>R-A SEM</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate ((\mu)mol/l)</td>
<td>27.3</td>
<td>5.2</td>
<td>25.9</td>
<td>6.0</td>
<td>26.0</td>
<td>7.2</td>
<td>17.8</td>
<td>3.3</td>
<td>0.48</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>4.50</td>
<td>0.2</td>
<td>4.73</td>
<td>0.3</td>
<td>4.55</td>
<td>0.1</td>
<td>4.53</td>
<td>0.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>104</td>
<td>40</td>
<td>106</td>
<td>43</td>
<td>80.0</td>
<td>9.8</td>
<td>121</td>
<td>50</td>
<td>0.51</td>
</tr>
<tr>
<td>GLP-1 (pmol/l)</td>
<td>3.50</td>
<td>0.7</td>
<td>4.2</td>
<td>1.1</td>
<td>2.83</td>
<td>0.5</td>
<td>3.50</td>
<td>0.7</td>
<td>0.69</td>
</tr>
<tr>
<td>PYY (pg/ml)</td>
<td>86.4</td>
<td>7.3</td>
<td>83.7</td>
<td>17</td>
<td>95.3</td>
<td>13</td>
<td>86.3</td>
<td>13</td>
<td>0.90</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>181</td>
<td>68</td>
<td>274</td>
<td>158</td>
<td>166</td>
<td>105</td>
<td>150</td>
<td>45</td>
<td>0.82</td>
</tr>
<tr>
<td>Adiponectin (pg/ml)</td>
<td>10.4</td>
<td>1.4</td>
<td>12.0</td>
<td>3.7</td>
<td>10.7</td>
<td>1.9</td>
<td>12.0</td>
<td>1.6</td>
<td>0.39</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>1.93</td>
<td>1.4</td>
<td>2.53</td>
<td>1.7</td>
<td>0.77</td>
<td>0.5</td>
<td>2.68</td>
<td>1.3</td>
<td>0.62</td>
</tr>
</tbody>
</table>

I-S, intravenous saline; I-A, intravenous acetate; R-S, rectal saline; R-A, rectal acetate; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

* For details of the four treatments, see Subjects and methods, study design section.
about 3:1 \textsuperscript{(20)}, the amount of acetate produced would be expected to be in the range of 130–460 mmol/24 h; thus, 20 mmol acetate, the amount we infused IV, would be produced within 1–4 h. We infused 60 mmol acetate rectally because we found that 25–30% of rectal acetate is absorbed into the circulation over 30 min \textsuperscript{(15)}.

After rectal acetate infusion, serum acetate reached a peak at about 45 min; this is consistent with previous studies in human subjects in which serum acetate peaked between 60 and 90 min after rectal infusion of 180 mmol acetate over 30 min, and between 30 and 60 min after rectal infusion of 90 mmol acetate over 30 min \textsuperscript{(21,22)}. However, the elimination rate constant of serum acetate after IV acetate infusion was over ten times that previously reported in normal subjects \textsuperscript{(23)}. This difference may be explained by differences in infusion protocol and analytical methods. In the previous study, acetate clearance may have been delayed because it was infused at a...
rate of 2.5 mmol/min for 60 min instead of only 8 min in the present study. In addition, serum acetate was measured using an enzymic method which resulted in nearly 10-fold higher fasting values (220 (SEM 10) v. 24 (SEM 4) mmol/l) but less than 2-fold higher peak values than those we obtained (1120 (SEM 20) v. 637 (SEM 62) mmol/l).

The lack of effect of acetate on plasma glucose and insulin is consistent with previous IV(23) and rectal infusion studies(21) and also in keeping with its lack of effect on glucose tolerance and glucose turnover in healthy subjects (24,25).

We found that both raising serum acetate and distending the rectum significantly raised plasma GLP-1 levels. This is not consistent with a previous report that failed to show an effect on plasma GLP-1 of rectal infusion of 54 or 90 mmol SCFA (37.8 and 63 mmol acetate, respectively)(26). However, we infused 300 ml fluid into the rectum over 8 min whereas Ropert et al. (26) infused 180 ml over 1 h (3 ml/min) into the proximal colon. Therefore our infusion rate probably produced a higher rise in serum acetate and greater colonic distension. The effect of acetate may be mediated via calcitonin gene-related peptide (CGRP). Acetate (1 mM) increases CGRP secretion from the rat colon(27), and CGRP, in turn, increases GLP-1 release from rat ileum (28,29). There may also be a direct effect of SCFA on GLP-1 secretion, since addition of SCFA to total parenteral nutrition feeding for periods of 6 to 72 h increased ileal proglucagon mRNA in rats(30,31), an effect which is not butyrate specific(32). The mechanism by which rectal distension increases GLP-1 is not clear; nevertheless, ileal infusion of saline increased GLP-1 from baseline in pigs(10).

Similar to GLP-1, PYY is produced and secreted from intestinal L-cells located primarily in the distal ileum and proximal colon. Expression of the SCFA receptor G-protein coupled receptor 43 has been found in enteroendocrine L-cells containing PYY in the rat intestine and human colon(33). Furthermore, CGRP(28) and SCFA(34) both increased PYY release from the isolated vascularity perfused rat ileum. Consistent with these results, in the present study infusion of acetate into the rectum elicited a gradual and sustained release of PYY. The less pronounced and transient rise in PYY seen after IV infusion of acetate is also consistent with this mechanism since less acetate would reach the gut and for a shorter period of time. Similar to previous studies(10,26,35), rectal saline infusion also elicited a transient rise in PYY, suggesting that mechanical distension of the rectum stimulates PYY secretion as well.

Ghrelin is an orexigenic factor released primarily from oxyntic cells of the stomach, but also from the duodenum, ileum, caecum and colon(36,37). Ingestion of carbohydrate and fat decreases plasma ghrelin in human subjects, whereas ingestion of protein increases postprandial ghrelin(38). We found that acetate tended to reduce ghrelin relative to saline but this effect did not reach statistical significance. It is of interest that distending the rectum with the rectal infusions increased plasma ghrelin significantly compared with IV infusions. This may have been due to mechanical stimulation of

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**Fig. 3.** Change in plasma (a) glucagon-like peptide-1 (GLP-1), (b) peptide YY (PYY), (c) ghrelin and (d) TNF-α in six subjects who were given intravenous (IV) acetate (- - - -), rectal acetate (- - - -), IV saline (- - - -) or rectal saline (- - - -). Values are means, with standard errors represented by vertical bars. There was a time × treatment interaction for PYY (P<0.001; ANOVA). * Rectal saline > IV saline (P<0.025; Tukey corrected). † Rectal acetate > IV saline (P<0.02; Tukey corrected). ‡ Rectal acetate > IV acetate and IV saline (P<0.01; Tukey corrected). § Rectal acetate > IV acetate, IV saline and rectal saline (P<0.001; Tukey corrected).
the colon or neuronal signalling to the stomach, since the greatest concentration of oxyntic cells is in the stomach. By contrast, distension of the stomach with a solution of guar gum had no effect on plasma ghrelin.\(^{33}\)

Adiponectin levels are decreased in obesity-related insulin resistance\(^{13}\) and this may be due, at least in part, to increased TNF-\(\alpha\).\(^{14}\) Chronic elevation of TNF-\(\alpha\), and other inflammatory markers, leads to insulin resistance whereas adiponectin promotes insulin sensitivity\(^{13}\). Both adiponectin and TNF-\(\alpha\) are produced and secreted by adipose tissue. In response to adipogenesis, TNF-\(\alpha\) and TNF receptor 2 are decreased\(^{39}\) and acetate is an agonist of adipogenesis\(^{40}\). However, we did not see any changes in plasma concentrations of adiponectin with acetate administration regardless of the route. Acetate decreased TNF-\(\alpha\) compared with saline, consistent with increased adipogenesis, and rectal infusion increased TNF-\(\alpha\) compared with IV. Similar to ghrelin, this may have been due to mechanical stimulation of the colon; the relative swiftness of its distension may have induced a slight stress response.

Previously we have shown that long-term ingestion of wheat fibre increases plasma levels of butyrate and acetate\(^{41}\) and others have shown increases in faecal bulk and stool weight\(^{42}\). Based on our current findings, both of these effects would be expected to raise GLP-1 and PYY, indeed as we have shown for GLP-1\(^{41}\). This would be of benefit, particularly for obesity, since both reduce food intake\(^{46–8}\). Conversely, increased colonic fermentation with fibre intake may lower, whereas faecal bulking may raise TNF-\(\alpha\) and ghrelin. Thus, there may be no overall effect of increased cereal fibre intake on these hormones.

The plasma acetate concentrations achieved after rectal infusion were within an achievable range. Previous research has shown that fasting plasma concentrations of acetate can rise to 100–300 \(\mu\)mol/l after ingestion of fermentable carbohydrate\(^{43,44}\). In the present study, peak plasma acetate levels were about 200 \(\mu\)mol/l after rectal infusion of acetate. By contrast, IV acetate resulted in peak plasma acetate concentrations of about 650 \(\mu\)mol/l. We gave acetate as a bolus to achieve as high a blood value as possible and determine if there was an effect. However, continuous infusion of acetate would probably be more appropriate to imitate the physiological production and absorption of acetate.

We conclude that both distending the rectum and infusing acetate acutely raised plasma PYY and GLP-1 in hyperinsulinaemic, overweight women. Furthermore, acetate suppressed TNF-\(\alpha\) and distending the rectum increased TNF-\(\alpha\) and ghrelin. These findings may be relevant to our understanding of the effect of dietary fibre on weight management and insulin sensitivity. However, it is important to note that our conclusions apply to the acute infusion of acetate; it is possible that the responses may vary with long-term ingestion of cereal fibre due to adaptation of colonic bacteria. With chronic ingestion of cereal fibre, plasma SCFA, particularly butyrate, and GLP-1 gradually rose over time\(^{41}\), but plasma acetate did not reach levels seen in the present study. Thus, whether the other hormone levels would change to the same extent with increased cereal fibre warrants investigation. In addition, because of the small number of subjects studied, further work is required to confirm these effects.

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K. R. F. conducted the research and wrote the manuscript under the supervision of T. M. S. W.

There are no conflicts of interest for either author.

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