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Colon luminal content and epithelial cell morphology are markedly modified in rats fed with a high-protein diet

Mireille Andriamihaja,[1] Anne-Marie Davila,[1] Mamy Eklou-Lawson,[1] Nathalie Petit,[1] Serge Delpal,[2] Fadhila Allek,[1] Anne Blais,[1] Corine Delteil,[1] Daniel Tomé,[1] and François Blachier[1]

[1]*Institut National de la Recherche Agronomique (INRA), AgroParisTech, Centre de Recherche en Nutrition Humaine-Ile de France, UMR 914 Physiologie de la Nutrition et du Comportement Alimentaire, Paris, France;* [2]*INRA, Unité de Nutrition et Régulation Lipidique des Fonctions Cérébrales, Jouy en Josas, France*

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Andriamihaja M, Davila A, Eklou-Lawson M, Petit N, Delpal S, Allek F, Blais A, Delteil C, Tomé D, Blachier F. Colon luminal content and epithelial cell morphology are markedly modified in rats fed with a high-protein diet. *Am J Physiol Gastrointest Liver Physiol* 299: G1030–G1037, 2010. First published August 5, 2010; doi:10.1152/ajpgi.00149.2010.—Hyperproteic diets are used in human nutrition to obtain body weight reduction. Although increased protein ingestion results in an increased transfer of proteins from the small to the large intestine, there is little information on the consequences of the use of such diets on the composition of large intestine content and on epithelial cell morphology and metabolism. Rats were fed for 15 days with either a normoproteic (NP, 14% protein) or a hyperproteic isocaloric diet (HP, 53% protein), and absorptive colonocytes were observed by electron microscopy or isolated for enzyme activity studies. The colonic luminal content was recovered for biochemical analysis. Absorbing colonocytes were characterized by a 1.7-fold reduction in the height of the brush-border membranes ($P = 0.0001$) after HP diet consumption when compared with NP. This coincided in the whole colon content of HP animals with a 1.8-fold higher mass content ($P = 0.0020$), a 2.2-fold higher water content ($P = 0.0240$), a 5.2-fold higher protease activity ($P = 0.0104$), a 5.5-fold higher ammonia content ($P = 0.0008$), and a more than twofold higher propionate, valerate, isobutyrate, and isovalerate content ($P < 0.05$). The basal oxygen consumption of colonocytes was similar in the NP and HP groups, but ammonia was found to provoke a dose-dependent decrease of oxygen consumption in the isolated absorbing colonocytes. The activity of glutamine synthetase (which condenses ammonia and glutamate) was found to be much higher in colonocytes than in small intestine enterocytes and was 1.6-fold higher ($P = 0.0304$) in colonocytes isolated from HP animals than NP. Glutaminase activity remained unchanged. Thus hyperproteic diet ingestion causes marked changes both in the luminal environment of colonocytes and in the characteristics of these cells, demonstrating that hyperproteic diet interferes with colonocyte metabolism and morphology. Possible causal relationships between energy metabolism, reduced height of colonocyte brush-border membranes, and reduced water absorption are discussed.

hyperproteic diet; colonic epithelial cells; ammonia; glutamine synthetase

IN A CONTEXT OF HIGH PREVALENCE of overweight and obesity (30), high-protein diets (HP) are commonly used for body weight control (27). However, the consequences of the consumption of such diets on the composition of large intestine content and on colonic epithelial cell characteristics have been little investigated.

Address for reprint requests and other correspondence: F. Blachier, UMR 914 PNCA INRA/AgroParisTech, 16 rue Claude Bernard, F-75005 Paris, France (e-mail: francois.blachier@agroparistech.fr).

Nitrogenous compounds can enter the large intestine through the ileocecal junction. This material, which originates from both dietary and endogenous sources, mainly consists of proteins and peptides (10), amounting to between 6 and 18 g per day in humans (23, 50). The protein source is known to modify the level of endogenous protein secretion and the digestibility of proteins in the intestine (2, 6, 19, 20). Proteins and peptides in the large intestine undergo intense proteolysis by residual pancreatic enzymes (21) and colonic flora proteases (25) resulting in the release of small peptides and free amino acids followed by the production of numerous bacterial metabolites including ammonia and short-chain fatty acids. Several among these bacterial metabolites are known to interfere with the colonic epithelium metabolism and physiology (4). However, the beneficial or deleterious effects of these metabolites on colonic mucosa remain largely unknown, and the maximal nondeleterious level of alimentary protein intake is still to be defined.

Short-chain fatty acids are produced from alimentary fibers, resistant starch, and amino acids (38, 39). They include acetate, butyrate, propionate, and iso-short-chain fatty acids. The latter, which are exclusively produced from amino acids, can be considered as a reflection of luminal protein breakdown in the large intestine. Short-chain fatty acids are known to be oxidized by the colonic epithelial cells (1, 48).

Luminal ammonia is produced through amino acid deamination and hydrolysis of urea produced in the liver and moving from the blood to the large intestine lumen (15, 37, 54, 56, 57). Ammonia (considered as the sum of NH_4^+ and NH_3) is present at millimolar concentrations in the human colonic lumen (34). In humans, an increase in the amount of ingested protein leads to a rise in fecal ammonia concentration (16, 22). In the rat model, a HP markedly increase ammonia concentration in the colonic lumen (42). Approximately 4 g of ammonia are absorbed daily by the human colon (51, 56). Ammonia absorption occurs through members of the ammonium transporter family, i.e., RhBG and RhCG (28) and of H^+-K^+-ATPase, which can function as NH_4^+-ATPase (12). In the rat distal colon, NH_3 is 400 times more permeant than NH_4^+ (13). NH_4^+ and NH_3 absorption is likely made through absorptive epithelial cells since the apical membranes of lower crypt colonocytes have a low permeability for NH_4^+/NH_3 (49). Since the pKa of NH_4^+/NH_3 is 9.25 (43), and since the luminal colonic pH in rats ranges from 6.2 to 7.0 (42), the vast majority of ammonia in the colon lumen is in the form of NH_4^+.

Ammonia at millimolar concentrations in the colon lumen has been shown to exert deleterious effects on the colonic epithelium (32) and to inhibit butyrate (17), acetate, and

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propionate oxidation by isolated colonocytes (14). In this context, this study aims at determining the consequences of HP diet ingestion on the colonic luminal composition and on the morphological and metabolic characteristics of colonic epithelial cells.

MATERIALS AND METHODS

Animals and diets. Male Wistar Lewis rats weighing 150 g were fed for 1 wk with a standard rodent diet containing 16% protein by weight. The animals then received for 15 days either a normoproteic or a hyperproteic isocaloric diet (Table 1) and water ad libitum. The animals were maintained in a 12:12-h light-dark cycle with the dark period beginning at 7:00 PM. The animals were then anesthetized between 10:00 and 11:00 AM with pentobarbital sodium (40 mg/kg body wt). The colon and small intestine were isolated and the luminal contents removed by expulsion and stored at −80°C until biochemical assay. The whole colon was utilized for colonocyte isolation. In some experiments, the distal colon was treated for observation of the absorbing colonocytes (see below). All aspects of the present protocol are in accordance with the guidelines of the French Committee for Animal Care and the European convention of vertebrate animals used for experimentation, under European council directive (86/609/EEC).

Water and ammonia measurement in the colon luminal content. For the measurement of the water content, colonic luminal contents were weighed before and after heating at 50°C. For ammonia measurement, the luminal contents were mixed with 10 ml perchloric acid (4%) per gram content and centrifuged at 10,000 *g* for 15 min. The supernatant was neutralized and ammonia measured after centrifugation on 100-μl aliquots of supernatant using the Sigma ammonia assay kit. Briefly, ammonia was assayed in the presence of α-ketoglutarate, glutamate dehydrogenase, and NADH, whose disappearance was followed at 340 nm.

Measurement of short-chain fatty acids in the colonic luminal content. Short-chain fatty acids were measured by a modification of the method of Kristensen (31) using gas chromatography and a capillary column (30 m, 0.32 mm ID, Restek Rtx 502.2). The amount of short-chain fatty acid was determined with reference to internal standards.

Measurement of protease activities in the colon and small intestine luminal content. Colonic luminal contents were mixed with borate buffer (pH 7.4) containing 43 mM NaCl, 7.3 mM disodium tetraborate, 171 mM boric acid, and 1 mM $CaCl_2$. The samples were then centrifuged at 200 *g* for 5 min to eliminate heavy particles in the pellet while maintaining bacteria in the supernatant (55). Aliquots corresponding to 0.75-mg luminal content were incubated for 30 min at 37°C in 50 μl borate buffer containing 0.48 mg purified caseins (Sigma Chemical, St. Louis, MO). The reaction was halted with 50 μl ice-cold trichloroacetic acid solution (7.06%), and after centrifugation (12,000 *g*, 5 min), 20 μl supernatants were mixed with 20 μl NaOH solution (0.3 M). Peptides were then measured at 650 nm by using a Bio-Rad kit against a bovine serum albumin standard curve. For the measurement of small intestine protease activities, aliquots of the mixture corresponding to 0.025 mg luminal content were incubated and treated by the same protocol. Kinetic measurement of protease activities for up to 60 min incubation revealed a correlation between time and protease activities ($R^2 = 0.99$, $n = 3$). Lastly, at 4°C, protease activities represented 5 ± 2% of the activities measured at 37°C ($n = 3$).

Table 1. *Composition of the experimental diets*

Ingredients, g/kg	NP	HP
Whole milk proteins	140	530
Corn starch	622.7	287.0
Sucrose	100	45.7
Cellulose	50	50
Soybean oil	40	40
Choline	2.3	2.3
Vitamin mixture, AIN 93-V	10	10
Mineral mixture, AIN 93-M	35	35
Energy, kJ/g	14.6	14.6

NP, normoproteic diet P14; HP, hyperproteic diet P53.

Intestinal epithelial cell preparation for enzymatic activity measurement. Colon and small intestine epithelial cells were prepared from the whole colon or the whole small intestine as described (24, 42).

Enzymatic activity measurement. For the measurement of glutamine synthetase activity in intestinal cells, its glutamyl transferase activity was used, a validated method of measurement (36). Colonic or small intestine epithelial cells were homogenized by sonication at 4°C in an imidazole buffer (50 mM, pH 6.8) and then centrifuged at 4,500 *g* for 15 min. The supernatants were incubated in 200 μl imidazole buffer containing 50 mM L-glutamine, 25 mM hydroxylamine, 25 mM sodium arsenate, 2 mM $MnCl_2$, and 0.16 mM ADP for 20 min at 37°C. The reaction was halted with 200 μl of a solution containing $FeCl_3$ (2.4%), trichloroacetic acid (1.45%), and HCl (1.82 N). The samples were then centrifuged at 4,500 *g* for 15 min and the absorbance was determined at 540 nm. The quantity of γ-glutamyl-hydroxamate formed was determined by using a standard curve with the product. For measurement of the phosphate-dependent glutaminase activity in intestinal cells, colonic or small intestine epithelial cells were homogenized by sonication at 4°C in a phosphate (150 mM)-Tris (50 mM) buffer (pH 8.5) containing 1 mM EDTA and 0.05% Triton X-100. The homogenates were preincubated for 20 min at 37°C then 160 μl samples were incubated for 5 min at 37°C in the absence or presence of 30 mM L-glutamine. The reaction was halted with 40 μl of a HCl solution (2 N) and then mixed with 60 μl of a KOH-Tris solution to obtain a pH equal to 8.0. The samples were centrifuged and 160 μl of the supernatant was incubated for 30 min at room temperature in the presence of 20 μl Tris buffer (50 mM, pH 8.0) containing 15 mM NAD, 1.25 mM ADP, 10 mM EDTA, 25% (vol/vol) hydrazine, and 20 μl (1.3 U) of a glutamate dehydrogenase solution (Sigma Chemical). L-Glutamate was measured by absorbance spectrometry at 340 nm to follow NADH formation and compared with a standard curve of L-glutamate.

Oxygen consumption by isolated colonocytes. For cellular oxygen consumption experiments, the cells were isolated by use of hyaluronidase (11), rinsed twice with Dulbecco's modified Eagle's medium, and then counted via a hemocytometer. Cells (5×10^6 cells/assay) were resuspended in HEPES buffer (20 mM, pH 7.4) containing 200 mM mannitol, 5 mM KH_2PO_4, 2.5 mM $MgCl_2$, and 0.5 mM EGTA and equilibrated against air. Colonocyte oxygen consumption was measured by polarography at 37°C with increasing concentration of NH_4Cl.

Electron and light microscopy. The distal colon was ligated and a cacodylate buffer (0.1 M, pH 7.0) containing 2% glutaraldehyde was endoluminally injected. The samples were postfixed in 2% OsO_4 in the same buffer and dehydrated in increasing concentrations of ethanol and embedded in Epon 812 (40). Semithin sections were observed with a light microscope to localize the absorbing colonocytes in the surface epithelium, and then thin sections were contrasted with uranyl acetate and lead acetate before observation with an EM-10 electron microscope (Zeiss).

Presentation of results. The results are expressed as means ± SE together with the number of independent experiments (*n*). The statistical significance of differences between mean values was assessed by the Student's *t*-test and mixed models for repeated-measure analysis (SAS Institute, Cary, NC). Differences with *P* values <0.05 were considered as statistically significant.

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Table 2. *Luminal content composition after NP and HP diet ingestion*

Colonic contents	NP	HP	*P*
Total mass, g	0.96 ± 0.12	1.69 ± 0.12	$P = 0.0020$
Water content, g	0.53 ± 0.01	1.15 ± 0.04	$P = 0.0240$
NH_4^+/NH_3, μmol	1.65 ± 0.39	9.04 ± 0.07	$P = 0.0008$
Acetate, μmol	24.90 ± 7,20	29.90 ± 4.10	NS
Propionate, μmol	5.52 ± 1.42	11.89 ± 2.40	$P = 0.0285$
Butyrate, μmol	2.09 ± 0.52	3.61 ± 0.48	NS
Valerate, μmol	0.51 ± 0.08	1.05 ± 0.21	$P = 0.0439$
Caproate, μmol	0.17 ± 0.04	0.21 ± 0.04	NS
Isobutyrate, μmol	0.27 ± 0.06	0.81 ± 0.11	$P = 0.0023$
Isovalerate, μmol	0.11 ± 0.02	0.24 ± 0.04	$P = 0.0155$

Values are mean ± SE of data obtained from 4 to 7 animals in both experimental groups.

RESULTS

Effects of high-protein diet on the biochemical composition of colonic luminal content. As indicated in Table 2, the whole colonic luminal content mass was increased 1.8-fold in rats fed HP diet compared with normal-protein diet (NP). This was associated with a 2.2-fold increase in the water mass. However, there was no significant difference between mean water consumption by NP rats (39.9 ± 5.8 ml/day) and HP (45.4 ± 4.8 ml/day, $n = 5$ in both cases). The amount of ammonia in the colonic content was 5.5-fold higher for HP than NP animals. As indicated in Table 2, no significant difference was found for the amounts of acetate and butyrate in the colonic luminal content recovered from NP and HP animals. In contrast, the amount of luminal propionate was increased 2.2-fold in HP animals. Furthermore, the amounts of valerate, isobutyrate, and isovalerate were significantly increased after HP diet ingestion.

Effect of high-protein diet on protease activities in the colonic luminal content: comparison with activities in the small intestine. Protease activities in the colonic lumen after HP diet ingestion, when expressed per gram content, were increased nearly threefold when compared with the activities measured in NP animals (Fig. 1*A*). When expressed as total luminal activities (54.62 ± 7.77 mg peptides released per 30 min in NP animals and 283.41 ± 49.85 mg peptides released per 30 min in HP); the protease activities were 5.2-fold higher in the colon of HP animals. This increase in protease activity was not associated with an increase in the amount of endogenous peptide in the colon lumen since it represented 9.1 ± 1.4 mg peptides/g content in NP animals ($n = 6$) and 9.9 ± 1.5 mg peptides/g content in HP ($n = 7$). For comparison, protease activities were also measured in the small intestine lumen. Protease activities when expressed per gram of luminal content were much higher in the small intestine content than in the colonic content. Furthermore, these activities were 1.6-fold higher in the small intestine content recovered from the HP animals than in the small intestine content obtained from NP (Fig. 1*B*). In the small intestine luminal content, the amount of endogenous peptide was nearly twofold higher in HP animals (27.9 ± 5.6 mg peptides/g content, $n = 8$) than in NP (14.5 ± 1.8 mg peptides/g content, $n = 8$; $P = 0.0490$ vs. HP animals).

Influence of HP on the height of brush-border membranes and on glutamine synthetase and glutaminase activities in colonocytes. As shown in Fig. 2*A*, the heights of the brush-border membranes were drastically reduced in a typical picture of absorbing colonocytes observed after 15 days HP diet ingestion compared with NP (0.82 ± 0.02 μm in NP animals and 0.49 ± 0.02 μm in HP; Fig. 2*B*). As indicated in Fig. 3, the morphology of tight junctions was apparently not affected after HP diet consumption when compared with NP diet. Also, the colonic mucosa morphology was similar in both experimental groups (Fig. 4).

To compare the glutamine synthetase activity in epithelial cells originating from the large and small intestines, the catalytic activity was measured in colonocytes and enterocytes isolated from NP animals. As indicated in Table 3, glutamine synthetase activity was 10-fold higher in colonocytes than in

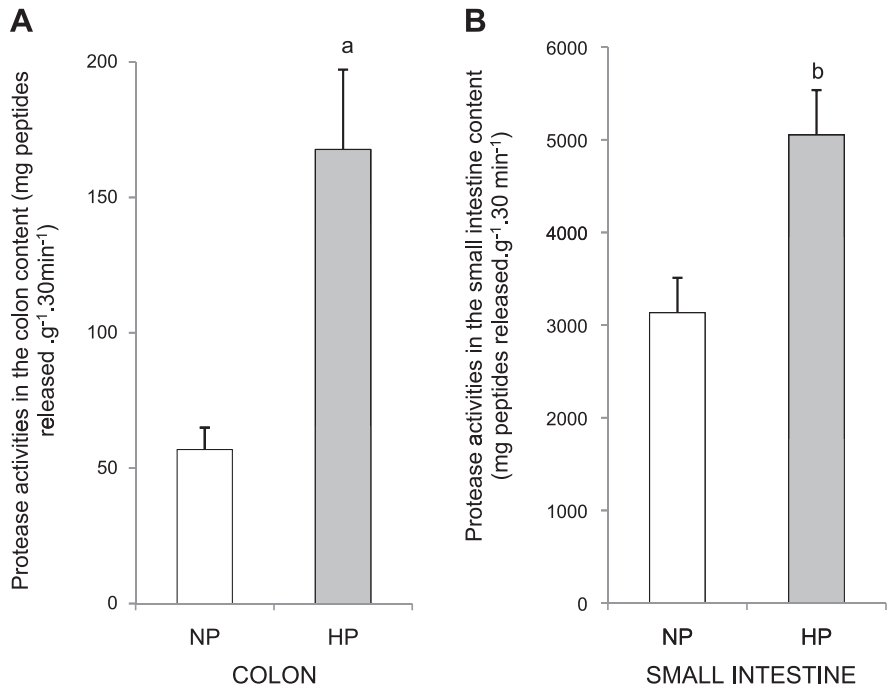


Fig. 1. Effect of hyperproteic diet (HP) vs. normoproteic diet (NP) ingestion on the protease activities measured in the colonic luminal content (*A*) and in the small intestine luminal content (*B*) after 15 days. Values are means ± SE of data obtained from 6 animals (*A*) and 8 animals (*B*) in both experimental groups. [a]$P = 0.0140$ and [b]$P = 0.0060$ vs. NP animals.

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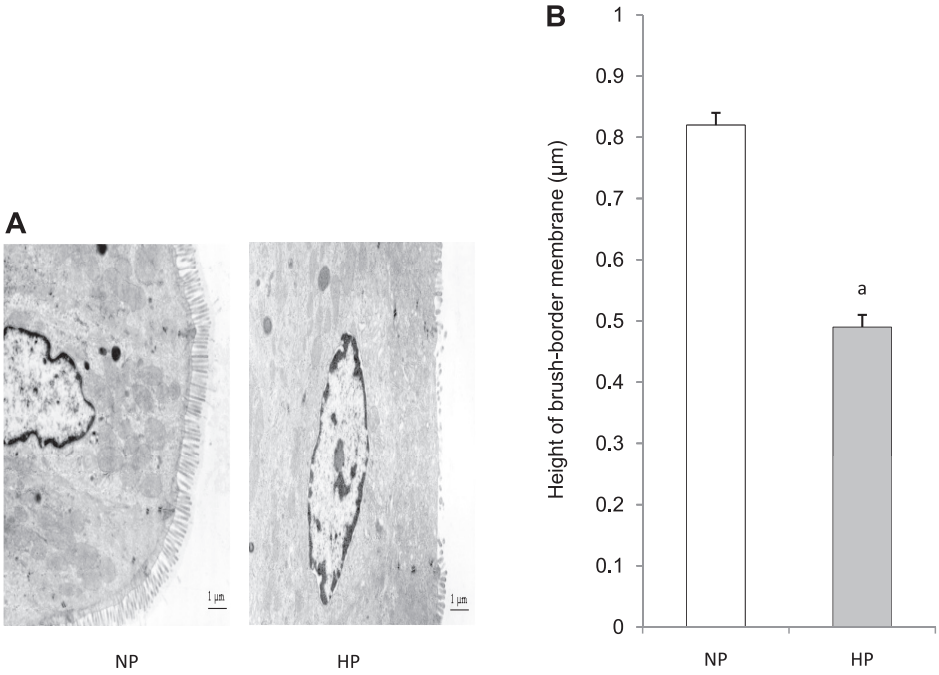


Fig. 2. Effect of HP vs. NP ingestion on the height of the brush-border membranes in absorbing colonocytes. *A*: typical electronic microscopy observation of absorbing colonocytes after 15 days ingestion of the experimental diets. *B*: comparison of heights of absorbing colonocyte brush-border membranes between NP and HP animals. Values are means ± SE of data obtained from 16 independent observations from 3 animals (NP) and 15 independent observations from 3 animals (HP). [a]$P = 0.0001$ vs. NP.

enterocytes. In contrast, glutaminase activity was found to be twofold lower in colonocytes than in enterocytes. Glutamine synthetase activity was increased 1.6-fold in colonocytes isolated from HP animals when compared with NP. In contrast, the activity of glutaminase was similar in colonocytes isolated from NP and HP animals. Finally, the L-glutamate contents were measured in isolated colonocytes. These contents were found to be similar in colonocytes from HP animals (34.2 ± 6.8 nmol/10^6 cells^{-1}, $n = 4$) and NP (38.0 ± 6.6 nmol/10^6 cells, $n = 4$).

Influence of ammonia on oxygen consumption by isolated colonocytes. The basal O_2 consumption in the absence of exogenous substrate was not significantly different in colonocytes isolated from NP animals (3.42 ± 0.57 nmol O_2·min $^{-1}$·10^6 cells^{-1}, $n = 9$) and HP animals (4.45 ± 0.65 nmol O_2·min $^{-1}$·10^6 cells^{-1}, $n = 6$). The relative oxygen consumption in the presence of the F1F0 ATPase inhibitor oligomycin (0.5 μg/μl) was significantly higher in the HP animal group (75.13 ± 5.21% of the oxygen consumption without oligomycin, $n = 5$) than in NP (56.53 ± 5.77%, $n = 5$, $P = 0.0430$). As indicated in Fig. 5, NH_4Cl when incubated in the presence of colonocytes isolated from NP or HP animals was found to cause a similar dose-dependent reduction in the cell oxygen consumption. NaCl used at a 50 mM concentration had no detectable effect on colonocyte oxygen consumption (data not shown), indicating that the effect of NH_4Cl was not due to an increase in the incubation medium osmolarity.

DISCUSSION

The present study indicates that the ingestion of a HP during 15 days is concomitant with a severe decrease of absorptive colonic epithelial cell brush-border height when compared with colonocytes originating from animals receiving a normoproteic diet. This result coincided with a marked increase of the mass

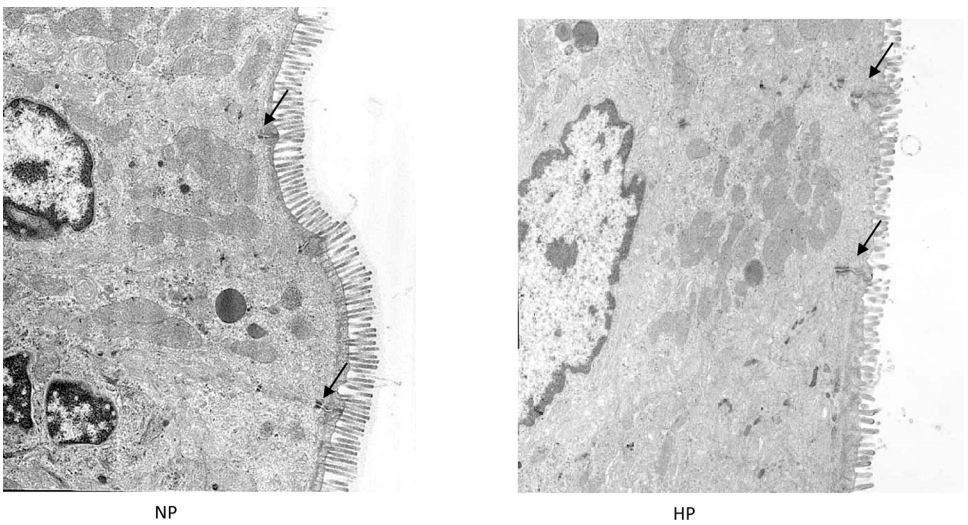


Fig. 3. Effect of HP vs. NP ingestion on the morphology of tight junctions between colonocytes.

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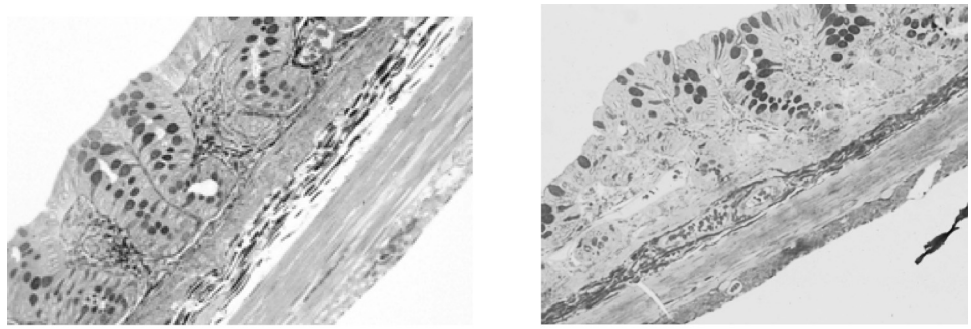


Fig. 4. Effect of HP vs. NP ingestion on the morphology of the colonic mucosa.

of the luminal content, of the amount of protease activities, and of the amount of ammonia in this content. The increase of the colonic luminal mass content in HP animals can be explained by an increase of the luminal water content. The concomitant decrease in the height of the colonocyte brush-border membrane induced by the HP diet is likely to play a role in the increased water and ammonia luminal content due to a decreased absorption of these compounds through the colonic epithelium. This is in accordance with previous work showing that when rats were shifted from a NP to an HP diet, ammonia concentration in the colonic vein transiently increases and returns back to the basal level whereas luminal ammonia concentration in the colon of HP animals remained high (42). In addition, since ammonia is able to inhibit Na^+ absorption by the colonocytes (9), this inhibition would amplify water retention as observed in HP animals. Another parameter that is presumed to play a role in the marked increase of the ammonia colonic luminal content is the increase of the protease activities in the colon lumen. These activities increase the speed of degradation of proteins and peptides, which will pass from the small to the large intestine, therefore increasing the release of amino acids for bacterial deamination. This proposition is in accordance with the fact that HP diet increases the amounts of isobutyrate and isovalerate (this study) that are produced exclusively from the amino acids L-valine and L-leucine, respectively (45, 47). However, this assay of luminal protease activities did not allow separation of activities associated with the bacterial and residual pancreatic activities. The fact that we measured a 1.6-fold higher protease activity in the small intestine content of HP animals than in the NP ones suggests that such an increase may play a role in the increase of the colonic protease activities. It is worth noting, however, that in our study colonic protease activities represent ~2% of the small intestine activities on a per gram content basis, indicating that, as previously reported (5), a large part of the small intestine protease activities is degraded and/or inactivated in the large intestine lumen. Many different types of proteolytic bacteria are found in the large intestine, and intracellular bacterial proteases are involved in proteolysis in the luminal content after cell lysis (33). Another interesting result is the doubling of the amount of propionate in the colonic luminal content following the HP diet ingestion. Propionate, which can be synthesized by the microbiota from the amino acids L-alanine and L-threonine (4), is known to represent a luminal fuel for colonocytes (48). However, butyrate and acetate in the colon content were not significantly modified after HP diet consumption. The amounts of these short-chain fatty acids represent the net result of production and utilization by the microbiota as well as absorption capacity by the colonic mucosa; further studies are required to determine whether any of these parameters is/are modified by the HP diet.

This study thus showed an effect of HP feeding on absorbing colonocyte morphology. Colonic epithelial cells are characterized by high energy demand due to the rapid colonic epithelium renewal (46) and thus intense anabolic metabolism (8) together with the process of electrolyte absorption. We used the working hypothesis that HP diet could have provoked changes in the colonic luminal environment and that these changes could have affected the metabolic pathways responsible for colonocyte energy production. The two main oxidative substrates for colonocytes are blood L-glutamine and luminal short-chain fatty acids (48). We focused on the effect of ammonia on colonocyte oxygen consumption since in the rat model, ammonia is increased in the colonic lumen after HP diet consumption (Ref. 42 and this study) and because ammonia decreases the oxidation of short-chain fatty acids in rat colonic epithelial cells (14, 17). The fact that acetate oxidation is partly inhibited by ammonia (14) suggests that the tricarboxylic acid cycle is somehow affected by this bacterial metabolite in colonocytes. We measured in HP and NP animals the level of oxygen consumption in the absence of exogenous substrate, which gives an indication of the colonocyte capacity to oxidize endogenous substrates, and found that basal oxygen consumption was not significantly different. However, and interestingly,

Table 3. *Glutamine synthetaze and glutaminase activities*

	Enterocytes	Colonocytes	
Enzymatic Activities	NP	NP	HP
Glutamine synthetase, nmol·mg protein^{-1}·20 min^{-1}	30.9 ± 4.5	312.2 ± 15.5*	503.3 ± 64.1†
Glutaminase, nmol·mg protein^{-1}·5 min^{-1}	375.3 ± 57.5	188.3 ± 32.2‡	197.2 ± 77.4

Enzymatic activities were measured in homogenates prepared from colonocytes and enterocytes. Values are means ± SE of data obtained from 4-7 animals; *P = 0.0001 vs. enterocytes NP; †P = 0.0304 vs. colonocytes NP; ‡P = 0.0293 vs. enterocytes NP.

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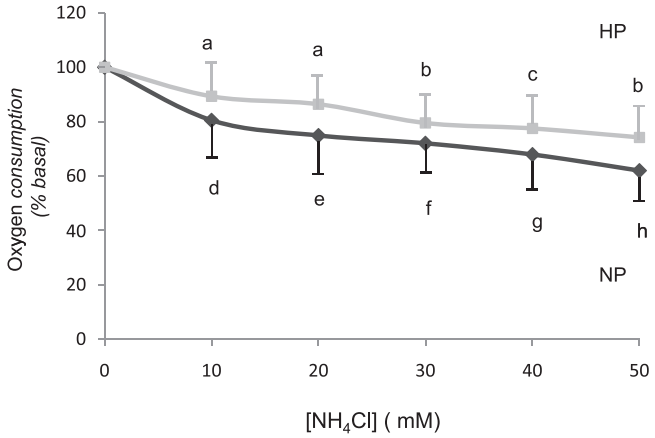


Fig. 5. Effects of NH_4Cl on oxygen consumption by colonocytes isolated from NP or HP animals. Values are means ± SE of data obtained from 6–9 animals. [a]$P = 0.0015$; [b]$P = 0.0010$; [c]$P = 0.0030$ vs. basal HP condition (no NH_4Cl added); [d]$P = 0.0007$; [e]$P = 0.0021$; [f]$P = 0.0008$; [g]$P = 0.0003$; [h]$P = 0.0001$ vs. basal NP condition. No significant difference on the inhibitory effect of NH_4Cl on colonocyte oxygen consumption between NP and HP groups was found ($P = 0.86$).

the rate of oxygen consumption in the presence of oligomycin, an inhibitor of the F1 F0 ATPase, was higher in colonocytes from HP rats than in colonocytes from NP rats. This result demonstrates in HP colonocytes an increased proton leak through the mitochondrial inner membrane. When leaking, protons pumped from mitochondrial matrix to intermembrane space can return into the matrix without energetic counterpart, implying a lower energetic efficiency in HP than in NP colonocytes. NH_4Cl was found to be able to dose dependently inhibit oxygen consumption by colonocytes isolated from both NP and HP animals. This suggests that ammonia in excess may represent a "metabolic troublemaker" toward colonocytes after HP diet. This together with the lower energetic efficiency in HP colonocytes than in NP colonocytes may lead to a decreased cellular energy production. It should, however, be kept in mind that in addition to ammonia, other bacterial metabolites derived from amino acids are known to act as inhibitors of oxygen consumption in colonocytes (4). However, despite increased concentration of ammonia in the colonic lumen, the integrity of isolated colonocytes was not different between NP and HP groups as judged from the lactate dehydrogenase leakage test, which allows to measure the percentages of necrotic cells (42). Also, the observation of the morphology of the colonic mucosa as well as the observation of tight junctions at the subcellular level indicated that no obvious difference could be detected between NP and HP animals. This indicates that in our experimental condition, HP diet consumption affect brush-border membrane rather specifically.

An effect of ammonia on cell respiration through the depletion of α-ketoglutarate in the tricarboxylic cycle can be excluded since in previous experiments, NH_4Cl did not modify α-ketoglutarate oxidation in rat colonocytes (41). In gastric mucosal cells, Tsujii and al. (52) showed that ammonia dose dependently inhibited mitochondrial respiration. As suggested by Tsujii et al. (53), gastric mucosal cell damage may be dependent on the concentration of the nonionized form of ammonia (NH_3). If both NH_3 and NH_4^+ can enter colonocyte apical membranes, only the nonionized form NH_3 can penetrate mitochondrial membrane (44). Ammonia supplied at the luminal side of colonocytes induces only slight modification of the overall intracellular pH (7, 29), but higher pH in mitochondria than in cytosol (thus increasing the NH_3/NH_4^+ ratio) has already been proposed as responsible for the ammonia-provoked decreased respiration in gastric mucosa cells (52).

Measurement in HP isolated colonocytes of the enzymatic activities involved in ammonia metabolism showed some modifications. Interestingly, glutamine synthetase activity was 10-fold higher in epithelial cells isolated from the rat colon than from the small intestine, suggesting a specific role of such an activity for colonocyte physiology in a context of high luminal ammonia concentration. Glutamine synthetase is a cytosolic enzyme that condenses NH_3 and L-glutamate for L-glutamine synthesis (26). We previously found in the pig model that

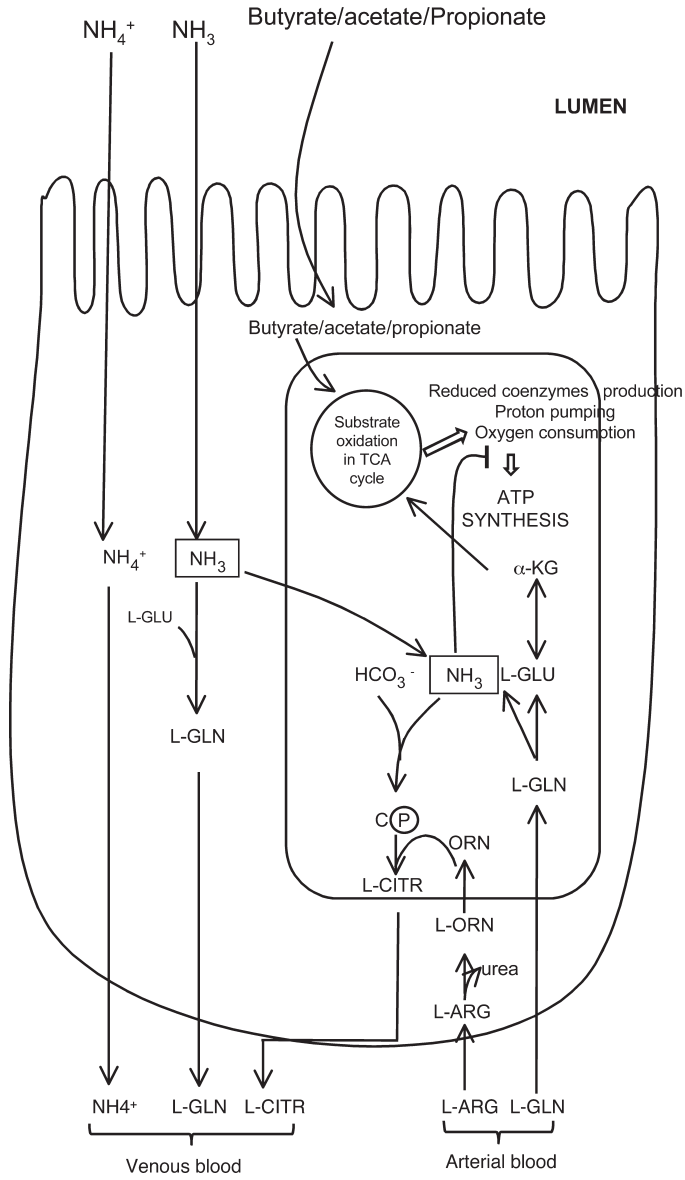


Fig. 6. Schematic view of the metabolism and effect of ammonia on the oxidative metabolism in colonocytes. L-ARG, L-arginine; L-CITR, L-citrulline; CP, carbamoylphosphate; L-GLN, L-glutamine; L-GLU, L-glutamate; TCA cycle, tricarboxylic acid cycle. This schematic view is drawn from data obtained in Refs. 18, 41, and 42 and data obtained from the present study.

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absorbing colonocytes are equipped with glutamine synthetase as evidenced by the measurement of catalytic activity and immunodetection (18). In that latter study, L-glutamine concentration in the portal vein was increased after colonic endoluminal injection of NH_4Cl. This result led us to propose that under high colonic ammonia concentration, and in a colon-liver metabolic axis, L-glutamine provided by the colon would be used by periportal hepatocytes for stepwise synthesis of L-glutamate and *N*-acetylglutamate. This latter compound is an allosteric activator for the first and rate-limiting enzyme in the urea cycle, i.e., carbamoylphosphate synthetase I (35).

In the mitochondria, NH_3 either originating from the luminal content or produced from arterial L-glutamine by the activity of the mitochondrial glutaminase can be metabolized to a limited extent into L-citrulline due to the presence of carbamoylphosphate synthetase I and ornithine carbamoyltransferase activities (41) (see Fig. 6). In the present study, we found that, following HP diet ingestion, the activity of glutamine synthetase in colonocytes was increased 1.6-fold. It is worth noting that the L-glutamate content was high in colonocytes recovered from both NP and HP animals, representing ~10-fold the content measured in rat enterocytes (3). This intracellular pool would allow a sustained L-glutamine synthesis in a situation of increased ammonia concentration in the colon lumen. Despite increased glutamine synthetase in colonocytes recovered from HP animals, the inhibitory effect of NH_4Cl on oxygen consumption was not significantly different in colonocytes isolated from NP and HP animals. This is consistent with the fact that the mitochondrial carbamoyl phosphate synthetase I and ornithine carbamoyl transferase enzymatic activities are relatively low and not induced following HP diet ingestion (42). It thus appears that, above a threshold concentration of ammonia in the mitochondria, this metabolite goes beyond the detoxification capacity of colonocytes. This emphasizes the relative vulnerability of colonic epithelial cells toward increased ammonia concentration in the colonic lumen. Indeed, it has been reported that ammonium chloride, when perfused at millimolar concentration in the rat colon lumen, exerts deleterious effects on the colonic epithelium (32). In fact, after treatment with NH_4Cl, the distal colon mucosa displayed histological damage: the epithelium was shown to be disorganized and epithelial cell sloughing off was evidenced.

In the present study, we thus found that HP diet ingestion led to a marked decrease of the height of the colonocyte brush-border membranes that was concomitant with an increased water content in the colon lumen; an increase which likely reflects decreased water absorption. This coincided with an increased ammonia content in the colon lumen, and this bacterial metabolite was found able to dose dependently inhibit colonocyte oxygen consumption. It appears conceivable that luminal ammonia in excess may result in energy deficiency in these cells. Whether inhibition of oxygen consumption and reduced height of brush-border membranes in colonocytes are related in a causal relationship remains to be proven.

According to the data obtained in the present study, we propose that HP diet consumption should be considered with some caution because of its ability to very markedly modify the composition of the luminal content and the morphology and metabolic oxidative capacity of colonic epithelial cells in this experimental model.

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DISCLOSURES

The authors have no potential conflict to disclose.

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