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Effects of a high soy protein diet on intestinal polyamines and ornithine decarboxylase activity in rats

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This study was performed to determine whether intestinal luminal polyamine concentrations are affected by a high soy protein diet when compared with a high casein diet or a normoprotein casein diet. We also determined the effects of these diets, with differences in polyamines content, on mucosal polyamines and ornithine decarboxylase (ODC) activity to assess cell proliferation. Three groups of eight male Wistar rats were fed either a 50% soy protein diet, a 50% casein diet, or an 18% casein diet as a control. After 4 weeks of feeding, both intestinal content and mucosa were recovered. Polyamines were assayed by high performance liquid chromatography. ODC activity was measured by the release of $^{14}CO_2$ from ^{14}C-L-ornithine. Luminal putrescine and cadaverine concentrations were higher in the jejunum than in the ileum, suggesting an absorptive process. The highest concentrations of intestinal polyamines were observed in rats fed the soy protein diet ($P < 0.05$). Only minor differences were observed in mucosal polyamines according to the diets. ODC activity was also higher in the intestinal mucosa of rats fed the high soy protein diet ($P < 0.05$). These results suggest that intestinal luminal polyamine concentrations and ODC activity are modulated by the dietary protein source. (J. Nutr. Biochem. 10:405–410, 1999)

Keywords: soy protein; casein; polyamines; ornithine decarboxylase; intestine; cell proliferation

Introduction

The polyamines putrescine, spermidine, and spermine are small polycationic molecules that are synthesized by both eukaryotic and prokaryotic cells. The polyamine cadaverine is produced only by prokaryotic cells. Due to their interaction with nucleic acids, polyamines are involved in a variety of cellular processes such as replication and transcription as well as cell growth and differentiation.[1,2] Mammalian cells possess two different pathways to regulate their polyamine contents: (1) enzymatic synthesis and interconversion and (2) membrane exchange.[3] Ornithine decarboxylase (ODC) is the first and the rate-limiting step in polyamine biosynthesis, catalyzing the conversion of ornithine to putrescine. ODC activity is considered a reliable marker to evaluate cell proliferation particularly in the intestinal and colonic mucosa.[4–6] Both de novo synthesis and absorption of polyamines from the extracellular environment are enhanced in tumor cells that exhibit a very high requirement for these molecules to sustain cellular growth.[7]

The intestinal tract is thought to play a central role in polyamine supply and homeostasis in the body. Polyamines enter the lumen of the gastrointestinal tract from several sources. These sources include the diet, gastrointestinal secretions, exfoliated cells, and digestive microflora synthesis.[8–10] Previous results indicate the existence of a polyamine secretion in the upper part of the intestine via bilio-pancreatic fluids.[8] Secreted endogenous polyamines and dietary polyamine are progressively absorbed along the intestine and contribute to an enterohepatic polyamine circulation. Luminal and endogenous polyamines could be used by the mucosal cells. Intraluminal infusion of putrescine stimulates small intestinal mucosal growth in rats.[11] In this regard, luminal polyamines may be the primary source of extracellular polyamines for tumor growth in tumor-bearing rodents.[12,13]

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Because of the evidence that indicates that polyamines are a prerequisite to cell proliferation and growth, further investigation of the role of dietary components in the modulation of polyamine intestinal levels and biosynthesis is important. Among nutrients, the specific role of dietary protein in intestinal polyamine metabolism is poorly studied. Protein, which is an essential nutrient, affects many metabolic processes that are directly and indirectly related to tissue growth. Furthermore, polyamines are metabolites of luminal protein degradation. The dietary protein source and particularly soy protein could influence small intestine morphology and function.[14–16] Soy protein also increases colonic cell proliferation.[17] The role of soy protein diet in colorectal cancer is not clear. Several studies suggest that a soy protein diet increases the incidence of chemically-induced intestinal cancers.[17–19]

This study was performed to determine whether intestinal luminal polyamines and mucosal cell proliferation were affected by the dietary protein source. For this purpose, both luminal and mucosal polyamines as well as mucosal ODC activity were measured in rats fed for 4 weeks with high-level casein or soy protein diet. High protein diets were used because we speculated that a part of luminal polyamines might originate from the microflora metabolism of dietary arginine or lysine.[20] Furthermore, high levels of soybean protein were associated with a greater incidence of colon tumors in rats treated with 1,2-dimethylhydrazine.[21]

Materials and methods

Diets

The experimental diets were prepared by INRA (APAE, Jouy en Josas, France). They were adjusted to 18% protein with casein or 50% protein with either isolated casein or soy protein, respectively. All the diets contained 12% sucrose, 2% cellulose, 2.5% peanut oil, 2.5% colza oil, 4.5% mineral mix, and 1.0% vitamin mix (AIN-76 vitamin mix and mineral mix, INRA). All diets were supplemented with 0.5% DL-methionine. The 18% casein, 50% soy protein, and 50% casein diets were adjusted to 57%, 25%, and 25% corn starch, respectively. These diets are isoenergetic (395 kcal/100 g of diet). Their amino acid composition is described in *Table 1*. The contents of putrescine, spermidine, and spermine were 0.28, 0.14, and 0.16 μmoles/g in the casein diets and 0.44, 0.42, and 0.13 μmoles/g in the soy protein diet, respectively. No cadaverine was detected in the diets.

Animals

Male Wistar rats, aged 4 weeks of age and weighing 80 to 90 g, were purchased from Charles River (Saint Aubin lès Elbeuf, France). The animals were housed two per wire cage. The holding room was controlled at 23 ± 2°C, with 50 ± 10% humidity, and a 12-hour light/12-hour dark cycle. The rats were initially given standard laboratory chow and tap water for 1 week. Three groups of eight rats received the 18% casein, 50% casein, or 50% soy protein diet during 4 weeks. The animals had free access to food and water. Food consumption was recorded every 2 days and the animals were weighed weekly. Food intake and growth were not significantly different according to the diet.

Table 1 Amino acid composition (g/100 g of protein) of casein and soybean proteins

Amino acid	Casein	Soy protein
Alanine	2.8	4.3
Arginine	3.4	7.6
Aspartic acid	6.3	11.6
Cystine	0.3	1.3
Glutamic acid	20.5	19.1
Glycine	1.6	4.2
Histidine	2.5	2.6
Isoleucine	4.7	4.9
Leucine	8.2	8.2
Lysine	7.2	6.3
Methionine	1.9	1.3
Phenylalanine	4.4	5.2
Proline	9.5	5.1
Serine	5.0	5.2
Threonine	3.8	3.8
Tryptophane	1.6	1.3
Tyrosine	4.7	3.8
Valine	6.0	5.0

Experimental procedure

After 4 weeks of feeding, the rats were euthanized by cervical dislocation at 12:00 PM, after 5 hours of fasting. The jejunum, ileum, cecum, and colon were removed. The intestinal contents were recovered by rinsing with ice-cold saline and stored immediately at −30°C before freeze-drying (Lyovac GT2, Finn-Aqua, Heraus, Germany) for polyamine measurement. All segments along the antimesenteric side were opened on ice, and the mucosa was scraped away from the underlying smooth muscle with a glass slide. Duplicate tissue samples were prepared from each segment. The first portion was fixed in 10% phosphate-buffered formalin for standard histologic examination. Mucosal scrapings of the second portion were weighed, divided into two portions, immediately frozen in liquid nitrogen, and then stored at −70°C for polyamine, ODC activity, and protein measurements.

Polyamines contents

Luminal and mucosal polyamine contents were determined by high performance liquid chromatography (HPLC), as previously described.[22] Mucosal scrapings and luminal content were homogenized in 2 mL of 10% perchloric acid and centrifuged at 3,000 g for 10 minutes. While stirring 10 μL of 1-6 diaminohexane as a standard (1 μg/mL), 0.5 mL of saturated Na_2CO_3, and 1 mL of an acetone solution containing 5 mg of dansyl chloride were added to 1 mL of the supernatant. Twelve hours later, 2 mL of cyclohexane were added. After mixing and centrifuging, the organic portion was collected and freeze-dried in a speed vac for 1 hour (Savant Instrument, New York, NY USA). The residue was dissolved in 250 μL acetonitrile, filtered through a 45 μm membrane, and then used for HPLC analysis (Waters, St. Quentin, Yvelinés France). Polyamines were separated on a reverse-phase column (column length was 150 mm with an inner diameter of 3.90 mm), filled with supersphere-encapped RP 18 (5 μm pellicular matrix; Waters) at a constant flow rate of 2 mL/min using a linear gradient (0–100%) of 50% acetonitrile in water. The amounts of polyamines were quantified by comparing the integrated peak areas of those of the known amounts of standards.

ODC activity

The activity of the enzyme ODC was assayed by a radiometric technique in which the $^{14}CO_2$ was liberated from L-[1-^{14}C]-

Table 2 Lumenal polyamines concentrations in the jejunum, ileum, cecum, and colon of rats

	Jejunum	Ileum	Cecum	Colon
Putrescine (μmoles/g DM)				
Casein 18	8.3 ± 2.5[a]	13.2 ± 4.1[a]	2.6 ± 0.7[a]	2.8 ± 0.6[a]
Casein 50	13 ± 7[a]	7.4 ± 4.8[a]	2.0 ± 0.5[a]	0.7 ± 0.3[a]
Soya 50	143 ± 133[a]	21.7 ± 9.4[a]	1.6 ± 0.4[a]	3.1 ± 1.6[a]
Cadaverine (μmoles/g DM)				
Casein 18	0.4 ± 0.1[a]	0.9 ± 0.4[a]	1.1 ± 0.3[a]	2.7 ± 1.0[a]
Casein 50	2.9 ± 1.1[a]	1.6 ± 0.4[a]	0.5 ± 0.0[a]	3.1 ± 0.1[a]
Soya 50	213 ± 143[a]	21.4 ± 8.8[b]	0.6 ± 0.2[a]	0.7 ± 0.2[a]
Spermidine (μmoles/g DM)				
Casein 18	23.7 ± 13[a]	63.3 ± 18[a]	26.1 ± 5.9[a]	26.3 ± 4.1[a]
Casein 50	65 ± 19[a]	100 ± 32.7[a]	39.6 ± 9.6[a]	15.0 ± 10.7[a]
Soya 50	491 ± 244[b]	1143 ± 677[a]	49.5 ± 15.0[a]	54.9 ± 14.2[b]
Spermine (μmoles/g DM)				
Casein 18	4.2 ± 1.7[a]	4.7 ± 1.2[a]	2.8 ± 0.6[a]	2.1 ± 0.4[a]
Casein 50	29 ± 7.4[a]	35.8 ± 25[a]	5.7 ± 1.6[a]	3.2 ± 1.5[a]
Soya 50	72.2 ± 18[b]	155 ± 64[b]	6.2 ± 1.5[a]	9.6 ± 3.5[b]

Note: Means ± SEM; statistical differences between diets are analyzed for each polyamine in each segment; means with different letters are significantly different in each group (Scheffe test, $P < 0.01$).
DM–dry matter.

ornithine, according to a previously described method.[23] Briefly, 50 to 60 mg of mucosal scrapings were homogenized at 0 to 4°C in 1 mL of 25 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM EDTA, 0.2 mM pyridoxal 5-phosphate, and 2.5 mM dithiothreitol with a polytron (Ultra Turax T25, IKA Labortechnik, Les Ulis, France) and centrifuged at 3,000 g for 20 minutes. Assays were performed in stoppered Erlenmeyer flasks fitted with a center well containing 0.5 mL of β-phenylethylamine to trap the released $^{14}CO_2$. A 200 μL aliquot of supernatant was placed in the outer compartment in the presence of carrier and labeled ornithine (10 μL of either 0.5 μCi of [^{14}C]-ornithine and 10 mM ornithine) and was incubated at 37°C for 1 hour. The reaction was stopped by injecting 80 μL of 0.4 M $HClO_4$. The flasks were shaken for an additional hour to ensure complete recovery of the released CO_2 in β-phenylethylamine. The well contents were then added to 6 mL of Dynagel and the radioactivity was determined in a Beckman LS 9000 scintillation counter (Beckman, Boston, MA USA). Values were corrected for the counts in blanks without enzyme. All assays were performed twice. Enzyme activity was expressed as pmoles of $^{14}CO_2$ formed per hour per milligram of tissue protein. Protein concentration was assayed according to the Bradford method using bovine serum albumin as the standard.[24]

Statistical analysis

Data are given as means ± SEM. The statistical significance of the results was evaluated using the analysis of variance procedure with a posteriori contrast using Scheffe's test. All of these procedures were performed with Statview (Abacus concept, New York, NY USA). Sheffe's test was used to compare the means, with the level significance preset at $P < 0.05$.

Results

Luminal polyamines

Independent of the diet, spermidine was the predominant polyamine in the lumen (*Table 2*). Higher concentrations of putrescine and cadaverine were observed in the jejunum than in the ileum of rats fed casein 50% and soy protein 50%, whereas spermidine and spermine concentrations were higher in the ileum than in the jejunum. The highest concentrations of polyamines (cadaverine, spermidine, and spermine) were observed in the jejunum and the ileum of rats fed soy protein diet compared with rats fed casein diet ($P < 0.05$). In the cecum and the colon, luminal polyamines concentrations were lower than in the intestine. No difference in polyamine concentrations was observed in the lumen of the cecum according to the diet. Spermidine and spermine concentrations were also slightly higher in the colon of rats fed the soy diet ($P < 0.05$).

Mucosal polyamines

Spermidine and spermine were present in higher concentration than putrescine in the mucosa (*Table 3*). Cadaverine was not detected in the mucosa. In the jejunum and ileum, putrescine and spermidine were lower in rats fed soy protein than in those fed casein diets, whereas no statistical difference was observed in the cecum and colon.

Mucosal histomorphology and ODC activity

Mucosa weight and protein content were not influenced by the dietary protein source (*Table 4*). Intestinal villus lengths (398 ± 10 μm) and crypt depths (181 ± 9 μm) did not differ between the diets. ODC activity was higher in both the jejunum and ileum in animals fed the soy protein diet than in those fed casein. No significant difference in ODC activity was observed in the colon according to the diet.

Discussion

The present study was performed to evaluate the influence of high casein or soy protein diets on polyamine levels and ODC activity in the rat intestine. The results showed high luminal polyamine concentrations, with the highest concentrations found in the jejunum and the ileum of rats fed the soy protein diet. A higher mucosal ODC activity also was observed in the intestinal mucosa in animals fed the soy protein diet.

Table 3 Mucosal polyamines concentrations in the mucosa of the jejunum, ileum, cecum, and colon of rats

	Jejunum	Ileum	Cecum	Colon
Putrescine (μmoles/g of mucosa)				
Casein 18	2.4 ± 0.4[a]	2.3 ± 0.4[a]	0.4 ± 0.2[a]	1.2 ± 0.1[a]
Casein 50	3.4 ± 0.4[b]	3.2 ± 1.1[a]	0.6 ± 0.2[a]	0.5 ± 0.1[a]
Soya 50	1.9 ± 0.3[a,c]	1.2 ± 0.2[b]	0.2 ± 0.1[a]	1.5 ± 0.9[a]
Spermidine (μmoles/g of mucosa)				
Casein 18	33 ± 4[a]	22.7 ± 3.4[a]	16.7 ± 9.6[a]	24.8 ± 10.0[a]
Casein 50	62 ± 25[a]	28.0 ± 1.6[a]	14.5 ± 9.0[a]	13.2 ± 3.7[a]
Soya 50	19 ± 2[c]	14.1 ± 2.6[b]	8.6 ± 1.5[b]	30.6 ± 18.5[a]
Spermine (μmoles/g of mucosa)				
Casein 18	43.9 ± 3.2[a]	37.4 ± 7.0[a]	45.2 ± 16.0[a]	41.6 ± 9.7[a]
Casein 50	62 ± 26.3[a]	31.1 ± 0.6[a]	33.7 ± 10.0[a]	35.2 ± 6.0[a]
Soya 50	45.8 ± 2.5[a]	39.2 ± 2.6[a]	38.0 ± 3.6[a]	74.0 ± 41.0[a]

Note: Results are expressed as means ± SEM; statistical differences between diets are analyzed for each parameter in each segment; means with different letters are significantly different in each group (Scheffe test, $P < 0.05$).

An interesting result was the high level of polyamines observed in the rat intestine. These luminal polyamine concentrations were closer in range than those previously reported in rats[8,25] and in healthy humans.[26] The putrescine and the cadaverine concentrations were higher in the proximal than in the distal part of the intestinal lumen. This pattern was analogous to those previously reported and indicated a polyamine supply in the upper part of the intestine with subsequent absorption along the intestine from the jejunum to the terminal ileum. This process is consistent with previous experimental data that have characterized polyamine uptake by the gut[27–29] and in brush-border membrane vesicles.[30–35] Several studies also have shown that putrescine was taken up by either isolated rat enterocytes, IEC-6, LoVo, or Caco-2 cells.[8,36–41] The polyamine in the upper part of the intestine is originated from both dietary intake and biliary and pancreatic secretions.[9] Moreover, Osborne and Seidel[8] also suggested the existence of an enterohepatic circulation of polyamines that were synthesized by the colonic microflora and transported to the proximal gut via the portal circulation and biliary tree.

Another result was that a higher level of luminal polyamines was observed in the jejunum and ileum in rats fed soy protein than in those fed casein. This difference could originate from either a higher polyamine supply from the diet, stimulation of polyamine secretion in the upper intestine, with a higher cell turnover, or stimulation of polyamine colonic microflora synthesis and subsequent enterohepatic transport to the proximal gut. The soy protein diet had a significant content of putrescine and spermidine but only twofold higher than the casein diet, whereas a tenfold higher level of luminal polyamine was observed with the soy protein diet. In addition, measurements were performed in the fasted state and dietary polyamines were probably already absorbed. Moreover, it was observed previously that polyamines are secreted continuously in the intestinal lumen.[26] Therefore, the effect of soy protein most likely originated from a stimulation of endogenous polyamine secretion. Soy diets have been demonstrated previously to stimulate bilio-pancreatic secretion due to the presence of the trypsin inhibitor.[42,43]

Despite the high number of luminal polyamines observed in the intestine of rats fed soy protein diet, the putrescine and the spermidine concentrations were decreased in the jejunum and the ileum mucosa. This result could be explained in part by the conversion of these two polyamines in spermine, by an increased use of these compounds by cells for their requirements, or by an increased use by the

Table 4 Mucosa weight, protein content, and ornithine decarboxylase (ODC) activity in the jejunum, ileum, cecum, and colon of rats

	Jejunum	Ileum	Cecum	Colon
Mucosa weight (mg/cm)				
Casein 18	30 ± 4[a]	30 ± 3[a]	80 ± 10[a]	20 ± 2[a]
Casein 50	40 ± 3[a]	38 ± 4[a]	110 ± 10[a]	20 ± 2[a]
Soya 50	38 ± 2[a]	40 ± 3[a]	90 ± 20[a]	22 ± 4[a]
Protein content (mg/mg of mucosa)				
Casein 18	58 ± 10[a]	65 ± 9[a]	60 ± 14[a]	59 ± 8[a]
Casein 50	65 ± 4[a]	58 ± 3[a]	51 ± 8[a]	45 ± 5[a]
Soya 50	60 ± 3[a]	60 ± 4[a]	70 ± 10[a]	60 ± 8[a]
ODC activity (pmoles/h/mg protein)				
Casein 18	31 ± 15[a]	—	—	10 ± 5[a]
Casein 50	35 ± 17[a]	19 ± 5[a]	—	11 ± 3[a]
Soya 50	78 ± 13[b]	36 ± 9[b]	—	16 ± 8[a]

Note: Means ± SEM; statistical differences between diets are analyzed for each parameter in each segment; means with different letters are significantly different in each group (Scheffe test, $P < 0.01$).

systemic circulation to contribute to the body polyamine pool in other part.

The changes in ODC activity indicate variations in cell proliferation associated with the soy protein diet. Previous in vivo experimental studies have suggested that both the level of protein intake and the source of dietary protein could affect intestinal cell proliferation and carcinogenesis.[17,18,21,44,45] However, the mechanisms of this soy protein diet effect remain to be established. The parallel increase in luminal polyamines and in mucosal ODC observed in this study suggest that the role of dietary protein in intestinal cell proliferation could be regulated, at least in part, by polyamines.

References

1 Pegg, A. and McCann, P. (1982). Polyamine metabolism and function. *Am. J. Physiol.* **243,** C212–C221

2 Jänne, J., Alhonen, L., and Leinonen, P. (1991). Polyamines: From molecular biology to clinical applications. *Ann. Med.* **23,** 241–259

3 Seiler, N., Delcros, J.G., and Moulinoux, J.P. (1996). Polyamine transport in mammalian cells. An update. *Int. J. Biochem. Cell. Biol.* **28,** 843–861

4 Luk, G.D., Moshier, J.A., and Ehrinpreis, M.N. (1988). Ornithine decarboxylase as a marker for colorectal polyps and cancer. *Prog. Clin. Biol. Res.* **279,** 227–239

5 McCormack, S.A. and Johnson, L.R. (1991). Role of polyamines in gastrointestinal mucosal growth. *Am. J. Physiol.* **260,** G795–G806

6 Rustgi, A.K. (1993). Ornithine decarboxylase: An oncogene. *Gastroenterology* **105,** 1255–1257

7 Pegg, A.E. (1988). Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* **48,** 759–774

8 Osborne, D.L. and Seidel, E.R. (1990). Gastrointestinal luminal polyamines: Cellular accumulation and enterohepatic circulation. *Am. J. Physiol.* **258,** G576–G584

9 Bardocz, S. (1993). The role of dietary polyamines. *Eur. J. Clin. Nutr.* **47,** 683–690

10 Bardocz, S., Duguid, T., Brown, D., Grant, G., Pusztai, A., White, A., and Ralph, A. (1995). The importance of dietary polyamines in cell regeneration and growth. *Br. J. Nutr.* **73,** 819–828

11 Seidel, E.R., Snyder, R.G., and Johnson, L.R. (1985). Ileal mucosa growth during intraluminal infusion of ethylamine or putrescine. *Am. J. Physiol.* **249,** G434–G438

12 Seiler, N., Sarhan, S., Grauffel, C., Jones, R., Knodgen, B., and Moulinoux, J.P. (1990). Endogenous and exogenous polyamines in support of tumor growth. *Cancer Res.* **50,** 5077–5083

13 Brachet, P., Quemener, V., Havouis, R., Tomé, D., and Moulinoux, J. (1994). Alterations in intestinal uptake of putrescine and tissue polyamine concentrations in tumor-bearing rats. *Biochim. Biophys. Acta* **1227,** 161–170

14 Seegraber, F.J. and Morrill, J.L. (1986). Effect of protein source in calf milk replacers on morphology and absorptive ability of small intestine. *J. Dairy. Sci.* **69,** 460–469

15 Makkink, C.A., Negulescu, G.P., Qin, G., and Verstegen, M.W. (1994). Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. *Br. J. Nutr.* **72,** 353–368

16 Tamura, M. and Suzuki, H. (1998). Effects of soy protein on the morphology of ileum and the ultrastructure of liver cells in adult mice. *Int. J. Vitam. Nutr. Res.* **68,** 73–76

17 Govers, M., Lapré, J., DeVries, H., and VanderMeer, R. (1993). Dietary soybean protein compared with casein damages colonic epithelium and stimulates colonic epithelial proliferation in rats. *J. Nutr.* **123,** 1709–1713

18 McIntosh, G., Regester, G., LeLeu, R., Royle, P., and Smithers, G. (1995). Dairy proteins protect against dimethylhydrazine induced intestinal cancers in rats. *J. Nutr.* **125,** 809–816

19 Hawrylewicz, E.J., Zapata, J.J., and Blair, W.H. (1995). Soy and experimental cancer: Animal studies. *J. Nutr.* **125(suppl 3),** 698S–708S

20 Tabor, C.W. and Tabor, H. (1985). Polyamines in microorganisms. *Microbiol. Rev.* **49,** 81–99

21 Reddy, B.S., Narisawa, T., and Weisburger, J.H. (1976). Effect of a diet with high levels of protein and fat on colon carcinogenesis in F344 rats treated with 1,2-dimethylhydrazine. *J. Natl. Cancer Inst.* **57(3),** 567–569

22 Saeki, Y., Uehara, N., and Shirakawa, S. (1978). Sensitive fluorimetric method for the determination of putrescine, spermidine and spermine by high-performance liquid chromatography and its application to human blood. *J. Chromatogr.* **145,** 221–229

23 Erwin, B.G., Seely, J.E., and Pegg, A.E. (1983). Mechanism of stimulation of ornithine decarboxylase activity in transformed mouse fibroblasts. *Biochem.* **22,** 3027–3032

24 Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochem.* **72,** 248–254

25 Iwami, K., Terabe, N., Kobayashi, T., and Ibuki, F. (1994). Postprandial changes in ornithine decarboxylase activity and the mucosal and intraluminal levels in the small intestine of rats concerning the significance of intestinal putrescine absorption. *Biosci. Biotech. Biochem.* **58,** 1357–1363

26 Benamouzig, R., Mahé, S., Luengo, C., Rautureau, J., and Tomé, D. (1997). Fasting and post-prandial polyamine levels in the human digestive lumen. *Am. J. Clin. Nutr.* **65,** 766–770

27 Bardocz, S., Brown, D.S., Grant, G., and Pusztai, A. (1990). Luminal and basolateral polyamine uptake by rat small intestine stimulated to grow by phaseolus vulgaris lectin phytohaemagglutinin in vivo. *Biochim. Biophys. Acta* **1034,** 46–52

28 Dumontier, A.M., Brachet, P., Huneau, J.F., and Tomé D. (1992). Transport of putrescine in the isolated rabbit intestine. *Eur. J. Physiol.* **420,** 329–335

29 Dorhout, B., Van Faassen, A., Van Beusekom, C., Kingma, A.W., de Hoog, E., Nagel, G.T., Karrenbeld, A., Boersma, E.R., and Muskiet, F.A. (1997). Oral administration of deuterium-labelled polyamines to suckling rat pups: Luminal uptake, metabolic fate and effects on gastrointestinal maturation. *Br. J. Nutr.* **78,** 639–654

30 Iseki, K., Kobayashi, M., and Miyazaki, K. (1991). Spermine uptake by rat intestinal brush border membrane vesicles. *Biochim. Biophys. Acta* **1068,** 105–110

31 Brachet, P. and Tomé, D. (1992). Putrescine uptake by rabbit intestinal brush-border membrane vesicles. *Biochem. Inter.* **27,** 465–472

32 Kobayashi, M., Iseki, K., Saitoh, H., and Miyazaki, K. (1992). Uptake characteristics of polyamines into rat intestinal brush-border membrane. *Biochim. Biophys. Acta* **1105,** 177–183

33 Kobayashi, M., Iseki, K., Sugawara, M., and Miyazaki, K. (1993). The diversity of Na(+)-independent uptake systems for polyamines in rat intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta* **1155,** 161–167

34 Milovic, V., Stein, J., Piper, A., Gerhard, R., Zeuzem, S., and Caspary W. (1995). Characterization of putrescine transport across the intestinal epithelium: Study using isolated brush border and basolateral membrane vesicles of the enterocyte. *Eur. J. Clin. Invest.* **25,** 97–105

35 Brachet, P., Prevoteau, H., Mathe, V., and Tomé, D. (1996). Modulation of putrescine transport in rat intestinal brush-border membrane vesicles by fasting and refeeding. *Digestion* **57,** 374–381

36 Kumagai, J. and Johnson, L. (1988). Characteristics of putrescine uptake in isolated rat enterocytes. *Am. J. Physiol.* **254,** G81–G86

37 Kumagai, J., Jain, R., and Johnson, L. (1989). Characteristics of spermidine uptake by isolated rat enterocytes. *Am. J. Physiol.* **256,** G905–G910

38 Scemama, J., Grabié, V., and Seidel, E. (1993). Characterization of univectorial polyamine transport in duodenal cell line. *Am. J. Physiol.* **265,** G851–G856

39 McCormack, S. and Johnson, L. (1989). Putrescine uptake and release by cancer colon cells. *Am. J. Physiol.* **256,** G868–G877

40 D'Agostino, L., Daniele, B., Pignata, S., D'Adamo, G., Ferraro, C., Silvestro, G., Tagliaferri, P., Contegiacomo, A., Gentile, R., and Tritto, G. (1990). Polyamine uptake by human colon carcinoma cell line caco-2. *Digestion* **46,** 352–359

41 Barlier, A., Benamouzig, R., and Tomé, D. (1996). Putrescine transport in differentiated Caco-2 cells. *Gastroenterol. Clin. Biol.* **20,** 178–184

42 Khalifa, F., Prost, J., Belleville, J., and Sarda, L. (1994). Pancreatic digestive hydrolase activities in growing rats fed alternately on raw and heated soya-bean flour. *Br. J. Nutr.* **72,** 533–544

43 Pusztai, A., Grant, G., Bardocz, S., Baintner, K., Gelencser E., and Ewen, S.W. (1997). Both free and complexed trypsin inhibitors stimulate pancreatic secretion and change duodenal enzyme levels. *Am. J. Physiol.* **272,** G340–350

44 Hawrylewicz, E.J., Moshovitis, C.K., Reger, C., and Madell, J. (1989). Dietary protein enhanced mammary ornithine decarboxylase activity and tumorigenesis in rats. *J. Nutr.* **119,** 547–552

45 Fleming, S., Youngman, L., and Ames, B. (1994). Intestinal cell proliferation is influenced by intakes of protein and energy, aflatoxin and whole-body radiation. *Nutr. Cancer* **22,** 11–30