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Interorgan movements of amino acid in the pig: effects of dietary fibres

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Summary. Six non-anaesthetized Large White pigs (mean body weight 59 ± 1.7 kg) were fitted with permanent catheters in the portal vein, the brachiocephalic artery and the right hepatic vein as well as with electromagnetic flow probes around the portal vein and around the hepatic artery. The animals were given a basal none-fibre diet (diet A) alone or together with 6% guar gum (diet B) or 15% purified cellulose (diet C). The diets were given for one week and according to a replicated 3 × 3 latin square design. On the last day of each such adaptation period test meals of 800 g were given prior to blood samplings. These samplings were continued for 8 h. Guar gum strongly reduced the amino acids (aa) and urea absorption as well as the hepatic production of urea. The aa profile of the absorbed mixture was not strongly modified by guar gum ingestion as well as the profile of the hepatic aa uptake. Cellulose at the consumed level had very few effects on the considered parameters.

It is suggested that the modulation of intestinal mechanisms by guar gum was sufficient to mediate the latter internal metabolic effects.

Keywords: Amino acids – Fibre – Absorption – Liver – Pig

Introduction

Dietary fibre influences events at all levels of the alimentary tract. There is considerable interest in the knowledge of nutritional physiological effects of dietary fibre (Laplace and Lebas, 1981; Heaton, 1983; Johnson, 1990). The possible mechanisms by which dietary fibre act and also improve some pathological states such as diabets, hyperlipaemia and hyperuraemia are more and more

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the subject of experimental work (Hagander et al., 1984; Gulliford et al., 1988; Rémésy and Demigné, 1989).

Better knowledge is needed in the effects of dietary fibre on the aa absorption and metabolism. Recently we studied the effects of wheat straw meal and of guar gum on the pig porto-arterial differences of amino-nitrogen and urea, (Malmlöf et al., 1988; 1989). The aim of the present work was to study the quantitative absorption and hepatic metabolism of aa in the growing pig after the ingestion of diets containing purified cellulose or guar gum.

The most reliable data for metabolite production and utilization in the splanchnic bed are obtained when measurements are made in vivo. Splanchnic metabolism in vivo can be studied if rate of blood flow through the liver (arterial and portal supplies) is recorded continously and if the metabolite concentrations can be measured in blood samples taken from one of the hepatic veins, the portal vein and from one artery. In the present work we used such an experimental model (Simões Nunes et al., 1989).

Materials and methods

Animals and diets

Six castrated male Large White pigs (59 ± 1.7 kg initial body weight) were used. Each animal was fitted with three catheters placed respectively in the portal vein, the brachiocephalic artery and the right hepatic vein as well as with electromagnetic flow probes around the portal vein and around the hepatic artery as described by Simões Nunes et al. (1989). The animals were given a basal none-fibre diet (diet A) alone or together with 6% guar gum (diet B) or 15% purified cellulose (diet C). The diets were given for one week and according to a replicate 3 × 3 latin square design. On the last day of each such adaptation period test meals of 800 g were given prior to blood samplings. These samplings were continued for 8 h. The diets composition and the ingested quantities of amino acids are shown in Table 1.

Table 1. Composition of experimental diets (%) and level of amino acid intake (mg) from each test meal

Dietary components (%)	A	B	C
Hydrochloric casein (UCCP)	17.3	17.3	17.3
Peanut oil	4	4	4
Maize starch	60.1	60.1	60.1
Guar gum	–	6	–
Purified cellulose	–	–	15
Mica powder	15	9	–
Mineral mixture[1]	2.5	2.5	2.5
Vitamin mixture[2]	1	1	1
Antioxidant	0.1	0.1	0.1
Level of amino acid intake (mg)	**A**	**B**	**C**
Σ essential aa	64368	68384	66672
Σ non essential aa	71288	75136	71384
Σ total aa	135672	143520	138056

[1] and [2] According to Simões Nunes et al. (1989)

Throughout the experimental period the animals were kept individually in cages which permitted easy access to the cannulae and the probes. To prevent obstruction of the cannulae by blood clots they were rinsed daily with heparinized 0.9% NaCl solution (100 IU/ml).

Measurements

Each test meal started at 09 h 00 after a fasting period of 24 h. On the day of each test meal, portal vein and hepatic artery blood flow-rates were recorded continously. Blood was sampled simultaneously from the hepatic vein, brachiocephalic artery and portal vein (3 ml/route) from time zero until 8 h after the begining of the meal, every 10 min during the first 40 min, at one hour, every 30 min during the 2nd hour and once every hour afterwards. Two ml of each blood sample were collected in ice-chilled tubes containing 10 μl heparin (50 IU) and immediately analyzed for its concentration in ammonia (Dropsy and Boy, 1961) and urea (Mather and Roland, 1969). The remaining ml of each blood sample was collected for amino acid analysis in a tube containing 2.75 ml of a solution of dihydrosulphosalicylic acid (64.7 g/l) and thiodiglycol (6 ml/l). This treatment ensured the breaking of erythrocytes and blood deproteinization prior to the measurement of both intracellular and extracellular circulating free amino acids. Free amino acids were extracted by ultrasonic grinding three times for 1 min, at discontinuous pulses followed by centrifugation (6000 g, 20 min), collection of the supernatant and adjustment to pH 2.2. The determination of amino acids was made by ion-exchange chromatography in a Beckman 6300 Amino Acid Analyser (Beckman, Palo Alto, Ca).

Calculations

The determination of the absorbed quantities or the intestinally produced quantities, the splanchnic input, the hepatic input, the hepatic output, the hepatic balance, the hepatic extraction coefficient, and the splanchnic balance or the non hepatic tissue balance were calculated acording to the formulae described by Simões Nunes et al. (1989).

Statistical analysis (Snedecor and Cochran, 1967) involved standard error of the mean as well as an analysis of variance followed by a Duncan's Multiple Range test. These calculations were performed with the Statistical Analysis System (SAS Institute, Cary, NC).

Results

Blood flow rates and hepatic and gut movements ammonia and urea

The nature of the test meal did not affect the blood flow-rates patterns. However the ingestion of the meal was accompained by an increase in the total hepatic blood flow. This increase appeared to be due to an increase in the portal flow. For all the animals and all the experiments the mean total hepatic blood flow rate was 3242 ± 48 ml/min (55 ± 0.8 ml/kg/min) and the arterial supply represented on an average 20% of the total blood received by the organ.

The highest ammonia absorption and hepatic uptake were noted after diet B (Table 2). For all the diets the hepatic ammonia uptake corresponded to the absorbed quantities and thus the ammonia extraction rate was very high. The ammonia absorption and hepatic uptake rates were very constant during the time and not modified by the ingestion of the meal.

Small quantities of urea were absorbed for the three diets, the lowest being observed after the guar gum ingestion as well as the lowest urea production (Table 2). The pattern of urea production was almost the same for the three diets

Table 2. Mean hourly (g) of absorbed quantities (AQ), hepatic balances (HB) and hepatic extraction coefficients (HEC) of ammonia and urea in the pig after ingestion of diets A, B and C

Parameters		A	B	C
Ammonia	AQ	$0.22 + 0.01^{1,a}$	$0.34 \pm 0.03^{b,c}$	$0.17 \pm 0.01^{b,f}$
	HB	0.24 ± 0.02^{c}	$0.37 + 0.04^{d}$	0.19 ± 0.03^{c}
	HEC	76 ± 10	82 ± 8	$72 + 10$
Urea	AQ	$0.35 \pm 0.10^{a,b}$	0.19 ± 0.05^{b}	0.51 ± 0.13^{a}
	HB	-2.1 ± 0.28^{a}	-1.3 ± 0.11^{b}	-1.7 ± 0.24^{a}

[1] Mean ± standard deviation of the mean of 6 determinations
[a,b] significantly different ($p < 0.05$)
[c,d] significantly different ($p < 0.01$)
[e,f] significantly different ($p < 0.001$)

with the largest production rates from the 2nd to the 4th hours after the beginning of the meal.

Amino acid absorption

The velocity by which amino acids were absorbed decreased significantly after ingestion of guar gum (Table 3). Thus, after 8 h the total amount of absorbed amino acids represented only 59% of the ingested amount for diet B whereas with the other two diets the same figures represented approximately the sum of ingested amino acids.

Table 3. Hourly means of absorbed quantities (mg) of amino acids (aa) and 8 h-absorption percentages (%) in the pig after ingestion of diets A, B and C

	A	B	C
Essential aa mg/h	6619 ± 1458^{1}	4120 ± 1063	6777 ± 1288
Essential aa %	$82 \pm 18^{2,a}$	48 ± 12^{b}	81 ± 15^{a}
Total aa mg/h	16839 ± 2698^{a}	10514 ± 886^{b}	17345 ± 2801^{a}
Total aa %	99 ± 16^{c}	59 ± 5^{d}	101 ± 16^{c}

[1] and [a,b,c,d] cf. legend of Table 2
[2] Mean ± standard deviation of the mean of 6 determinations expressed as a percentage of the ingested quantity

The absorption profile was similar for the three diets with a maximum hourly absorption during the first four hours after the meal. The quantities of individual essential amino acids absorbed and the absorption percentages (except for HIS and ARG) after ingestion of guar gum diet were significantly lower than those measured after the other two diets (Table 4). There were no significant differences between the quantities of essential amino acids absorbed after ingestion of diets A and C as well as between the absorption percentages. Very high absorption percentage was noted for HIS after diet B. Irrespectively of the diet, the lowest

Table 4. Hourly means of absorbed quantities (mg) of essential amino acids and 8h-absorption percentages (%) in the pig after ingestion of diets A, B and C

	A	B	C
VAL mg/h	$1001 \pm 283^{1,a}$	511 ± 140^{b}	1155 ± 155^{a}
%	$91 \pm 26^{2,a}$	45 ± 12^{b}	100 ± 13^{a}
LEU	1289 ± 228^{a}	599 ± 66^{b}	1093 ± 154^{a}
	84 ± 15^{a}	38 ± 4^{b}	73 ± 10^{a}
ILE	679 ± 130	354 ± 78	835 ± 193
	74 ± 14^{a}	38 ± 8^{b}	91 ± 20^{a}
THR	658 ± 131^{a}	236 ± 74^{b}	665 ± 100^{a}
	93 ± 19^{a}	31 ± 10^{b}	91 ± 14^{a}
PHE	758 ± 173^{a}	383 ± 100^{b}	713 ± 179^{a}
	87 ± 20^{a}	41 ± 11^{b}	81 ± 20^{a}
LYS	$988 \pm 208^{a,b}$	600 ± 66^{a}	1135 ± 244^{b}
	79 ± 17^{a}	44 ± 5^{b}	86 ± 18^{a}
MET	366 ± 68^{a}	206 ± 48^{a}	$255 \pm 48^{a,b}$
	71 ± 13^{a}	37 ± 9^{b}	$45 \pm 8^{a,b}$
HIS	389 ± 108	860 ± 388	364 ± 100
	80 ± 22	159 ± 72	70 ± 19
ARG	491 ± 129	371 ± 103	544 ± 155
	72 ± 19	50 ± 14	76 ± 16

[1] and [a,b] cf. legends of Table 2
[2] cf. legend of Table 3

absorption percentage was observed for MET. The absorbed quantities of some non essential amino acids are shown in Table 5. The absorbed quantities of TYR, GLN, PRO, OH-PRO and TAU as well as the absorption percentages of TYR and PRO appeared significantly lower after guar gum diet than after the other two diets. For all the diets the absorption percentages of ASP and GLU appeared to be very low. Opposite to that, those of ALA and GLY appeared to be very high.

Amino acids hepatic input

The quantity of amino acids entering the liver after ingestion of diet B was approximately 78% of that measured after diets A and C. Whatever the diet, the essential amino acids accounted for about 35% of the mixture of total amino acids entering the liver. The quantities of VAL, LEU, ILE, THR, CYS, SER and ORN entering the liver after ingestion of guar gum diet were significantly lower than those noted after ingestion of the other two diets.

Amino acids hepatic uptake

The quantities of total amino acids taken up by the liver after ingestion of guar gum diet were significantly lower than those taken up after diets A and C. This

Table 5. Hourly means of absorbed quantities (mg) of some non essential amino acids and 8h-absorption percentages (%) in the pig after ingestion of diets A, B and C

	A	B	C
TYR mg/h	$826 \pm 243^{1,a}$	385 ± 100^{b}	$654 \pm 169^{a,b}$
%	$89 \pm 26^{2,a}$	36 ± 10^{b}	$67 \pm 18^{a,b}$
ASP	506 ± 206	288 ± 130	381 ± 176
ASN	939 ± 229	829 ± 281	1600 ± 634
GLU	205 ± 44	190 ± 65	138 ± 38
GLN	175 ± 79^{a}	6 ± 4^{b}	166 ± 45^{a}
ALA	2025 ± 536	1224 ± 425	1991 ± 279
	385 ± 102	220 ± 77	373 ± 52
GLY	1014 ± 359	980 ± 335	851 ± 226
	304 ± 107	263 ± 90	252 ± 67
TAU	341 ± 60^{a}	155 ± 38^{b}	843 ± 353^{a}

[1,2] and [a,b] cf. legends of Tables 2 and 3

was true in terms of real amounts of amino acids but not as a percentage of the absorbed quantities (Table 6). Within the mixture of amino acids taken up by the liver, the essential amino acids represented 44%, 52% and 45% respectively of the mixture of total amino acids for A, B and C. Among the essential amino acids the quantities of PHE, LYS, MET and ARG retained by the liver were significantly lower after intake of guar gum diet than after diets A and C whilst that of HIS was significantly higher than that retained by the hepatic parenchyma after cellulose diet (Table 7).

The kinetic profile of the hepatic uptake of essential amino acids showed some differences depending on which of the diets that had been ingested. The hepatic balance of VAL, LEU, THR, PHE, LYS, HIS and ARG was always positive whatever the diet; that of ILE was always positive for diets B and C and negative during the first, the seventh and eighth hours of observation for diet A; that of MET was always positive after diet A, negative during the third hour after diet B as well as during the fourth, seventh and eighth hours after diet C.

Table 6. Hourly means (mg) of the quantities of amino acids (aa) taken up by the liver and percentage (%) of absorbed amino acids taken up by the liver in 8 hours in the pig after ingestion of diets A, B and C

	A	B	C
Essential aa mg/h	2714 ± 996^{1}	1653 ± 596	2245 ± 837
Essential aa %	41 ± 15^{2}	40 ± 14	33 ± 12
Total aa mg/h	6203 ± 1440^{a}	3162 ± 1090^{b}	$4942 \pm 1475^{a,b}$
Total aa %	37 ± 9	30 ± 10	28 ± 9

[1,2] and [a,b] cf. legends of Table 2 and 3

Table 7. Hourly means (mg) of the quantities of essential amino acids taken up by the liver (HB) and mean hepatic extraction coefficients (HEC) in the pig after ingestion of diets A, B and C

	A	B	C
VAL HB mg	328 ± 151[1]	207 ± 56	260 ± 122
HEC %	3 ± 1	3 ± 1	2 ± 1
LEU	319 ± 154	310 ± 91	404 ± 120
	4 ± 2	5 ± 2	5 ± 2
ILE	176 ± 106	156 ± 57	339 ± 129
	3 ± 2	4 ± 2	7 ± 3
THR	187 ± 44	113 ± 52	194 ± 64
	3 ± 1	3 ± 2	3 ± 1
PHE	390 ± 107[a]	128 ± 51[b]	278 ± 74[a]
	± 2	4 ± 2	7 ± 2
LYS	553 ± 181[a]	182 ± 86[b]	366 ± 179[a,b]
	4 ± 1	2 ± 1	3 ± 2
MET	206 ± 47[a]	68 ± 29[b]	70 ± 24[a,b]
	9 ± 2	4 ± 2	4 ± 1
HIS	294 ± 95[a]	374 ± 129[a]	58 ± 56[b]
	9 ± 3	11 ± 4	2 ± 2
ARG	261 ± 111[a,b]	115 ± 45[a]	276 ± 69[b]
	7 ± 3	4 ± 1	9 ± 2

[1] and [a,b] cf. legends of Table 2

However, the hepatic extraction coefficient of essential amino acids was almost similar for all individual amino acids as well as for that of the sum of essential amino acids. Taking into account the small differences observed in the hepatic extraction coefficient of essential amino acids, the non hepatic tissues received and utilized similar proportions of absorbed amino acids. Thus, the fraction of essential amino acids taken up in the free form by the non hepatic tissues accounted for 59%, 60% and 67% respectively of the mixture of essential amino acids absorbed after ingestion of diets A, B and C.

The quantity of amino acids leaving the liver after intake of the guar gum diet was approximately 78% of those measured after diets A and C. This was also due to a proportionally similar hepatic amino acid uptake for all the diets. Inside the amino acid mixture leaving the liver, the essential amino acids accounted for 38% of the sum of amino acids after ingestion of A, 34% after that of B and 33% after that of C.

The hepatic uptake of non essential amino acids after intake of guar gum feeding was slightly lower than that observed for diets A and C (Table 8). The hepatic extraction coefficients of non essential amino acids were respectively 2.2%, 1.2% and 1.7% for diets A, B and C. The hepatic balances of ASP and GLU were negative after all the three diets. Kinetic profiles of hepatic uptake

of non essential amino acids were very similar for all the diets except for CYS, OH-PRO, SER and TAU.

Table 8. Hourly means (mg) of the quantities of some non essential amino acids taken up by the liver (HB) and mean hepatic extraction coefficients (HEC) in the pig after ingestion of diets A, B and C

	A	B	C
TYR HB mg	416 ± 121[1]	212 ± 46	271 ± 116
HEC %	10 ± 3	5 ± 1	5 ± 2
ASP	−139 ± 83	−291 ± 196	−121 ± 106
	−7 ± 4	−20 ± 14	−6 ± 5
ASN	208 ± 49	386 ± 132	473 ± 194
	6 ± 1	13 ± 5	11 ± 5
GLU	−743 ± 180	−839 ± 334	−799 ± 213
	−13 ± 3	−19 ± 8	−13 ± 3
GLN	124 ± 184	137 ± 31	164 ± 124
	2 ± 3	2 ± 0.4	2 ± 2
ALA	1137 ± 337	708 ± 192	929 ± 193
	10 ± 3	7 ± 2	7 ± 2
GLY	136 ± 102	296 ± 300	325 ± 79
	1 ± 0.6	2 ± 2	2 ± 0.4

[1] cf. legend of Table 2

The fraction of non essential amino acids taken up by the non hepatic tissues represented 66%, 76% and 74% respectively of the absorbed mixture of non essential amino acids after A, B and C feeding respectively.

Discussion

The effects of fibre on postprandial blood glucose and insulin levels have often been investigated (Gulliford et al., 1988; Torsdottir et al.,1989) but little is known about the effects of fibre on the dietary aa absorption and the mechanisms of such effects as well as on the fibre effects on the hepatic aa metabolism. The latter were the main aim of the present study.

Ingestion of guar gum strongly reduced amino acid absorption. At our knowledge this was the first time that the measurement of the diminished amino acid absorption after ingestion of a viscous fibre was described. However, that reduction in absorption appeared to be rather uniform for almost all amino acids and not selective for some groups of amino acids. The question arising from those observations was related to the possible mechanisms of the reduced amino acid absorption in presence of guar gum since the ingestion of cellulose failed to modify the amino acid absorption. The slower aa absorption induced by guar gum does not seem to be explained by delayed gastric emptying (Holt et al.,

1979), but rather support the theory of a slower digestion or absorption (Hagander et al., 1984). The importance of viscosity with regard to the physiological effects of guar gum has been reported to be fundamental (Rainbird and Low, 1986; Torsdottir et al., 1989; Cherbut et al., 1990). Thus, the effect of guar gum at least on the glucose absorption disappeared when the property of the fibre to increase viscosity of the meal was destroyed by hydrolysis or processing of the guar gum (Jenkins et al., 1978; Torsdottir et al., 1989). Moreover, the decreased enzymic hydrolysis in small intestine induced by viscous dietary fibre which limits further steps of digestion and absorption has appeared to be quite important in the mechanisms of action of viscous dietary fibre such as guar gum (Isaksson et al., 1982; Hamberg et al., 1989).

The absorbed aa were primarily extracted by the liver confirming that there was a direct aa uptake by the liver during postabsorptive state (Barrett et al., 1986; Simões Nunes et al., 1991). The hepatic aa uptake rate was not modified by the ingestion of guar gum or cellulose.

The ingestion of guar gum didn't induce modifications in the profile of hepatic amino acid uptake. This suggested that the influence of guar gum on amino acid metabolism only took place at the luminal level. Conversely, the smaller urea production observed after guar gum ingestion seemed to be the result of a sufficient and equilibrated hepatic input mixture of amino acids.

Cellulose at the level used in this study, didn't influence the aa absorption rate and kinetic profile of absorption. Cellulose appears also to have on one hand a weak capacity in reducing glucose absorption in man (Jenkins et al., 1983) and on the other hand a lack of effect on the digestion and intestinal absorption of lipids in the rat (Borel et al., 1989) whilst guar gum also reduced lipidic absorption (Turner et al., 1990).

Ammonia concentration in the portal blood was rather high, but was very small in the systemic blood, meaning that almost all absorbed ammonia was taken up by the liver (Simões Nunes et al., 1989). The origin of ammonia in portal vein blood is complex since some arise from the intestinal metabolism of glutamine and some from microbial metabolism in the hindgut including the breakdown of indigested proteins and of bacterial proteins (Windmueller, 1982; Wrong and Vince, 1984). The comparatively high ammonia absorption after guar gum ingestion could result at least in part from a higher bacterial hydrolysis of urea and from other nitrogenous materials having as a result a lower urea uptake from the gastrointestinal tract. Liver ammonia uptake could result in the formation of purines, pyrimidines, non essential amino acids and urea (Powers-Lee and Meister, 1988). In the present work guar gum ingestion appeared to reduce the hepatic urea production as well as the blood urea concentrations whilst in a previous work (Malmlöf et al., 1989) we noted higher blood levels of urea after ingestion of a complex diet supplemented with guar gum than after the same diet gave alone. The only possible explanations for this contradiction are on one hand the higher level of guar gum in the first study (9.3% versus 6%) and on the other hand the interactions of guar gum with the other complex components of the diet while in the present work we used a synthetic one.

In conclusion, our results demonstrated that guar gum strongly inhibited the in vivo absorption of aa without changing their hepatic extraction coefficients.

The latter demonstration suggested that the intestinal mechanisms of action of guar gum were sufficient to regulate the latter observed metabolic effects.

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