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ORIGINAL CONTRIBUTION

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Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig.
Significance for man

Summary *Background* During the processing of foods, the Maillard reaction may occur contributing to altering the nutritional value of proteins. In dairy products the formation of lactuloselysine reduces the availability of lysine but the effects on the other nutrients are not very well known. *Aim of the study* Determination of the consequences of a high level of lactuloselysine in milk on the bioavailability of skim milk nutrients and the kinetics of their appearance in the portal blood and of their urinary and faecal excretions and extrapolation to lower heat treatments and to man, using the pig model. *Methods* Sub-adult pigs were fitted, under anaesthesia, with permanent catheters in the portal vein, carotid artery and urethra, and with an electromagnetic flow probe around the portal vein. Each animal was successively fed with two experimental meals containing an equal amount of dried skim milk (SM), either lyophilised or heat treated to obtain an intense Maillard reaction, (M-SM) resulting in a 50 % lysine blockage. Portal and arterial concentrations and flux of individual amino acids (AA), glucose, galactose and fructoselysine were measured for a period of 12h after the meals. Lysine, fructoselysine and AA excreted in the urine and faeces within 72h were also determined. *Results* In M-SM containing 50 % blocked lysine, no other AA was chemically modified. Fructoselysine appeared in the portal blood very late compared to amino acids resulting from a very slow release and corresponded to 8.2 and 18.6 % of the ingested amount after 12 and 72h, respectively. Significant changes of the appearance in the portal blood were observed only for lysine (–60 %), alanine (–17 %) and cystine (+37 %). A small decrease in the digestibility of most AA during the same period was observed, which was significant after 48h for lysine, phenylalanine, cystine, aspartic acid, glycine and total AA (–6 %). *Conclusion* It was confirmed that lactuloselysine was not bioavailable. The loss in protein nutritive value was mainly due and proportional to the deterioration of lysine and, to a lesser extent, to the decrease in the digestibility of some essential AA. Taking into account the very high level of lactuloselysine in the M-SM sample studied, it may be concluded that in common foods such as milk, infant formulas, biscuits, bread, pasta, containing lower levels of blocked lysine, the nutritional loss is primarily due to the loss of lysine and to a less extent to the decrease in the digestibility of other essential AA.

Key words Maillard reaction – lactuloselysine – absorption – excretion – pig

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Introduction

During processing, foods undergo chemical changes leading to an alteration of the nutritional value of proteins. This is the case for the Maillard reaction, occurring between reducing sugars and lysine, which takes place in dairy foods, eggs and cereals [1–3]. The "early" Maillard reaction modifies lysine into fructose- or lactuloselysine (in dairy products), which are both biologically unavailable. Under severe heat treatments, "blocked lysine" generates reactive carbonyls which may react with other AA or polymerise into brown melanoidins, affecting other nutritional parameters than lysine.

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Lysine availability is well controlled in foods for infants and children who have a high lysine requirement, using the analytical methods developed for reactive lysine [4, 5] or blocked lysine [6, 7]. The nutritional effects and metabolism of Maillard compounds have been investigated mainly in rats and reported in reviews [8, 9] and international congresses [10–14]. The few human studies relate to the urinary excretion of blocked lysine [15]. The rat is not the best model for man for studying the kinetics of the absorbed nutrients because of its differences in terms of feeding behaviour (nibbler vs mealeater, nocturnal vs diurnal eating). In this study, the pig was chosen because its intestinal physiology is very close to that of man and because it can be cannulated to follow the kinetics parameters in the portal and arterial bloods and in the urines, i.e (i) to determine the consequences of feeding a protein with a very high level of blocked lysine on the kinetics of nutrient absorption and excretion, which cannot be obtained in rats and humans and (ii) to extrapolate to man for lower blocked lysine levels. The test materials chosen were skim milk with no or 50 % blocked lysine.

Materials and methods

Test materials

Skim Milk powders (SM) prepared especially for this study were used as models of protein sources without or with Maillard reaction products (M-SM). Samples were prepared from fresh cow milk centrifuged and pasteurised under standard conditions. One fraction was lyophilised (SM) and contained no detectable level of blocked lysine and the other was roller-dried (M-SM) to block about 50 % lysine as lactuloselysine, as determined by the furosine method (Table 1). Dried samples were stored at room temperature and under nitrogen. M-SM was very slightly yellow, indicating a very low extent of the "advanced" Maillard reaction with no loss of other amino acids [1, 16], as confirmed by AA analyses of both samples which were identical. If other side reactions have taken place, they should be negligeable: lysinoalanine is not produced in large amounts during the very short heat treatment applied at low water activity and the absence of destruction of other amino acids is a good indication that reactions with reactive carbonyls like glyoxal or methylglyoxal are absent or negligeable compared to the reaction of lysine with lactose. The M-SM sample can therefore be considered as a good model of the "early" Maillard reaction and thus the nutritional consequences depend essentially on the presence of blocked lysine, as it is in most of the processed milks and infant formulas.

Animals

Three sub-adult Large White female pigs from the herd of the Nutrition Department of INRA (La Minière, France) were used. Each animal (52.2±2.7kg) was anaesthetized and fitted with an electromagnetic flow probe previously validated [17, 18] to measure the portal vein blood flow rate. Catheters were placed in the portal vein and in the left brachiocephalic artery through the carotid route as already described [17, 19, 20]. A permanent cannula was also placed into the urethra. They were given penicillin (1,200,000 IU/d) and streptomycin (1 g/d) for 3d, intramuscularly to have no direct effect on the digestion / absorption process and recovered their normal growth rate after 6 d. Blood and urine were therefore sampled in conscious animals placed in metabolic cages. Throughout their experimental life, the animals were maintained according to the principles for care of laboratory animals.

Experimental design and feeding conditions

Two weeks before surgery, the animals received a cereal based preexperimental diet (17 % protein) as already described [21], twice daily to maintain a growth rate of 600 g/d. This diet was progressively reintroduced 1–2d after surgery up to 800–1000 g per meal on day 5 or 6. Experimental trials began 8–10d after surgery when the pigs had recovered a normal appetite and growth rate. Each animal was subjected to two 72h trials at one week intervals. Three days before the trials, each animal was progressively adapted to skim milk, being fed a mixture (1kg/meal twice daily) of diminishing amounts of the preexperimental diet at the expense of a mixture (50/50) of a protein free diet, as already described [20] and lyophilised skim milk (SM). Each trial started at 09.00h after a fasting period of 24h [17]. The two trials differed in composition of the skim milk offered: a protein free diet (400 g) plus SM (400 g) or M-SM (400 g). The only differences in their chemical composition were the level of reactive lysine and the presence of blocked lysine in the M-SM diet. The experimental diets were alternated and the second trial performed as described before.

During each trial, the portal blood flow rate was recorded continuously; the mean values were not different, i. e. 2304±116 and 2352±51 ml min^{-1} for SM and M-SM diets, respectively. Blood was sampled from the portal vein (PV) and the carotid artery (CA) at time 0 and every 30 min for 12h. Urine was sampled at time 0, and then every hour until 4h, every two hours until 24h, and four times per day until 72h. Faeces were collected at 09.00 and 16.00 h from day 1 to 5.

Analytical methods

Determination of individual AA in the free form (blood) or after hydrolysis (skim milk, faeces, urine for lysine) was performed according to methods already described [22]. The level of blocked lysine was measured according to the furosine method [5–7] (Table 1). Reactive lysine estimated by the furosine method is a good indicator of biologically available lysine. Furosine determination in milk, plasma, urine and faeces was done by ion exchange chromatography after acid hydrolysis (boiling 6N HCl for 24 h). It has been established that free fructoselysine (or lactuloselysine) generates 20 % furosine [4, 5, 23, 24] while protein bound lactuloselysine (or fructoselysine) generates 32 % furosine [4–7, 25]. The level of blocked lysine was therefore calculated from the furosine values using the multiplication factors of 5 or 3,1 according to whether blocked lysine was considered as free (urine, plasma) or bound to proteins (skim milk, faeces) respectively (Table 1).

Free lactoselysine was not evaluated because no standard was available. Free fructoselysine present in the plasma was evaluated using the synthetic molecule [26] as a standard and by the furosine level. It was recorded as lysine equivalent (or lysine as fructoselysine). Blood glucose, galactose, total sugars and amino nitrogen, were determined using the standard procedures of glucose oxidase (EC1.1.3.4) [27], galactose oxidase (EC1.1.3.9) [28], total reducing sugars [29] and trinitrobenzenesulphonate TNBS [30].

Calculations and limits of the methods

Net intestinal absorption was defined as the amount of ingested nutrients appearing in the portal blood as opposed to the amount disappearing from the lumen (true absorption) [17, 19]. It was quantified according to equations 1 and 2:

Table 1 Protein content and levels of blocked and reactive lysine in skim milk (SM) and maillardised skim milk (M-SM) samples as estimated by the furosine method [6, 7]

	SM	M-SM
Protein (6.25 N)	32.12	32.06
Total lysine (g/16 gN)	8.40	6.40
Blocked lysine as lactuloselysine		
– in g/16 gN	0	4.27
– in % of initial lysine	0	50.8
Reactive lysine (g/16 gN)	8.40	4.13

Total lysine: lysine content measured after acid hydrolysis corresponding to the sum of reactive lysine and of lysine regenerated from lactuloselysine upon acid hydrolysis.
Blocked lysine: biologically unavailable
Reactive lysine: biologically available

equation 1: $q = (Cp - Ca)\, D\, dt$ equation 2: $Q = \int_{t_0}^{t_1} q$

where q is the quantity appearing in the portal blood during the period of time dt; Cp and Ca are the portal and arterial blood concentrations respectively; D is the blood flow in the portal vein; dt is the short period of time (5 min) during which variables can be considered as constant, and Q the net absorption during the postprandial period between times t_0 and t_1. Because of the metabolisation and uptake by the gut wall of nutrients coming from the lumen or from the arterial blood, Q corresponds to the net influx of nutrients in the portal blood and is equal to the true absorption minus the gut wall uptake.

Statistical analysis [31] involved SEM and comparison of two groups of data by matched pair Student's t test and blocked one-way ANOVA. Differences were considered significant at $P < 0.05$.

Results and discussion

Differences between experimental diets

The experimental diets differed only by the levels of reactive lysine in the dried milk (2.70 and 1.32 g/100 g for SM and M-SM respectively) and of blocked lysine present only in M-SM (1.37 g/100 g as lactuloselysine). The level of blocked lysine in the M-SM diet was very high (50.8 % of the initial lysine level) as compared to the levels generally found in processed milk (0–10 %), infant formulas (10–15 %), baby cereals (15–20 %) or pasta (7–15 %) depending on the process used. However, the highly maillardised M-SM can be considered as a valuable experimental model to better estimate the nutritional consequences of using blocked lysine and to extrapolate to lower and more normal levels.

Appearance of glucose and of galactose in the portal blood

The net glucose absorption, calculated according to equations 1 and 2, reached a maximum after 4h of the SM diet (37.1±6.5 g/h) and after 2h of M-SM diet (35.3±7.4 g/h). It decreased more quickly with M-SM than with SM diets and reached a plateau at 5.1–9.4 g/h between 9 and 12h. The total 12h absorption yield (absorbed/ingested glucose x 100) of M-SM diet (55.0±10.6 %) was 78 % of that of SM diet (69.1±8.1 %). None of the differences were statistically significant.

The net galactose absorption reached a maximum after 2 h for both SM (10.3±3.2 mg) and M-SM (5.8±1.4 g) diets. The net total galactose absorption within 12h was

57.7±14.1 g after SM and 31.5±10.0 g after M-SM diet. The yield of net galactose absorption within 12h was 46 % lower with M-SM (30.9 %) than with SM (56.6 %) diet. None of these differences were statistically significant.

The differences in the absorption rate and levels of carbohydrates may be explained by i) the lower level of dietary lactose due to its transformation into lactuloselysine (7.6 %) and into lactulose [32], ii) a possible influence of the Maillard reaction products on the intestinal disaccharidase activities [33, 34], or iii) a different emptying rate or intestinal transit time of the M-SM diet.

Pattern of blood amino acids

The concentration of total AA in the portal blood followed the same pattern in SM and M-SM fed rats. The initial concentration (320 mg/l) increased abruptly to reach a maximum (670 mg/l) after 2–3 h and then decreased until 7h and stayed around a plateau (370–430 mg/l) until 12h. The arterial concentration of total AA followed the same trend with lower values (maximum at 560–585 mg/l). The majority of individual AA presented the same portal and arterial concentration pattern as that of total AA except for lysine (see below). The still high level of AA in the portal blood after 12h was formerly observed when the amounts of ingested proteins were rather large [35, 36] pointing out that protein digestion was not finished.

The net absorption of total AA, as calculated according to equations 1 and 2, followed the same kinetics pattern: maximum at 2h and then a gentle diminution until 12h irrespective of the diet (Fig. 1). This pattern was different from that observed during former experiments with fish meal diets which gave a maximum absorption after 4h [35] due to different kinetics of gastric emptying and intestinal hydrolysis. In the present study, the large amount of blocked lysine did not modify these gut transit parameters.

However, the net absorption of total AA was lower in M-SM than in SM fed animals (Table 2). The total amounts absorbed within 12h were 114.1±16.2 g after SM and 93.4±10.5 g after M-SM feeding (NS difference). The yields of absorption (absorbed/ingested AA x 100) of total AA over 12h were 78.2 and 66.2 % for SM and M-SM respectively. Concerning the amounts of total individual AA absorbed, none of the differences observed were statistically significant except for lysine, alanine and ornithine which were lower in M-SM and for cystine which was higher in M-SM. The extrapolation to processed foods containing much lower levels of blocked lysine, leads to conclude on a very small reduction, if any, in the AA absorption due to the "early" Maillard reaction, except for lysine.

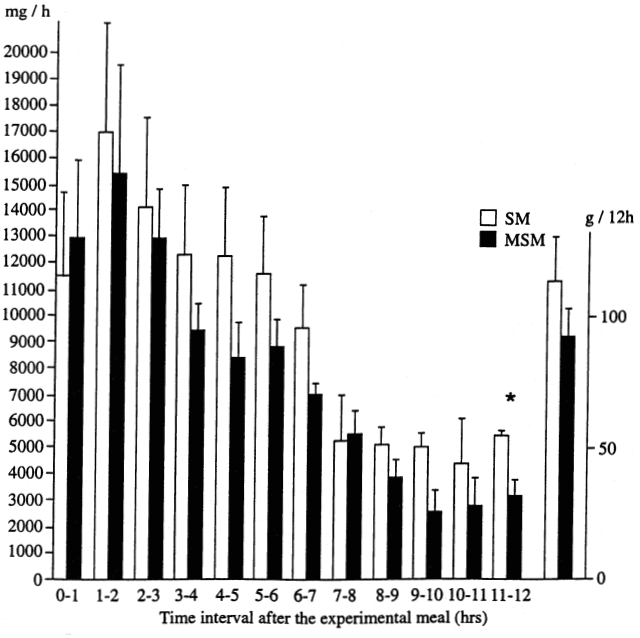

Fig. 1 Changes in hourly amounts of total amino acids (g/h) appeared in the portal blood according to time following intake of a lyophilised skim milk (SM) vs a maillardised skim milk (M-SM) diet. Statistical significance of differences: * $P < 0.05$.

The yield of net absorption of the individual AA after 12h, expressed in % intake ($\%I_{SM}$ and $\%I_{M\text{-}SM}$) varied to a very large extent depending on the AA (Table 2). For most of them it was below 100 % in both SM and M-SM diets. This apparent low net absorption levels of most AA were due to their uptake by the gut wall. Interestingly, the gut uptake was very important for aspartic acid, glutamic acid and glutamine. By contrast, the gut generated a large amount of alanine (251–220 %) coming from transamination reactions with other AA which were oxidised [22].

The net absorption yield of individual AA was always lower after M-SM than after SM feeding. The ratio ($I\%_{M\text{-}SM}/I\%_{SM}$) was within a narrow range for most of AA (mean value = 0.85) except for valine (0.64) and cystine (1.36) (Table 2). For both diets, the absorption ratio of (reactive) lysine was similar to that of the other AA (0.82).

A decrease in the absorption of AA has already been reported by Erbersdobler et al. [37] in rats fed a mixture of protein plus sugar heated at 110°C for 24h. This decrease may either be due to a decrease in the transport of some AA (threonine, proline, glycine) or to an inhibition of the proteolysis both being related to the presence of some Maillard reaction products [38]. After a drastic heat treatment (105°C, 24h, open air), this reduction, as evidenced by a poorer appearance of AA in the rat portal blood, concerned lysine and arginine and also large and variable proportions of the other AA (except tyro-

Table 2 Cumulative amounts of individual and total amino acids (g ± SEM) appeared within 12h in the portal blood of animals fed skim milk diet (SM) or maillardised skim milk diet (M-SM) and percent absorption compared to intake ($\%I_{SM}$ and $\%I_{M\text{-}SM}$)

	SM		M-SM		Ratio	
	Amounts	$\%\ I_{SM}$	Amounts	$\%\ I_{M\text{-}SM}$	M-SM/SM	$\%I_{M\text{-}SM}/\%I_{SM}$
HIS	2.98±0.48	79.2	3.19±0.26	78.2	1.07	0.99
LYS	7.36±0.09	68.1	2.97±0.55*	56.2	0.40	0.82
PHE	6.66±0.99	102.1	5.79±0.64	83.7	0.87	0.82
LEU	11.97±1.79	82.0	9.78±1.52	65.4	0.82	0.80
ILE	6.82±1.29	89.3	5.22±0.81	67.6	0.76	0.76
MET	3.97±0.62	134.1	3.48±0.40	124.1	0.88	0.93
VAL	6.60±1.09	70.5	4.38±0.74	45.2	0.66	0.64
THR	4.95±0.90	80.3	4.02±0.53	64.8	0.81	0.81
ARG	4.70±0.27	94.7	4.81±0.71	90.4	1.02	0.95
CYS	0.67±0.25	52.3	0.91±0.21*	71.1	1.37	1.36
TYR	7.03±1.24	96.0	6.40±1.09	88.3	0.91	0.92
ASP	0.96±0.21	9.4	1.06±0.20	10.4	1.10	1.11
GLU	0.08±0.07	0.3	0.06±0.05	0.2	0.76	–
PRO	13.00±1.49	97.6	10.81±0.36	82.1	0.83	0.84
SER	6.68±0.97	83.1	5.93±0.37	77.6	0.89	0.93
GLY	2.46±0.19	86.6	2.32±0.50	77.3	0.94	0.89
ALA	11.87±1.83	251.5	9.87±1.45*	220.3	0.83	0.86
ASN	6.98±0.66	–	6.04±0.56	–	0.86	–
GLN	0.50±0.22	–	0.79±0.29	–	1.58	–
CIT	5.10±1.52	–	3.78±0.09	–	0.74	–
ORN	1.78±0.07	–	1.26±0.09*	–	0.71	–
TAU	0.43±0.04	–	0.25±0.08	–	0.58	–
AAT	114.05±16.19	78.2	93.40±10.49	66.2	0.82	0.85

SEM Standard error of the mean
%I: Percentage of ingested amino acids
* Significantly different from the SM values

sine). After a rather moderate treatment (90°C, 24h, open air), the reduction concerned only lysine [39]. In the present experiment, the absorption was moderately reduced with M-SM diets for most AA, and more largely for lysine (–60 %) and valine (–34 %). Opposite to the Erbersdobler data [39], arginine was not affected by the Maillard reaction since its absorption rate was the same with M-SM and SM diets, confirming the absence of advanced Maillard reaction on the side chain of arginine. The significantly higher absorption rate of cystine after M-SM feeding is more difficult to interpret; the most likely hypothesis being a further denaturation of milk proteins in M-SM samples improving cystine availability. These differences in the absorption yield will be further dicussed in the following chapter on the faecal excretion.

Pattern of blood lysine

After SM feeding, the portal blood concentration of lysine (Fig. 2) increased up to 47 mg/l after 2–3 h and decreased slowly to reach a plateau at about 24 mg/l at the end of the experimental period, while after feeding M-SM, it increased to only 21 mg/l after half an hour and decreased rapidly for 3–4 h below the initial level (13.4 mg/l) to reach a plateau at about 6.5 mg/l.

The arterial concentrations of lysine followed the same trend as that of the portal concentrations but with lower concentrations (maximum at 38 mg/l for SM and 17 mg/l for M-SM feeding).

Hourly amounts of absorbed lysine (Table 3), were always lower after M-SM than after SM feeding, the difference being significant ($P < 0.05$) between the 2nd and the 5th h.

Total amounts of lysine absorbed (g/12h) (Table 2) with M-SM (2.97±0.55) was 40 % of that of SM (7.36±0.09), although the reactive lysine reduction was 50 %. This difference appeared also in the yields of absorption (%I, expressed as a percentage of reactive lysine intake) (Table 2) within 12h, which were 68.2 and 56.3 % (NS), respectively after SM and M-SM feeding.

The very marked reduction of lysine absorption in M-SM was due to its transformation (50 %) during processing into lactuloselysine which is not bioavailable because the covalent bond between lysine and the sugar is not split by any pancreatic, gut or tissue enzymes. These data confirm the previous observation that any of the Amadori compounds of lysine was found to promote growth in rats [25]. The observed absorption yield of lysine (40 %) (Table 2, column 4) in M-SM lower than the level of lysine blocked (50 %), can be explained by the inability of gut enzymes to release lysine adjacent to a residue of blocked lysine. Indeed, the sequence -lysyl-lysyl- exists in several milk proteins (sequences 102–103 of α_{S1}-casein, 136–137, 149–150, 165–166 of α_{S2} casein, 111–112 of kappa-casein and 69–70 and 100–101 of β-

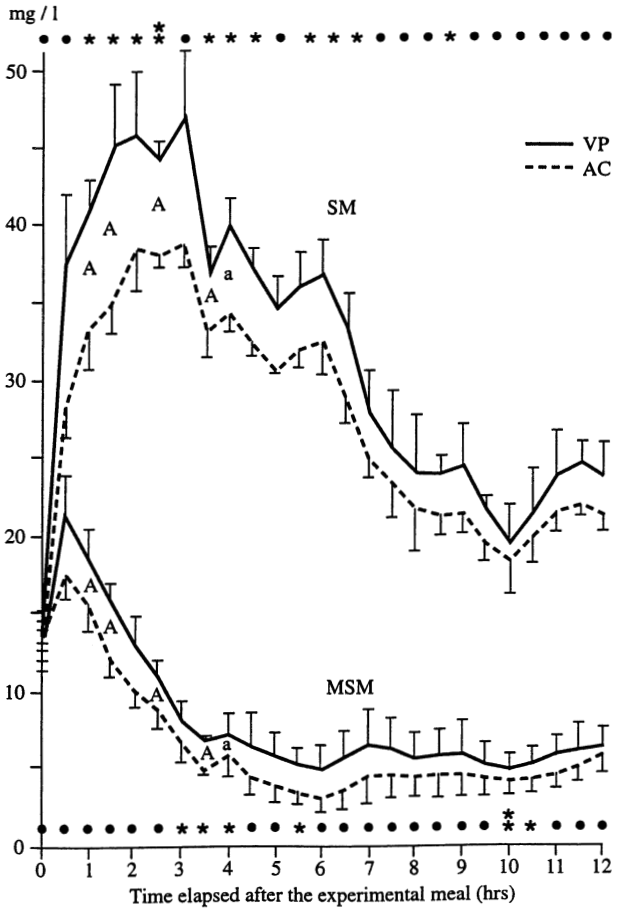

Fig. 2 Changes in portal (VP —) and arterial (AC ----) lysine blood concentrations (mg/l) according to time following intake of a lyophilised skim milk (SM) vs a maillardised skim milk (M-SM) diet. Statistical significance of differences: between portal and arterial concentrations for each heat treatment * $P < 0.05$ ** $P < 0.01$; between the two treatments for porto-arterial differences: A $P < 0.05$, a $P < 0.01$.

Table 3 Changes in the flux (mg/h) of free lysine and fructoselysine (expressed as lysine equivalent)[1] appeared in the portal blood within 12h after the meal (Skim milk diet SM vs maillardised skim milk diet M-SM)

Time interval after the meal (h)	Free Lysine		Lysine[1] as fructoselysine
	SM	M-SM	MSM
0–1	1006±289	432±144	
1–2	1216±190	481±164[2]	
2–3	949±212	303±37[2]	
3–4	749±180	228±21[2]	2.2±2.2
4–5	685±169	199±60[2]	10.9±7.2
5–6	592±127	252±33	29.0±15.6
6–7	534±100	263±78	29.0±15.6
7–8	355±183	225±82	26.9±17.6
8–9	381±92	170±80	33.3±14.1
9–10	317±106	130±66	25.4±8.1
10–11	233±117	141±26	24.7±6.4
11–12	346±69	146±38	32.5±12.6
0–12 (mg/12h)	7363±90	2970±550[2]	214.0±94.2

[1] fructoselysine x 47.4 %
[2] Significantly different from SM

lactoglobulin) and the risk of blocking a lysine residue of such a -lysyl-lysyl- sequence is not negligeable.

Pattern of blood fructoselysine

Lactuloselysine originally present in M-SM cannot be detected as such in the plasma because no synthetic reference exists. However, some free fructoselysine was detected in the plasma and quantified, using the synthetic molecule [26] as a reference. Its level was estimated in parallel from the level of furosine generated after acid hydrolysis (Table 4). Values estimated by the furosine method were about 2,6 times higher at the beginning of the absorption than those obtained by the direct titration of fructoselysine and identical after about 10 h. This would show the existence of non measurable free lactuloselysine and/or of blood peptides containing fructose- or lactuloselysine which are absorbed rapidly. Only traces of fructoselysine were found in the blood of animals fed SM, coming probably from endogenous production. In animals fed M-SM, traces were found during the first 4 h; afterwards, the concentrations increased slowly to a maximum at the 11th h, showing that the absorption was not achieved after 12 h. The arterial concentrations of fructoselysine followed the same trend as the portal ones but at lower levels.

Using the furosine conversion coefficients, the net amounts of fructose- or lactuloselysine absorbed after M-SM feeding (expressed as lysine equivalent), are given in Table 3. The total amount absorbed between the 4th and the 12th h, when absorption was not achieved, was 214.0±94.2 mg (compared to 2.60 g ingested) representing 8.2 % of the ingested quantity.

Table 4 Mean blood concentrations of fructoselysine (as lysine equivalent in mg/100 ml)[1] in animals receiving maillardised skim milk (M-SM) diet

Time after the meal (hours)	Direct measurement*		From the furosine content**	
	Portal blood	Arterial blood	Portal blood	Arterial blood
0–3	Traces			
3.5	0.025	0.022		
4	0.028	0.026	0.073	0.074
5	0.063	0.060		
6	0.093	0.062	0.132	0.120
7	0.136	0.125		
8	0.191	0.166	0.205	0.200
9	0.250	0.228		
10	0.250	0.237	0.267	0.268
12	0.233	0.210	0.240	0.209

[1] lysine equivalent = fructoselysine x 47.4 %
* direct measurement using lactose-lysine as a standard
** Calculated from the furosine value obtained after acid hydrolysis (fructoselysine=Fur X 5)

Urinary excretion of total lysine

The hourly amounts of total urinary lysine (as measured after acid hydrolysis) (Fig. 3) (Table 6) in the SM fed group ranged between 10 and 15 mg/h for 72h. In the M-SM fed group, there was a rapid increase in the excretion of lysine from an initial level of 10 mg/h to a peak of 32 mg/h at the 14th h, followed by a rapid decrease until the 22nd h back to the mean level of the SM fed group. Differences of excretion of lysine between M-SM and SM were significant between the 8th and 14th h. During the first 24h, total urinary excretion of lysine was thus significantly higher (178 %, $P < 0.05$) after M-SM than after SM feeding due to its release from hydrolysed lactulose-lysine.

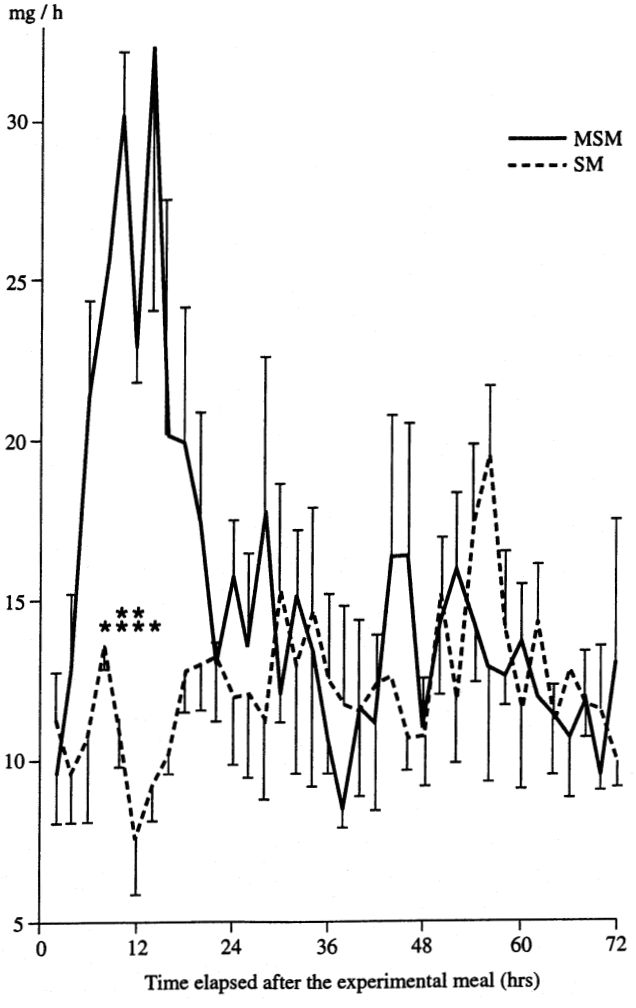

Fig. 3 Changes in urinary hourly amounts (mg/h) of lysine according to time following intake of a lyophilized skim milk (SM ----) vs a maillardised skim milk (M-SM) diet. Statistical significance of differences: * $P < 0.05$, ** $P < 0.01$.

Urinary excretion of fructoselysine

Fructose (or lactulose-lysine) excreted hourly by the SM fed group (Fig. 4) (Table 6) was almost null during the first 18 h and then increased to about 4–5 mg/h. After M-SM feeding, there was a rapid increase in the excretion of fructoselysine from a very low initial value to a maximum of 24 mg/h, followed by a rapid decrease at the 26th h to values similar to those of the SM fed group. Differences of fructoselysine excretion were significant ($P < 0.05$ and $P < 0.01$) from the 4th to the 20th h.

The pattern of the urinary excretion of fructoselysine was indicative of its rate of release and absorption. The peak of excretion occurred between 8 and 14 h, i. e. long after the peak of absorption of dietary AA which occurred between 2 and 3 h after the meal and when the dietary AA have been nearly completely absorbed. The ap-

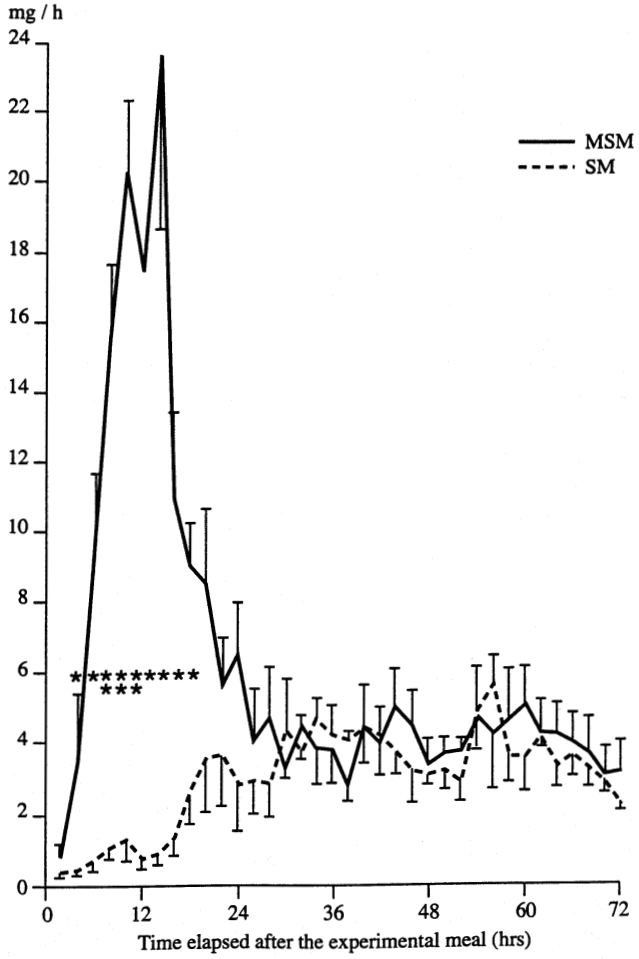

Fig. 4 Changes in urinary hourly amounts of fructoselysine excreted as lysine equivalent (mg/h) according to time following intake of a lyophilized skim milk (SM ----) vs a maillardised skim milk (M-SM) diet. Statistical significance of differences: * $P < 0.05$, ** $P < 0.01$.

pearance of fructoselysine in the portal blood started after 3–4 h and reached a plateau between 4 and 12h while it was not completely absorbed.

Considering that the transit time of proteins in the small gut of the pig ranges between 4–5h for initial fractions of feeds and 14–16h for terminal fractions arriving at the ileo-coecal junction [40], the release of lactuloselysine from the protein matrix appeared to be very late in the intestine; its absorption, resulting from a long process, lasting more than 8 hours. The presence of free fructoselysine in plasma indicates that the galactose residue of lactuloselysine is released by some enzyme present in the gut lumen or in the brush border. Fructose (or Lactuloselysine) is most likely absorbed passively opposite to "normal" AA.

Small cumulated amounts of urinary fructoselysine were found in animals fed the SM diet which however did not contain any measurable lactuloselysine. These positive values may be due to the presence of traces of lactuloselysine in the SM diet, to its endogenous production or to artefacts. In this experiment, the endogenous production cannot be estimated because of possible interference of trace amounts of blocked lysine in the diets.

The urinary excretion of fructoselysine during the first 24 h corresponded to 10.7 % of the ingested amount, i. e. slightly higher than that measured in the portal blood (8.2 %) during the first 12 h. This excretion level increased to 18.6 % for the whole period of 72 h, The fructoselysine excretion level depends on its chemical form and seems to be species specific. In the rat [41], the excretion level of the free synthetic fructoselysine was 60–72 %; it was in the range of 3 to 13.4 % when fructoselysine was bound to proteins. When fructoselysine was estimated after acid hydrolysis (as furosine), these values were slightly higher probably because some fructoselysine was excreted after phosphorylation by the kidney [42]. In human beings, the urinary excretion of protein bound fructoselysine ranged from 1.4 to 3.5 % of the amount ingested [15].

Table 5 Amounts of amino acids (expressed as a percentage of AA intake in the experimental diet) in faeces collected within 48h after feeding a meal containing lyophilised skim milk (SM) vs maillardised skim milk (M-SM)

	SM	M-SM	Difference
HIS	29.9	37.4	7.5
LYS	36.8	45.6*	8.8
PHE	36.9	48.1*	11.2
LEU	30.9	35.9	5.0
ILE	47.1	53.8	6.7
MET	31.0	38.2 (P < 0.06)	7.2
VAL	44.5	50.2	5.7
THR	59.1	66.7	7.6
ARG	58.2	65.4	7.2
CYS	68.6	83.0*	14.4
TYR	26.3	32.1	5.8
ΣAAE	39.8	47.1*	7.3
ASP	59.2	71.7*	12.5
GLU	29.4	33.7	4.3
PRO	22.0	24.9	2.9
SER	49.5	57.2	7.7
GLY	106.5	119.0*	12.5
ALA	81.4	93.2	11.8
Lysine as lactose lysine[1]	–	1.8	
ΣAANE	41.2	47.7	6.2
ΣAAT	40.9	47.4*	6.5

[1] fructoselysine = furosine x 3.1
* Significant differences (P < 0.05)

Table 6 Changes in urinary and faecal excretion (mg) of lysine and of fructoselysine[2] expressed as lysine equivalent[1] within 72h following intake of diets containing lyophilised skim milk (SM) or maillardised skim milk (M-SM)

Time interval after the meal (h)	Lysine				Lysine[1] as fructoselysine[2]			
	Urine		Faeces		Urine		Faeces	
	SM	M-SM	SM	M-SM	SM	M-SM	SM	MSM
0–12	126.9±13.0	243.4±21.7			9.7±3.6	145.3±19.4		
12–24	140.8±11.9	233.7±43.0			31.7±12.3	134.9±24.6		
0–24	267.7±24.5 *	477.2±103.3	1885.7±56.8	2098.0±301.1	41.5±16.1 **	280.1±38.6	12.8±6.5	15.0±3.7
24–36	157.7±29.7	166.4±30.6			48.3±10.2	51.4±11.6		
36–48	140.1±16.4	150.4±19.6			48.6±7.3	51.7±6.9		
24–48	297.8±46.0	316.8±46.3	2084.3±282.2 *	2810.0±300.6	96.9±17.6	103.1±17.3	13.2±3.4	32.5±2.9
48–60	180.2±10.3	168.1±14.2			50.0±6.4	55.7±10.0		
60–72	143.8±8.6	141.5±19.4			41.9±3.3	47.9±10.0		
48–72	324.0±18.6	309.6±30.0	2651.0±164.8	2594.3±368.5	92.0±9.7	103.8±19.9	24.5±8.7	23.2±4.1
72–96	–	–			–		25.7±14.4	33.9±2.3
Tot 0–72	889.6±84.7 (NS)	1103.7±131.4	6621.0±465.2	7502.3±747.4	230.6±42.6 *	482.1±69.7	50.5±18.1	70.7±5.7.9
Tot 0–96			9083.3±830	11633.7±1.050			76.3±31.4	104.6±10.1

[1] fructoselysine measured from the content of furosine (x 5 in urine; x3.1 in faeces) after acid hydrolysis
[2] lysine equivalent = fructoselysine x 47.4 %
* Significant difference (P < 0.05)
** Significant difference (P < 0.01)

Faecal excretion of fructoselysine

The faecal excretion of fructoselysine as estimated by the furosine method was very poor, corresponding to 2.7 % after 72 h and 4 % after 96h of the ingested amount (Table 6). Similar values were found in the rat [7] and in man [15]. Indeed, fructoselysine was found to be metabolised by the intestinal microflora and to release lysine which is not bioavailable for the body [2, 5, 25].

Faecal excretion of amino acids

Total faecal AA were measured after acid hydrolysis, over the periods 0–24, 24–48 and 48–72 h. For the 48h period, which is the most representative of the transit time in the pig (33h) [43], the individual AA amounts excreted in the faeces, expressed in % of intake, were higher after M-SM than after SM feeding, the differences being significant ($P < 0.05$) for lysine, phenylalanine, valine, cystine, aspartic acid, glycine, the sum of essential AA and total AA. For methionine the significance was $P < 0.06$ (Table 5). Such a decrease was already reported especially for lysine and for almost all essential AA [39]. This results from disturbed protein hydrolysis and AA transport by the enterocyte.

The presence of lactuloselysine in food proteins reduces the action of trypsin and of peptidases as the AA adjacent to the lactuloselysine residues cannot be released enzymatically. It can be expected that the speed of hydrolysis is reduced for two reasons: a) the high level of lysine blockage (50.8 %) which diminishes by a factor 2 the number of hydrolysis sites by trypsin, and b) the peptides containing lactuloselysine are longer than usual and would take more time to be hydrolysed by the other enzymes. No reduction in the speed of hydrolysis was however observed, as the peak of the portal AA appeared at the same time after M-SM and SM feeding. The only difference was in the efficiency of hydrolysis due to the inability of the AA adjacent to lactuloselysine to be released. Thus, as indicated in the following list, AA which were excreted in larger amounts in the faeces were found to be adjacent to lysine residues in most abundant milk proteins, caseins (Cas), β-lactoglobuline (BLG) and α-lactalbumine (ALA); for phenylalanine: $Lys^{91\&173}$-Phe in α-S2-Cas at lysine positions 91 and 173 respectively, Lys^{135}-Phe and Phe-Lys^{83} (BLG); Lys^{79}-Phe (ALA); for valine: $Lys^{14\&91}$-Val (BLG), $Lys^{97\&169}$-Val (β-Cas), $Lys^{36\&105}$-Val (α-S1-Cas) and Lys^{199}-Val (α-S2-Cas); for cystine: Cys-Lys^{41} (α-S2-Cas), Lys^{5}-Cys and Cys-Lys^{62} (ALA); for aspartic acid: $Lys^{13,26,112}$-Asp (κ-Cas) and Lys^{42}-Asp (α-S1-Cas); for methionine: Lys^{60}-Met (α-S1-Cas) and Met-Lys^{8} (BLG).

Many other AA were adjacent to lysine; they were also excreted in larger amounts after M-SM than after SM feeding, but not significantly. The steric availability of lysine residues to the Maillard reaction should be taken into consideration, but it is not predictable.

By analysis of the portal AA, it was not possible to show any significant difference in the AA absorption between M-SM and SM groups, except for lysine, cystine and alanine. Analysis of the faecal excretion of AA appeared to be more sensitive to detect a lower AA absorption in the M-SM fed group.

The faecal AA content of the animals fed SM diet ranged from 22 to 68 % of the AA intake (Table 5). It corresponded mainly to endogenous losses. The difference in the faecal excretion of AA between the two groups of animals fed M-SM and SM diets ranged from 2.9 % (proline) to 12.5 % (aspartic acid) of the AA intake, depending on the AA, with a mean value of 6.2 % for all AA. This decrease in AA digestibility can be considered as low for an intentionally high degree of lysine blockage (50 %) which is much higher than that usually found in normal human foods and processed milks. If the AA loss is proportional to the level of lysine blockage, it can be concluded that for conventional human foods containing a maximum level of 15 % of blocked lysine, the decrease in AA digestibility would be around 2 %, which can be considered as negligible, except, of course, for lysine.

Conclusion

Feeding pigs with milk proteins containing 50 % lysine blocked as lactuloselysine modifies the following nutritional parameters as compared to milk proteins containing no Maillard products. There is a lower absorption of glucose and of galactose due to a loss of lactose partly transformed into lactuloselysine and lactulose, and probably also due to the lactase inhibition. The kinetics of AA absorption were quite similar after M-SM and SM feeding showing that the Maillard reaction did not significantly change the speed of AA release by the proteases. The amounts of AA absorbed and appearing in the portal blood were lower for most of them in the group fed the M-SM diet, but not significantly except for lysine – confirming that lactuloselysine is not bioavailable –, cystine and alanine. The faecal excretion of AA was higher in the M-SM group and significantly different for many of them confirming their lower digestibility. It is likely that the amino acids adjacent to the lactuloselysine residues were uneasily released, reaching the colon and being therefore excreted in the faeces. Fructoselysine appeared in the portal blood at a very low level, long after the dietary AA and was excreted in the urine mostly between 8 and 14 h after the meal due to its slow release. Its urinary excretion corresponded to 18.6 % of the ingested quantity within 72 h. A very small proportion escaped from the action of the intestinal microflora was excreted in the faeces. Except the decrease in lysine availability due to the formation of lactulosely-

sine, the modifications observed in sugar and AA absorption were rather small for a highly maillardised milk protein. In human foods, the nutritional consequences of mild Maillard reactions may thus be considered as negligible.

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