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Effects of monosodium glutamate supplementation on glutamine metabolism in adult rats

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1. ABSTRACT

Monosodium glutamate (MSG) is a worldwide used flavor enhancer. Supplemental glutamate may impact physiological functions. The aim of this study was to document the metabolic and physiological consequences of supplementation with 2% MSG (w/w) in rats. After 15 days-supplementation and following the ingestion of a test meal containing 2% MSG, glutamic acid accumulated for 5h in the stomach and for 1h in the small intestine. This coincided with a significant decrease of intestinal glutaminase activity, a marked specific increase in plasma glutamine concentration and a transient increase of plasma insulin concentration. MSG after chronic or acute supplementation had no effect on food intake, body weight, adipose tissue masses, gastric emptying rate, incorporation of dietary nitrogen in gastrointestinal and other tissues, and protein synthesis in intestinal mucosa, liver and muscles. The only significant effects of chronic supplementation were a slightly diminished gastrocnemius muscle mass, increased protein mass in intestinal mucosa and decreased protein synthesis in stomach. It is concluded that MSG chronic supplementation promotes glutamine synthesis in the body but has little effect on the physiological functions examined.

2. INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of glutamic acid, is a worldwide used flavor enhancer. The consumption of this compound has increased 3 times between 1979 and 2001 in human (1). Then it appears mandatory to document the consequences of MSG consumption in terms of beneficial over deleterious effects of this compound. Some animal studies have reported that MSG is able to induce overweight and/or obesity (2, 3) but mechanisms which would be responsible for such an effect remain unclear. Different physiological effects of glutamate on the gastro-intestinal tract and on the endocrine and exocrine pancreas have been reported. Some of them are believed to occur through binding to receptors which are present in numerous cell types. Glutamate is well known to represent the predominant excitatory neurotransmitter in the central nervous system (4) and appears to also act as a peripheral neurotransmitter. Indeed, mGluR1 glutamate receptors are present in the fundic gland of stomach (5), N-methyl-D-aspartate (NMDA) glutamate receptors have been identified in the myenteric plexus of the ileum (6-8) and mGluR2/3 glutamate receptors have been characterized in the enteric nervous system between muscularis mucosa and the circular muscular layer (9).

Table 1. Composition of the experimental diets based on NaCl (C) or MSG (MSG)

	C	MSG
MSG (g/100g dry matter)	0	2
NaCl (g/100g dry matter)	0.87	0
Total Milk protein (g/kg)	140	140
Starch (g/kg)	538.1	538.1
Sucrose (g/kg)	87.6	87.6
Cellulose (g/kg)	50	50
Soy oil (g/kg)	137	137
Minerals (AIN-93M) (g/kg)	35	35
Vitamins (AIN-93M) (g/kg)	10	10
Choline (g/kg)	2.3	2.3
Total protein (% of energy)	15	15
Total carbohydrates (% of energy)	55	55
Total fat (% of energy)	30	30
Metabolizable energy (kJ/g)	16.8	16.8

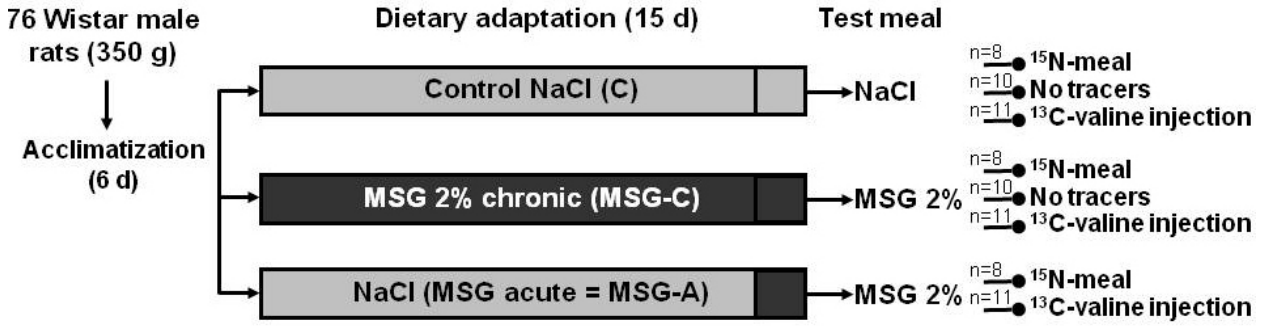

Figure 1. Experimental design

Glutamate was shown to induce tonic contractions of rat fundus (10), to elicit contraction of longitudinal muscle in ileum (8, 11) and to increase pancreatic flow and protein output from exocrine pancreas (12, 13). The effect of glutamate as a stimulator of insulin secretion is much controversial and have been highly debated (14). In conscious rats, the intragastric administration of glutamate at 200 mg/kg elicited a transient insulin response in the fed state but had no effect in the fasted state (15); and the infusion of the oral cavity with MSG was found to transiently increase blood insulin level (16).

From a metabolic point of view, glutamate is oxidized, converted to other amino acids or used as precursor for the synthesis of different bioactive compounds (17-21) and oxidation in the small intestine absorbing epithelial cells (enterocytes) appears to represent the main metabolic fate of glutamate during its transcellular journey from lumen to blood (22). As a consequence, the quantity of glutamate appearing in the portal blood is usually very low with normal or moderately high level of dietary glutamate and is more likely originating from glutamine catabolism through the action of intestinal glutaminase than from the absorption of dietary glutamate itself (17). In healthy human volunteers, nearly all of the enterally delivered glutamate is removed by the splanchnic bed on the first pass (23). A transient portal and arterial hyperglutamatemia was however observed in pig fed a diet supplemented with very high quantity of MSG (24).

In that overall context, the aim of the present study was to determine the consequences of MSG supplementation on the kinetics of luminal concentration of glutamate in the gastro-intestinal tract, on the body composition, on gastrointestinal and pancreatic functions, on the plasma aminoacidemia and on the postprandial protein metabolism in rats receiving MSG for the first time (acute conditions) or after a 2-wk adaptation (chronic conditions).

3. MATERIALS AND METHODS

3.1. Animals and diets

Experiments were carried out in accordance with the guidelines of the French Committee for Animal Care and the European Convention on Vertebrate Animals Used for Experimentation. Wistar male rats (Harlan, Horst, The Netherlands) weighing 325-350 g at the beginning of the experiment, were housed with a 12:12-h light-dark cycle (light 8:00 PM-8:00 AM). They were fed a standard chow diet for 6 days before starting dietary adaptations. Then rats were randomly assigned to one of three groups (Figure 1). Each group received for 15 days a normoenergetic diet (INRA, Jouy en Josas, France) providing 15% of energy as protein, 30% as fat and 55% as carbohydrate (Table 1). The diets only differed by the MSG concentration: 0 or 2% w/w (Ajinomoto Co., LTD, Tokyo, Japan). For control group (C), an isosodic amount of sodium chloride (NaCl) was added to the food. Supplemental MSG in diet adds 7% more nitrogen than in the control group (only NaCl) i.e. a modest relative increase. One of the three groups received the MSG supplemented diet only on the last day to test the acute effect of MSG supplementation (Figure 1). All diets were moistened (powdered diet/water: 1:2) to prevent spillage. A feeding pattern, designed to accustom the rats to

eat in a short time a standard meal, was applied: a first meal (6 g dry matter) was given between 08:00 and 09:00h and then removed. Rats had then ad libitum access to food between 13:00 and 17:00h.

3.2. Experimental protocol

The rats were euthanatized with sodium pentobarbital (50 mg/kg BW) (Sanofi Synthélabo Santé Animale, Libourne, France) 1h, 2h or 5h after ingestion of the test-meal. Within each of the three groups, a subgroup of rats served to measure the fed-state tissue protein synthesis rates. Another subgroup of rats were given a ^{15}N-labeled protein meal in order to determine the postprandial fate of dietary N and ^{13}C-sodium acetate supplementation (10 mg per rat) to determine gastric emptying rate by the recovery of ^{13}C in expired breath, as described below. Blood was collected from the vena cava and samples were centrifuged to recover plasma.

3.3. Determination of protein synthesis rate in tissues

Tissue synthesis rates were assessed using the flooding dose method. Rats were injected subcutaneously with 300 µmol/kg BW of a flooding dose of L-[1-^{13}C]-valine (50 mol %, Cambridge Isotope Laboratories, Andover, MA, USA) 20 minutes before the organ collection following the method described by Mosoni et al. (25). Tissues were promptly removed, rinsed, weighed and frozen for further analysis. The ^{13}C-valine enrichment in free and protein-bound amino acids of tissues was measured by gas chromatography mass spectrometry (GC-MS) (Hewlett-Packard 6890 N) and by gas chromatography combustion isotopic ratio mass spectrometry (GC-C-IRMS) (HP5890/Isoprime, VG Instruments, Manchester, UK) using a 50 m apolar column (HP5MS, Hewlett-Packard) after sample preparation as previously described (26). Fractional synthesis rates (FSR, %/d) of tissue proteins were calculated as $FSR = E_{bound\ val} / (E_{free\ val} \times t) \times 100$ where $E_{bound\ val}$ and $E_{free\ val}$ are protein-bound and free ^{13}C-valine enrichments in tissues. Absolute synthesis rates (ASR, in g protein per day : gP/d) were calculated as ASR = FSR x P where P is the tissue total protein content.

3.4. Determination of oro-fecal transit time and gastric emptying rate

The oro-fecal transit time was measured in each rat according to the Evans blue (Sigma) method. Rats received immediately before the test meal, a 3-g meal colored with Evans blue. Oro-fecal transit time was calculated as the time between meal ingestion and apparition of blue color in feces. The same protocol was used to determine the progression of the meal in intestine. Gastric emptying rate was measured after ingestion of ^{13}C-sodium acetate within the meal, by collecting expired breath every 20 min for 5 hours. The rats were housed in air-proof cages linked to an open-circuit flow-through calorimetric device (27). A fifth cage was maintained empty to serve as zero control. Air from each cage was sampled in turn to oxygen (Siemens) and carbon dioxide (Maihak) gas analyzers. The ^{13}C enrichment of CO2 in breath was determined using gas chromatography-isotope-ratio mass spectrometry (Multiflow/Isoprime, Micromass). The digestive and nitrogen metabolic fate of dietary N in tissues and urea was assessed in the rats undergoing expired breath monitoring. Rats were anesthetized 5h after the ingestion of the ^{15}N-labeled meals which were identical to the one described above except that milk proteins were uniformly ^{15}N-labeled. The urine was collected throughout the postprandial period. The gastrointestinal tract was removed and the contents of the stomach, small intestine, colon and cecum were collected. The intestinal mucosa, stomach, liver, kidneys, and gastrocnemius muscle were promptly removed, rinsed, weighed and frozen until for analysis.

3.5. Determination of glutaminase and glutamine synthetase activity in tissues

Glutamine synthetase activity was determined in rat tissues according to Minet et al., 1997 (28). Briefly, this method uses the capacity of glutamine synthetase to convert L-glutamine into gamma-glutamyl-hydroxamate. After mixing tissues with imidazole buffer (50 mM pH 6.8) and sonication (3 times, 10 sec at 4°C), supernatants were collected after centrifugation (4500 g, 15min). The supernatants were incubated 10 min at 37°C with or without L-glutamine (50mM) in the presence of hydroxylamine 25 mM, sodium arsenate 25 mM, $MnCl_2$ 2 mM and ADP 0.16 mM. Enzymatic reaction was stopped with a MIX-Stop solution ($FeCl_3$ 2.42 % in weight, TCA 1.45 % in weight and HCl 1.82 N). After centrifugation (4500 g, 15 min), γ-glutamyl-hydroxamate was read at 540 nm against a gamma-glutamyl-hydroxamate standard curve performed in exactly the same conditions than the experimental samples.

Glutaminase activity in intestinal mucosa was measured in a phosphate 150 mM - Tris 50 mM buffer containing EDTA 1mM and Triton X100 0.05% (pH 8.0) after homogeneization by sonication, pre-incubation of the homogenates for 20 min at 37°C and 5 min incubation at 37°C with L-glutamine (30 mM) or without L-glutamine (blank value). For glutaminase activity measurement in liver homogenate, 2 mM NH_4Cl was added to the incubation medium. The reaction was stopped with HCl 2 N and the pH was adjusted to 8.0. After centrifugation (10 min, 12000 g), aliquotes of the supernatants were incubated for 30 min at room temperature in the presence of glutamate dehydrogenase (1.3U) dissolved in a Tris 50 mM buffer solution (pH 8.0) containing 15 mM NAD, 1.25 mM ADP, 10 mM EDTA, 25 % (v/v) hydrazine (pH 8.0). The NADH formed in the reaction was read at 340 nm and the quantity of L-glutamate formed was determined using a L-glutamate standard curve obtained in exactly the same conditions that for the experimental samples.

3.6. Measurement of gene expression of glutaminase and glutamine synthetase activity

After extraction and quantification of total RNA, synthesis of first strand complementary DNA (cDNA), real time PCR was carried out as previously described (29). Sequence of PCR primers used were: 5'-GAAGGGCTACGCTGCAAGAC-3' (up) and 5'-TTCCACTCGGGTAACTCTTCTACA-3' (down) for glutamine synthetase (NM-017073-3), 5'-CGACTTGGTGACCTGCTTTTCT-3' (up) and 5'-TTCAGGGCCGTGGTGAAC-3' (down) for hepatic

glutaminase (NM-138904-1) and 5'-ACCGCCATTAGCCAAGGTT-3'(up), 5'-CACAGACATGGTTGGGATACTAGATT-3' (down) for renal glutaminase (NM-012569-2) and 5'-CGGAAGGGCACCACCAGGAG-3' and 5'-CACCACCACCCACGGAAACG-3' for 18S. Gene expression was determined using $2^{-\Delta Ct}$ formula where 2 represent an optimum efficiency of the PCR and $\Delta Ct = Ct_{target\ gene} - Ct_{18S}$. PCR efficiency was determined in each plate using a serial dilution of reverse transcribed RNA.

3.7. Protease activity and peptides contents

Protease activities and peptide contents in the small intestine luminal fluid were assessed by mixing 12.5 mg of intestinal luminal content with 250 µL of a borate buffer (pH 7.5) containing 171 mM boric acid, 7.3 mM disodium tetraborate, 10 mM $CaCl_2$ and 43 mM NaCl. Then, protease activities and peptide amounts were determined by incubating for 1h at 37°C respectively 0.025 mg and 1.5 mg luminal content in 50 µL borate buffer in the presence or absence of 0.48 mg purified casein (Sigma-Aldrich INC., St Louis, USA). The reaction was halted with 50 µL of a TCA solution (7.06% in weight) and the samples were centrifuged at 12,000 g for 5 min. Then 20 µL supernatant were mixed with 20 µL of a NaOH solution (0.3M) and peptides released from casein were assayed in the supernatant against a bovine serum albumin (BSA) standard curve prepared exactly the same way that the experimental samples using the Biorad kit.

3.8. Assessment of the postprandial kinetics of dietary N

The ^{15}N-enrichment and total N in digesta, tissues, plasma and urine were measured by elemental analyzer isotopic ratio mass spectrometry (EA-IRMS) after freeze-drying and separation of N fractions (plasma urea, AA and protein) in the sampled pools as previously described (30). The ^{15}N-enrichment was measured by IRMS (Isoprime, GV Instruments, Manchester, UK) coupled to the EA (Euro Elemental Analyser 3000, EuroVector, Redavalle, Italy). Urea concentrations were determined using a commercial kit (Bio-Mérieux, Marcy l'Etoile, France). Tissue dry matter (DM) was determined after freeze-drying of the tissue and served to calculate tissue hydration. The total protein content (g) of tissues was determined as P = TM x %DM x %N x 6.25 / 1000 where TM refers to dry tissue mass (g), %N refers to percentage of nitrogen and %DM refers to percentage of DM. The incorporation of dietary N (% of ingested) in samples was calculated as $N_{inc} = [(E_{sample}-E_{basal})/(E_{meal}-E_{basal}) \times N_{total}] / (N_{ing} \times 100)$ where E refers to enrichment (Atom%) and N_{ing} refers to ingested nitrogen. The total deamination (% of ingested) was calculated as Deamination = $N_{exo\ urine}$ + ([plasma urea] x TBW / 0.92 x ($E_{plasma\ urea\ 5h}$ - $E_{plasma\ urea\ basal}$)/(E_{meal} - $E_{plasma\ urea\ basal}$)), where $N_{exo\ urine}$ refers to nitrogen incorporation in urine (mmolN), TBW refer to total body water (g), [plasma urea] the plasma urea concentration and E enrichment (Atom%).

3.9. Hormones, glucose and amino acid concentrations

Plasma leptin, insulin and glucagon concentrations were analyzed in duplicates using a rat endocrine panel (Linco Research, St-Charles, MI, USA) on a Bioplex 200 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Plasma glucose concentrations were determined using a commercial kit (Bio-Mérieux, Marcy l'Etoile, France).

Amino acid concentrations were determined in plasma, urine, gastric and luminal content after acid precipitation (5% trichloroacetic acid for plasma and urine and 10% trichloroacetic acid for gastric and luminal content), centrifugation and supernatant collection. The supernatants were transferred to 10 kDa cut-off filter (Microcon Ultracel YM-10 Regenerated Cellulose 10,000 MWCO) and centrifuged for 5,000g at 4°C during 40 min. Amino acids were quantified in the filtrate using an automatic amino acid analyzer (L-8800, Hitachi, Tokyo, Japan) with ninhydrin reactant post-column reaction as previously described (31). Amino acids were separated by HPLC using an Hitachi ion exchange resin column with a sample injection volume between 0.1 and 100 µL, a flow rate between 0.05 and 1.0 mL/min and a column temperature between 20 and 70°C. Amino acids were treated with o-phtalaldehyde (OPA) before detection. Finally, 3-methyl-histidine concentrations in urine were determined using the amino acid analyzer Hitachi L-8800. In addition, concentration of creatinine in urine was determined using the spectrophotometric method of Jaffe by the "Creatinine test Wako kit" (Wako Pure, Chemicals, Osaka, Japan).

3.10. Statistics

Data are expressed as means +/- standard deviation (SD). The effects of MSG supplementation were analyzed by a 1-way ANOVA (SAS 9.1, SAS Institute, Cary, USA). Differences were considered significant at $P<0.05$.

4. RESULTS

4.1. Food intake, body weight and body composition

After 15d of dietary adaptation, no significant differences were found between C and MSG-C groups for initial and final weight and weight gain (Table 2). Food intake and food efficiency ratio (weight gain/energy ingested) were not significantly affected by MSG supplementation. The body composition was not influenced by the diet excepted for the gastrocnemius muscle mass which was slightly reduced (-4% for MSG-C vs C P=0.01). The absence of MSG supplementation effect on body composition was consistent with the absence of significant difference in plasma leptin. Indeed, plasma leptin concentration was 694 +/- 99pM and 637 +/- 91pM for C and MSG-C rats respectively. For these results, data from MSG-C and MSG-A rats were pooled because these data mainly reflected 2-wk meal ingestion effect and not adaptation effect.

4.2. Gastro-intestinal transit and luminal intestinal proteases activity

Two parameters of gastric emptying rate were measured following meal ingestion: (1) time-course of gastric content masses and (2) $^{13}CO_2$ excretion in expired breath after an oral dose of ^{13}C sodium acetate. Kinetic of the gastric content was not influenced by MSG

Table 2. Effect of MSG supplementation on growth, dietary consumption and body composition after ingestion of test meal containing NaCl (C) or MSG in chronic condition (MSG-C) in rats (n=76)

	C	MSG-C	Stat effect[1]
Weight			
Initial weight (g)	353 ± 13	354 ± 15	NS
Final weight (g)	416 ± 20	423 ± 15	NS
Weight gain (g/15day)	63.5 ± 16.1	68.7 ± 16.0	NS
Consumption			
Food intake (kcal)	1325 ± 105	1366 ± 144	NS
Food efficiency (g/kcal)	5.1 ± 1.3	5.6 ± 1.2	NS
Tissue masses (g)			
Adipose tissues			
Mesenteric	9.38 ± 1.52	10.08 ± 1.69	NS
Perirenal	12.53 ± 2.56	13.71 ± 3.00	NS
Epididymal	12.58 ± 1.97	12.45 ± 2.58	NS
Lean tissues			
Small intestine	3.51 ± 0.52	3.38 ± 1.08	NS
Liver	12.50 ± 1.16	12.77 ± 1.18	NS
Stomach	1.66 ± 0.12	1.66 ± 0.12	NS
Kidney	2.26 ± 0.18	2.28 ± 0.17	NS
Gastrocnemius muscle	1.42 ± 0.09 [a]	1.36 ± 0.09 [b]	P=0.01

Results are expressed as means ± SD,[1] One way ANOVA; NS: not significant, Means in a row with different letters are significantly different (P<0.05, Tukey post-hoc test)

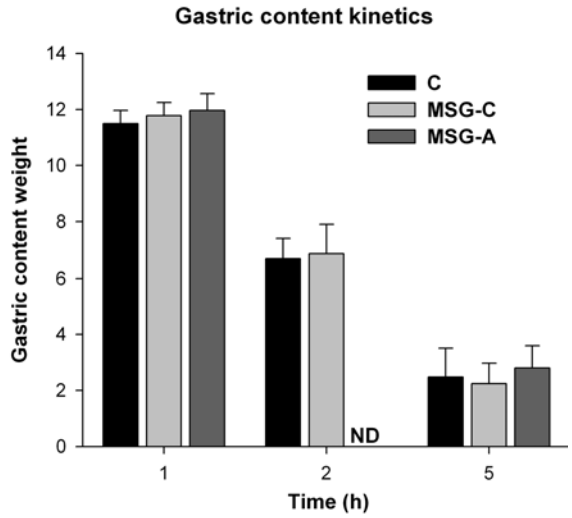

Figure 2. Effect of MSG supplementation on gastric content kinetics 1h, 2h and 5h after the meal (n=76) after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats. Results are expressed as means +/- SD. ND: Not determined. Bars with different letters are significantly different (P<0.05, Tukey post-hoc test).

supplementation (Figure 2). This was confirmed using the ^{13}C-sodium acetate/$^{13}CO_2$ test which showed no significant difference between MSG-supplemented groups and control group (data not shown). These results were represented as tracer appearance in expired breath. Areas under the curve reached 282 +/- 122, 337 +/- 200 and 338 +/- 104 Atom% Excess/5h for C, MSG-C and MSG-A rats, respectively. The progression of the meal in the small intestine tended to be lower after MSG acute supplementation (-15% with MSG-A vs MSG-C, P=0.06), without any significant impact in overall oro-fecal transit time. Oro-fecal transit time was 497 +/- 88 min, 545 +/- 84 min and 559 +/- 89 min for C, MSG-C and MSG-A rats respectively. Moreover MSG supplementation displayed no significant effect on luminal intestinal proteases activity and peptides. Luminal intestinal proteases activities were 4.1 +/- 1.0 mg peptides released/mg of luminal content/h for C group and 4.2 +/- 1.3 and 5.0 +/- 0.9 mg/mg of luminal content/h for MSG group in chronic and acute conditions, respectively with no significant difference between experimental groups. Peptides in the intestinal lumen represented 13.40 +/- 2.55 mg/g of luminal content for C group and 13.65 +/- 2.54 and 11.08 +/- 4.23 mg/g of luminal content for MSG group in chronic and acute conditions respectively with no significant difference.

4.3. Amino acid and enzymes related to glutamine metabolism

Not surprisingly glutamate was found at relatively high concentrations in the gastric contents after MSG ingestion (Figure 3A). Furthermore glutamate concentration in intestinal contents was affected by MSG supplementation in acute and chronic situation (+44% MSG-A vs C and +40% MSG-C vs C) 1h after the meal (Figure 3B). These significant differences were not observed 2h and 5h after the meal. Interestingly, the MSG supplementation affected glutamine concentration in plasma 2h after the meal without effect on any other amino acid concentration (Figure 4). Indeed, circulating glutamine concentration was 19% higher after MSG-C supplementation vs. C 2h after the meal. To gain insight into this increase, we determined glutamine urine concentration and enzymatic activities related to glutamine metabolism (glutaminase and glutamine synthetase) in liver, intestinal mucosa and muscle. Glutamine concentration was 41% higher after MSG-A supplementation vs. C in urine 5h after the meal but MSG-chronic supplementation failed to modify this concentration (Figure 5). Glutamine synthetase activity in muscle and glutaminase activity in intestinal mucosa were modified by MSG supplementation 1h after the meal but no effects on liver enzymatic activities were recorded (Figure 6). In fact, glutamine synthetase activity in muscle was significantly higher after MSG-A supplementation than in the MSG-C group. Glutamine synthetase in intestinal mucosa was equally low in the three experimental groups. Glutaminase activity in intestinal mucosa was respectively 17% lower after MSG-A supplementation and 21% lower after MSG-C supplementation than in the control group. When gene expression of glutaminase and glutamine synthetase was determined in liver, muscle and intestinal mucosa, no significant effect of MSG supplementation was recorded (data not shown).

4.4. Dietary and whole body protein metabolism

Five hours after the meal, dietary nitrogen transit was similar in stomach, intestinal, cecum and colon content when comparing MSG supplementation and control experimental groups (data not shown). The incorporation of dietary nitrogen into plasma amino acids and in tissues was not significantly affected by MSG acute or chronic supplementation. Five hours after the meal the dietary nitrogen total losses were similar in the three experimental groups of animals (data not shown). MSG chronic

Table 3. Effect of MSG supplementation on tissue protein content 1h or 5h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=56)

g protein /100 g tissue	C	MSG-C	MSG-A	Stat effect[1]
1h after the meal [2]				
Intestinal mucosa	13.58 ± 0.85 [a]	15.36 ± 2.17 [b]	13.93 ± 1.88 [a]	P<0.05
5h after the meal [3]				
Intestinal mucosa	12.43 ± 2.40	12.07 ± 0.73	12.03 ± 1.18	NS
Liver	20.52 ± 0.79	20.70 ± 1.53	21.22 ± 1.25	NS
Stomach	19.75 ± 2.57	19.48 ± 2.40	18.84 ± 0.82	NS
Kidney	19.69 ± 0.44	19.89 ± 0.86	20.28 ± 0.89	NS
Muscle	22.65 ± 0.41	21.97 ± 1.07	22.37 ± 1.06	NS

Results are expressed as means ± SD, [1] One way ANOVA; NS: not significant, Means in a row with different letters are significantly different (P<0.05, Tukey post-hoc test), [2] Determined by spectrophotometer, [3] Determined by EA-IRMS with total nitrogen

Table 4. Effect of MSG supplementation on fractional synthesis rate (FSR) and absolute synthesis rate (ASR) of tissue proteins measured 1h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=24)

	C	MSG-C	MSG-A	Stat effect [1]
Stomach				
FSR (%/d)	66 ± 12	56 ± 9	68 ± 14	P<0.05
ASR (gP/d)	0.22 ± 0.05	0.19 ± 0.04	0.22 ± 0.05	NS
Intestinal mucosa				
FSR (%/d)	190 ± 25	171 ± 20	174 ± 28	NS
ASR (gP/d)	0.90 ± 0.22	0.78 ± 0.13	0.77 ± 0.09	NS
Liver				
FSR (%/d)	88 ± 12	94 ± 12	97 ± 9	NS
ASR (gP/d)	2.27 ± 0.36	2.40 ± 0.34	2.53 ± 0.21	NS
Total muscle				
FSR (%/d)	18.1 ± 2.3	18.1 ± 2.0	18.7 ± 2.1	NS
ASR (gP/d)	8.05 ± 1.49	7.72 ± 0.99	8.04 ± 0.99	NS

Results are expressed as means ± SD,[1] One way ANOVA; NS: not significant

supplementation led to a significant increase of the intestinal mucosa protein content (+12% in MSG-C vs C P<0.05) 1h after the meal (Table 3). This effect was however only observed shortly after the meal since it was not observed 5h after the meal.

Five hour after the meal, the rates of protein degradation were not modified by the MSG supplementation as determined by the 3-methyl-histidine concentration in urine. Before the meal, urinary concentrations of 3-methyl-histidine concentration ranged from 30.9 to 33.6 nmol/g creatinine, and after the meal from 41.7 to 42.7 nmol/g creatinine with no difference between groups. Tissue fractional synthesis rates (FSR) and absolute synthesis rates (ASR) were determined after ^{13}C-valine injection 20 min before rats sacrifice. The FSR and ASR of intestinal mucosa, liver and skeletal muscle were not significantly influenced by MSG supplementation (Table 4). A chronic, but not an acute MSG supplementation significantly reduced FSR in stomach 1h after the meal (Table 4).

4.5. Glucose and insulin

MSG supplementation had no significant effect on glycemia. Glucose concentrations were 1.91 +/- 0.18 g/L for C group and 1.82 +/- 0.14 and 1.87 +/- 0.20 g/L 1h after the meal for MSG group in chronic and acute conditions, respectively (NS). Two hours after the meal, it was 1.86 +/- 0.15 and 1.96 +/- 0.14 g/L for C and MSG-C groups respectively (NS). Finally, 5h after the meal it was 1.70 +/- 0.24 g/L for C group and 1.66 +/- 0.42 and 1.86 +/- 0.17 g/L for MSG-C and MSG-A groups respectively (NS). Insulinemia (Figure 7A) was significantly affected by MSG supplementation 1h after the meal (40% higher in MSG-C vs C; P<0.04). However, 2h and 5h after the meal, no significant difference in insulinemia were recorded when comparing the 3 experimental groups. In contrast, glucagon concentration was not affected by MSG supplementation 1h, 2h and 5h after the meal (Figure 7B).

5. DISCUSSION

This study was performed to evaluate the influence of MSG supplementation on food intake, body composition, gastrointestinal transit, protease activities in the small intestine content, endocrine parameters, glutamine-related enzymatic activities, aminoacidemia and postprandial protein metabolism in rats. To test the importance of adaptation to MSG on the response, different groups of animals were used either receiving MSG for the first time (acute conditions) or after a 2-wk adaptation (chronic conditions).

MSG-chronic supplementation had no effect on food intake, body weight, adipose tissue mass, dietary nitrogen incorporation in gastrointestinal and other tissues and protein synthesis in intestinal mucosa, liver and muscles. Some animal studies have reported that MSG is able to induce overweight and/or obesity (2, 3). For instance, it is worth noting however that most of the evidence on weight gain was provided from animals that were injected subcutaneously with MSG at neonatal age with large dose of MSG i.e. in a situation which does not

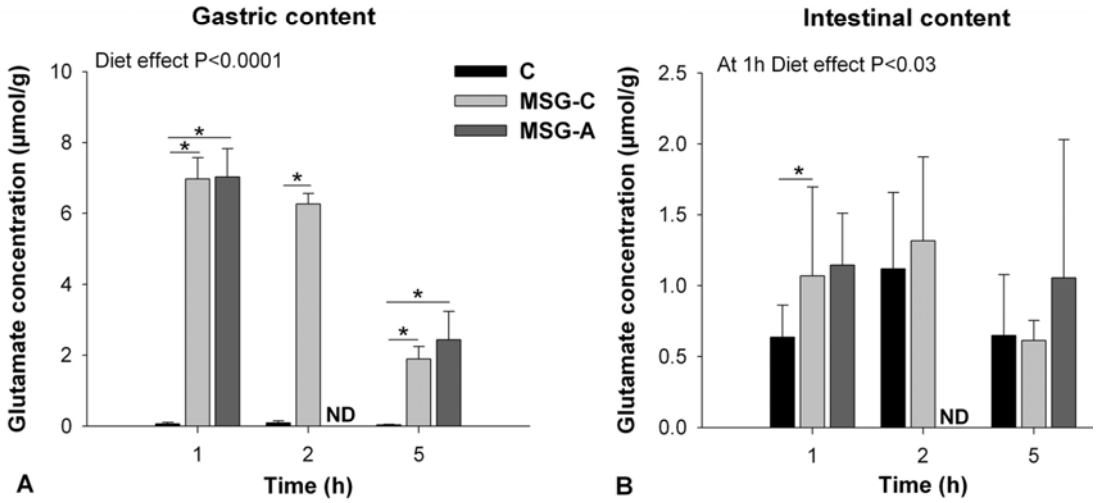

Figure 3. Effect of MSG supplementation on glutamate concentration in gastric (A) and intestinal contents (B) 1h, 2h and 5h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=76). Results are expressed as means +/- SD. ND: Not determined. *: significant difference between groups at each time (P<0.05, Tukey post-hoc test).

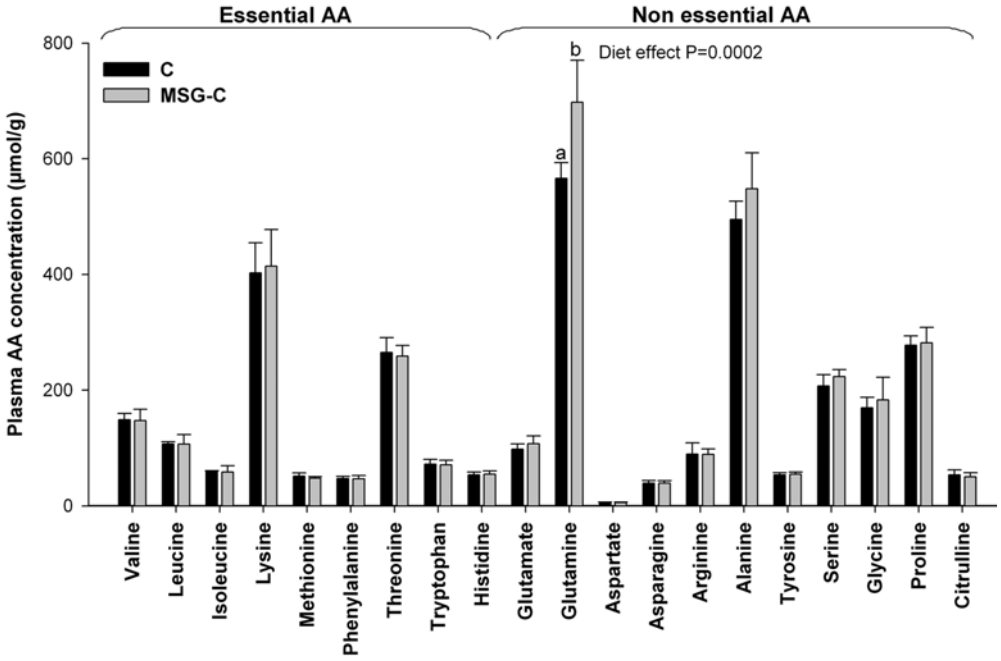

Figure 4. Effect of MSG supplementation on plasma concentrations of amino acids 2h after ingestion of test meal containing NaCl (C) or MSG in chronic condition (MSG-C) in rats (n=20). Results are expressed as means +/- SD. Circulating amino acid concentrations were not determined for MSG-A supplemented rats. Bars with different letters are significantly different (P<0.05, Tukey post-hoc test).

allow the intestine to catabolize the dietary glutamate during its transfer from the lumen to the portal blood. Interestingly and in contrast, MSG dissolved in the drinking water was found able to reduce weight gain, body fat mass, and plasma leptin concentrations in rats (32). Since these changes were not concomitant with modified food intake, it was proposed that increased energy expenditure would explain the reduction in weight gain and body fat mass (33). The mechanism underlying MSG-induced obesity in non physiological situation remains unclear and controversial. Some studies have reported a lower lipolytic activity in adipocytes as well as higher

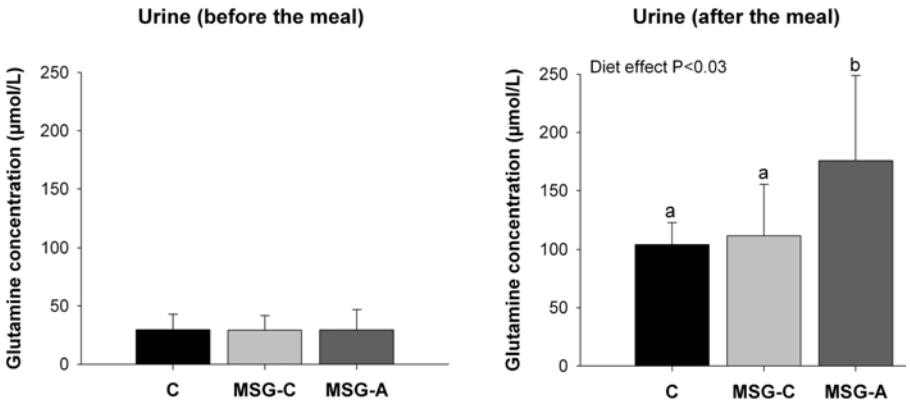

Figure 5. Effect of MSG supplementation on urine glutamine concentration before the meal and 5h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=24). Results are expressed as means +/- SD. Bars with different letters are significantly different (P<0.05, Tukey post-hoc test).

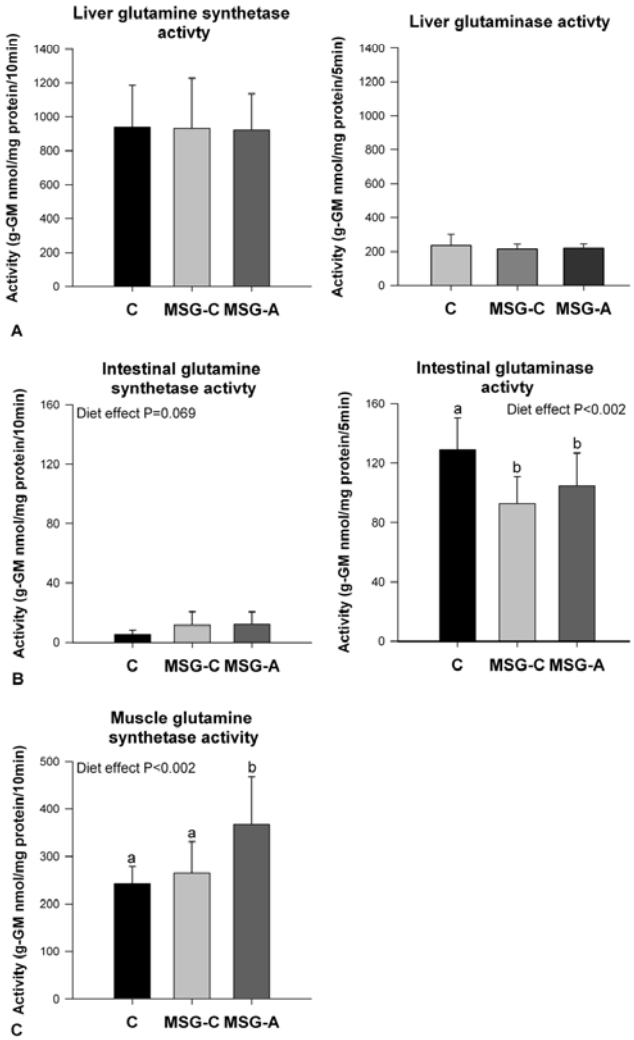

Figure 6. Effect of MSG supplementation on glutaminase and glutamine synthetase activity in liver (A), intestinal mucosa (B) and muscle (C) 1h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=24). Results are expressed as means +/- SD. Bars with different letters are significantly different (P<0.05, Tukey post-hoc test).

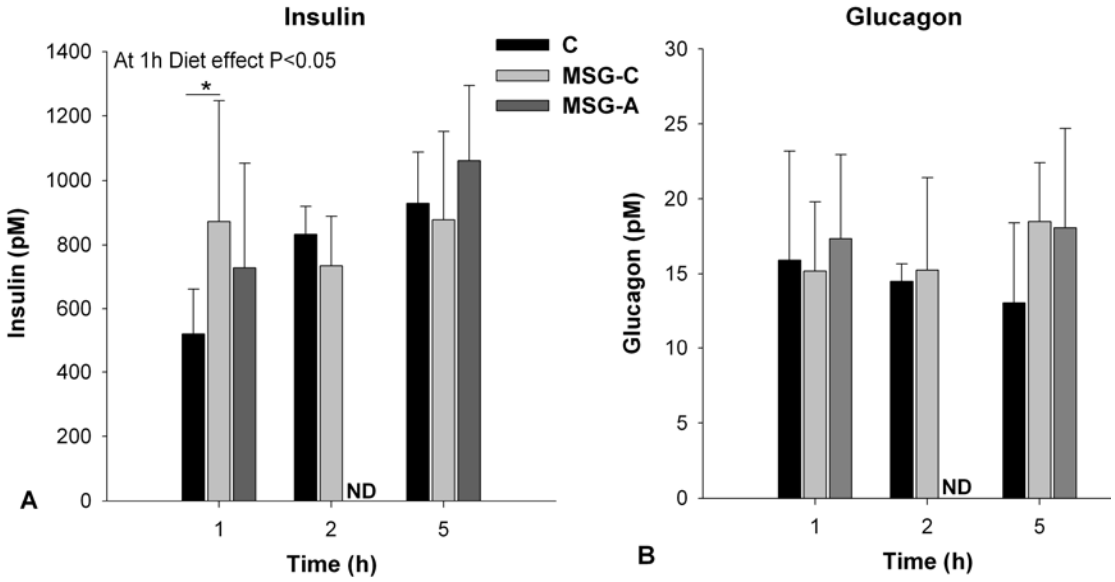

Figure 7. Effect of MSG supplementation on insulin (A) and glucagon (B) concentration 1h, 2h and 5h after ingestion of test meal containing NaCl (C) or MSG in chronic (MSG-C) and acute condition (MSG-A) in rats (n=76). Results are expressed as means +/- SD. ND: Not determined. *: significant difference between groups at each time (P<0.05, Tukey post-hoc test).

insulin sensibility in adipose tissues from MSG-administrated animals (34-36). In addition Hermanussen et al. have reported that chronic hyperglutamataemia exerts deleterious effects on the arcuate nucleus neurons, thereby disrupting the hypothalamic signaling cascade of leptin action and causing hyperphagia, obesity and hyperleptinaemia (37, 38). In supranutritionnal dose (i.e. 5g MSG per day in young rats), this compound has been reported to exhibit significant neuronal toxicity and to have an effect on appetite regulation (1).

Our study clearly demonstrates that MSG chronic supplementation increased circulating glutamine concentration. This increase was specific since glutamine was the only amino acid to be modified. Glutamic acid supplementation has been previously shown to noticeably enhance glutamine arterial concentration (39-41); although in these experiments, glutamate supplementation was at much higher doses than in the present study. Intestine represents a major site for glutamine catabolism in the body whatever its luminal or circulating origin through glutaminase activity (42, 43). The glutaminase isoform expressed in intestinal epithelial cells has been shown to be strongly inhibited by one of its product i.e. glutamate (44). In isolated enterocytes, glutamate is able to markedly reduce both glutamine utilization and oxidation indicating a sparing effect of glutamate on glutamine catabolism (24). Accordingly, the present study showed that blood glutamine increase was concomitant with a marked decrease of glutaminase activity in the intestine. Decrease of glutaminase activity in intestine together with the glutamate sparing effect on glutamine utilization are likely to play an important role in the increase of circulating glutamine concentration. In contrast, intestinal glutamine synthetase activity remained low after MSG chronic supplementation. Glutamine synthetase is known to be expressed at low level in intestine in contrast with the situation in liver and muscles (45). In these latter tissues, glutamine synthetase activity was not modified by MSG-chronic supplementation but MSG-acute supplementation resulted in a significant increased of this activity. The interpretation of this result remains difficult since both stimulating and suppressive effect of glutamine on glutamine synthetase activity in muscles have been reported (46, 47). Our study also showed a significant increase of the concentration of glutamine in urine after acute MSG supplementation suggesting an increased glutamine glomerular filtration which remains to be tested in future work.

Other effects of dietary MSG supplementation were a decreased protein synthesis in stomach, a transient increased protein mass in the intestinal mucosa and a slightly decreased gastrocnemius muscle mass. These changes in protein metabolism could be related to the change in glutamine metabolism and in insulin but remain to be more precisely analyzed. Indeed, the increased glutamine concentration in circulating blood is likely to be involved in the transient increased insulin concentration in blood following MSG-chronic supplementation and test meal. Glutamine is well known to be rapidly taken up and metabolized by islet cells with enhancing effect of leucine-induced insulin secretion (14, 48). In contrast there was no significant change of the small intestine luminal protease activities and of the oro-faecal transit time indicating that in our experimental conditions, modifications of pancreatic exocrine secretion and of intestinal peristaltism were not detectable. The accumulation of glutamate in the stomach following chronic supplementation and test meal was, as expected, tremendous and still visible five hours after the

meal. In our initial working hypothesis, since glutamate has been shown to produce tonic contractions of rat fundus (10), we envisaged that supplemental MSG through sharp increase of its gastric concentration (before glutamate release from alimentary proteins through the action of pepsin in the stomach) could have modify gastric emptying rate. However, such an effect was not detected in our study.

In conclusion an important finding of this study was the effect of MSG supplementation on plasma glutamine concentration which likely results –at least in part- from a sparing effect of glutamate on glutamine utilization in intestinal epithelial cells. This increase capacity of MSG to increase circulating glutamine may prove to be useful in situation of decreased circulating glutamine concentrations like the one occurring in some hypercatabolic situations (49) and require new experiments to be tested. The present work also demonstrated that chronic MSG supplementation used at the relatively high dose of 2% did not result in measurable change in food ingestion, weight gain and adiposity in the rat arguing against a promoting effect of MSG towards overweight and/or obesity.

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Abbreviations: MSG: monosodium glutamate, GC-MS: gas chromatography mass spectrometry, GC-C-IRMS: gas chromatography combustion isotopic ratio mass spectrometry, EA-IRMS: elemental analyzer isotopic ratio mass spectrometry

Key words: Monosodium Glutamate, Glutamine, Protein Metabolism, Digestion, Insulin

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