EFFECTS OF CHRONIC DIETARY EXPOSURE TO MONOSODIUM GLUTAMATE ON FEEDING BEHAVIOUR, ADIPOSITY, GASTROINTESTINAL MOTILITY AND CARDIOVASCULAR FUNCTION IN HEALTHY ADULT RATS

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Running title: Monosodium glutamate: effects in adult rat

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ABSTRACT

Background: Monosodium glutamate (MSG) is a flavor-enhancer widely used as a food additive. However, its safe dietary concentration and its toxicity, including its possible implication in the recent metabolic syndrome pandemia, is still a controversial issue. Therefore, a deep knowledge of its effects upon regular dietary use is needed. Our aim was to evaluate the effects of chronic exposure to MSG on feeding behavior, abdominal fat, gastrointestinal motility and cardiovascular function in rats. **Methods**: Two groups of adult male Wistar rats were used: control and treated with MSG (4 g L^{-1} in drinking water) for 6 weeks. Different functional parameters were determined and the histological structure was analyzed in tissues of interest. **Key Results**: Compared to control animals, chronic MSG increased water intake but did not modify food ingestion or body weight gain. Neither the abdominal fat volume nor the fat fraction, measured by magnetic resonance imaging, was modified by MSG. MSG did not alter general gastrointestinal motility, but significantly increased the colonic response to mechanical stimulation. It slightly reduced endothelium-dependent relaxation in aorta, without significantly modifying any other cardiovascular parameters. No significant histological alterations were detected in salivary glands, intestinal wall, aorta, heart and kidney. **Conclusions & Inferences**: Chronic treatment with MSG in the adult rat increased water intake. This supports its potential to improve acceptance of low-fat regimens and to increase hydration in the elderly and sportspeople, often at risk of dehydration. Changes in colonic contractility and cardiovascular function could have some long-term repercussions warranting further research.

Keywords: monosodium glutamate, MSG, fat, gastrointestinal motility, cardiovascular function.

KEY MESSAGES

- MSG is a widely-used food additive and flavor-enhancer earlier suggested to induce toxic effects and to be involved in the induction of obesity and metabolic syndrome. Due to its low oral bioavailability, this has recently been questioned, and MSG possible therapeutic potential (and safety) is currently the focus of intense research.
- Thus, we analyzed, in rats, the effects of chronic exposure to MSG in drinking water. We used magnetic resonance imaging to measure abdominal fat, conventional recordings to assess cardiovascular function, X-rays and intracolonic pressure recordings to evaluate gastrointestinal motility, and conventional histological techniques to detect structural alterations in tissues of interest.
- We confirmed that MSG included in drinking water enhances drinking, does not alter body weight gain or abdominal fat distribution, increases colonic contractility *in vivo* and slightly reduces endothelium-dependent relaxation in aorta, without producing any remarkable histological alteration.
- MSG might be useful to increase hydration or colonic movement, but should be used with care in patients suffering or prone to suffer cardiovascular diseases or colic gut pain.

Monosodium glutamate (MSG) is the sodium salt of L-glutamic acid (or glutamate), an aminoacid naturally found in many foods (1,2). Glutamate participates in different functions, including synthesis of neurotransmitters (gamma-aminobutyric acid, nitric oxide), collagen, glutathione and folic acid (3,4), immunomodulation (5), and excitatory neurotransmission (6).

MSG produces a special taste called umami ("delicious"), and it has been suggested to favor protein ingestion, in the same way as sweet taste induces carbohydrate intake or salty taste induces that of minerals (1,2). MSG has long been used as a flavor-enhancer in the food industry and it is currently a widely consumed food additive, also under other names (7,8). Interestingly, the clinical use of MSG as a food flavor-enhancer in low-fat or low-salt diets (for obese and/or hypertensive patients) has recently been proposed (9), and it could also be useful to increase food palatability and appetite in aged and malnourished patients (10-12).

Although some short-term transient and generally mild symptoms may be induced in some sensitive individuals after acute intake of extremely high doses of MSG without food (13,14), more important from a public health point of view, and still under high scientific debate, are the effects of regular MSG dietary use. Average daily MSG intake has been estimated as 0.55 $gday^{-1}$ (13) but this figure might be too conservative, due to the wide consumption of processed food with added MSG, and in Asian people it may reach 3-4 $gday^{-1}$ (8,15,16).

It has been suggested that chronic ingestion of high amounts of MSG as a food additive could contribute to the current epidemics of metabolic syndrome and obesity (8,16,17). MSG may in fact induce obesity in rodents after parenteral administration to neonates, due to neurotoxic effects in key brain regions controlling appetite and adiposity (18,19,20). However, although this is a useful model in obesity studies, it does not reproduce the effects of MSG added to the

diet, since the oral bioavailability of MSG is lower than 5% (21), which reduces the likelihood of significant systemic effects, including neurotoxicity. Therefore, normal dietary MSG use is unlikely to influence energy intake, body weight or fat metabolism, and the proposed link between MSG intake and weight gain is likely explained by co-varying environmental factors (22). Nevertheless, actions of MSG on the alimentary canal either direct (glutamate is essential to maintain gut integrity and function: 21) or mediated via the activation of (vagal, gustatory) nerve afferents (23) or after the release of enteric hormones (24) might be significant. Furthermore, the effects of the proportional sodium content in MSG, which could have a role in triggering cardiovascular or renal alterations, should not be disregarded.

Therefore, the aim of the present work was to study, in healthy adult rats, the effects of MSG chronic dietary ingestion on different functional parameters, particularly those related to the gastrointestinal and cardiovascular systems, and the possible occurrence of histological alterations in some tissues of interest. The effect of MSG ingestion on abdominal fat was also studied, using magnetic resonance imaging.

MATERIALS AND METHODS

The experiments were designed and performed in accordance with the European and Spanish legislation on care and use of experimental animals (EU Directive 2010/63/EU for animal experiments; R.D. 53/2013), and were approved by the Ethics Committees at both Universidad Rey Juan Carlos (URJC) and Hospital General Universitario Gregorio Marañón (HGUGM).

Animals and protocol

Thirty-six 10-12 weeks old male Wistar rats (250-300 g, at the beginning of the experiment) were used. Animals were housed in cages (2-4 animals each cage) and were maintained in environmentally controlled conditions (temperature 20°C, humidity 60%) with 12 h light/darkness cycles. Standard diet (2014 Tekland Global 14% Protein Rodent Maintenance Diet, Harlan-Ibérica, Barcelona, Spain) (diet composition is shown in table 1), and tap water were provided *ad libitum*. Seventeen rats were supplied with MSG (4 gL^{-1}) in their drinking water for 6 weeks. This dose of MSG corresponds to approximately 0.45 $gkg^{-1}day^{-1}$, which in turn corresponds to 5.1 $gday^{-1}$ for a 70 kg man (25).

During treatment, general health and weight were regularly recorded. Food and water intake were recorded 3 times per week, in a combined manner for the 2-4 rats in each cage; at least 3 cages were included in each group. Nine control rats and 17 animals treated with MSG were used to evaluate body weight, food and water intake, as well as the effects of MSG on cardiovascular function, colonic motor function and histology. Magnetic resonance studies (to analyze abdominal fat) and radiographic analysis of gastrointestinal motor function were performed in 5 rats per experimental group.

Abdominal fat volume and fat fraction: Magnetic resonance imaging (MRI)

Images were obtained at two time points: baseline (before administration of MSG) and endpoint (after treatment). Abdominal fat volume and fraction were determined in a BrukerBiospec 70/20 scanner. Animals were anesthetized with sevofluorane (5% for induction and 3% for maintenance in 100% O_2) and placed in an MRI-adapted stereotaxic holder with a water-circulating blanket to maintain body temperature. Respiration and body

temperature were continuously monitored, and to avoid breathing artifacts, the image acquisition was gated to the respiratory signal.

MRI acquisition protocol included shimming with FASTMAP, a T2-weighted RARE sequence in axial and coronal orientation with and without fat suppression (matrix size = 256x256, FOV = 10x7 cm, 23 slices, slice thickness = 1 cm, TR/TE= 2067/12 ms, RARE factor = 8, NA =3, acquisition time = 4 m 10 s). Anatomical images were acquired using a linear volume coil.

Calculation of abdominal fat volume was made using 4 procedures, i.e., slice selection, fat extraction, segmentation and voxel counting. For this, we determined a difference image from the studies with and without fat suppression. This new image provides the real information on the spatial distribution of fat. A thresholding-based segmentation obtained the abdominal volume of fat in a coronal section located at half the height of the kidney.

After a dedicated shimming (water FWHM lower than 30 Hz), non-localized proton spectroscopy was performed (PRESS technique, CHESS water suppression, 6 mm^3 voxel size, TR/TE=2500/16 ms). Spectroscopy studies were acquired using a 1H surface array.

The fat fraction was determined as an estimation of the total lipids/water ratio. Quantification was performed using the software MestRe Nova (MestrelabResearch). For this, we corrected phase artifacts and baseline of each spectrum, and performed a global fit to a Lorentzian-Gaussian sum. Lipid content typically is expressed relative to the water or creatine peak used as internal standards. As a result, the areas under water and fat peaks were measured to determine their ratio (fat/water).

Gastrointestinal motor function

At the end of treatment (end of week 6), two different kinds of studies were performed. General gastrointestinal motility was evaluated by radiographic techniques (26). Briefly, barium contrast (2 gml^{-1}; 2.5 ml) was administered by gavage and serial X-rays were taken (0-8 h after contrast) with a digital X-ray apparatus (Siemens; 60kV, 7mA) coupled to NPG Real DVD Studio II software. Animals were not fasted and were conscious during X-ray sessions. No anesthesia was used, so that natural gastrointestinal motility was not altered. For each X-ray exposure (60 ms) each animal was briefly immobilized in prone position using an adjustable hand-made transparent plastic tube to which they were previously accustomed. A researcher, blind to the treatment received by the animals, semiquantitatively analyzed the X-rays. In each temporal point, a value of 0-12 points was given to each gastrointestinal region as previously described (26).

Colonic motor function was analyzed in more detail as shown in a previous work(27). Under light anesthesia (ketamine 50 $mgkg^{-1}$, i.p., Imalgene 500, Merial, Lyon, France; and equithesin 2.1 mL kg^{-1}, i.p.), a latex balloon was inserted 7 cm into the colon. This balloon was connected to a pressure transducer (Transpac IV 61HF-0698-03; Abbot, Sligo, Ireland) and a perfusion pump (55-2222; Harvard Apparatus, Holliston, MA, USA), so that the balloon could be inflated and the colon mechanically stimulated. Intracolonic pressure was recorded by means of a Power Lab/8sp (ADInstruments, Oxford, UK). The number of colonic contractions produced at 0, 30 and 60 mmHg was recorded.

Cardiovascular function

For measuring cardiovascular function, we anaesthetized the animals with pentobarbital (50 mg kg^{-1}, ip) (28), which, compared to other anesthetics with distinct effects on cardiovascular

function (29), is considered to have only a modest influence on the cardiovascular system (blood pressure level and its maintenance by the main vasoactive systems) (30). After anesthesia, a catheter coupled to a pressure transducer was inserted into the right carotid artery of the animals for direct measurements of mean arterial blood pressure (MAP) and heart rate (HR) using a PowerLab/4e system (PanLab S.L., Barcelona, Spain). Recording of these cardiovascular parameters lasted for 15 min (31). After blood pressure measurements, the rats were euthanized by decapitation and different preparations were isolated.

For basal cardiac function measurements, hearts were rapidly removed and immediately mounted on the Langendorff set-up for the recording of the following cardiac parameters for 30 minutes: coronary perfusion pressure (CPP), left ventricular developed pressure (LVDP), end diastolic pressure (EDP) and heart rate (HR) (32).

For aorta vascular reactivity measurements, the abdomen was opened by a midline incision, and the aorta was carefully excised and cleaned, with caution taken not to disrupt the endothelium. Transverse vascular rings 3-4 mm long were prepared and mounted in an organ bath. After the equilibration period, the following experiments were carried out: a) aorta contractile function was evaluated by the vessel response to a concentration response curve of phenylephrine 10^{-9} M - 10^{-5} M; b) aorta vasorelaxant function was evaluated by the precontracted vessel (phenyleprine 10^{-6} M) response to a concentration response curve of carbachol 10^{-9} M - 10^{-4} M (endothelium-dependent vasodilation) and/or to a concentration response curve of sodium nitroprusside 10^{-9} M - 10^{-6} M (endothelium-independent vasodilation). Contraction responses of the aorta's ring are expressed as mean absolute values and relaxation responses are expressed as the percentage relaxation of the tone induced by phenylephrine by the concentration-response curves of carbachol or sodium nitroprusside.

Finally, the mesenteric bed reactivity was also measured. For this, the abdominal cavity was opened and the superior mesenteric artery was identified, cleaned of connective tissue and cannulated. The mesenteric vascular bed was then separated from the gut and perfused at a constant flow rate of 5 ml/min, using a peristaltic pump (Gilson S.A.) Mesenteric vascular responses were detected as changes in perfusion pressure (mmHg). The experiments were performed in intact mesenteric beds. The evaluation of the functionality of the vascular bed was carried out following two different procedures: 1) mesenteric bed contractile function (33) was evaluated by a concentration response curve of phenylephrine 10^{-8} M – 8 x 10^{-8} M; 2) mesenteric bed vasorelaxant function was evaluated by the response to the precontracted bed (phenyleprine 50 x 10^{-6} M) to a concentration response curve of carbachol 5 x 10^{-13} M – 5 x 10^{-8} M (endothelium-dependent vasodilatation) and to a concentration response curve of sodium nitroprusside 0.01 - 1000 nM (endothelium-independent vasodilatation) (34). Contraction responses of superior mesenteric artery bed are expressed as mean absolute values (in mmHg) and relaxation responses are expressed as the percentage relaxation of the tone induced by phenylephrine by the concentration-response curves of carbachol or sodium nitroprusside.

Histological analysis

At the end of treatment, samples of submaxilar salivary gland, ileum, colon, aorta, heart and kidney were obtained, fixed in 4% formaldehyde, and embedded in paraffin in the following week. Then, 5 μm sections were cut, stained with haematoxylin-eosyn and observed with a Zeiss Axioskop 2 microscope, equipped with the image analysis system AxioVision 4.6.

Compounds and drugs

MSG was obtained from Productos Químicos Manuel Riesgo SA (SS061/1000). Barium sulphate (Barigraf® AD, Juste SAQF, Madrid, Spain) was suspended in tap water and continuously stirred until administration. Carbachol, sodium nitroprusside and phenylenilephrine (Sigma Chemical Company, Poole, Dorset, United Kingdom) were dissolved in distilled water.

Statistical analysis

Data is presented as the mean values ± SEM. Differences were analyzed using Student's t-test with Welch's correction, or one or two-way ANOVA followed by adequate *post-hoc* tests. Values of $p<0.05$ were considered as statistically significant.

RESULTS

Body weight at the beginning of the experiments was similar in control (week 0 = 286 ± 6 g, n=8) and treated (week 0 = 281 ± 6 g, n=12) groups; weight gain after the six weeks of treatment was 29.3 ± 3.8 and 31.9 ± 2.3 %, reaching a final body weight of 371 ± 16 and 370 ± 7 g, respectively ($p>0.05$). In each group, food intake was not modified throughout treatment. The mean food intake was 19.7 ± 0.3 $gday^{-1}rat^{-1}$in control rats, and 20.7 ± 0.3 $gday^{-1}rat^{-1}$for MSG-treated animals ($p>0.05$). In contrast, water intake was significantly higher in those animals exposed to MSG in drinking water compared to control rats (water: 35.9 ± 0.68; MSG: 42.4 ± 1.01 mL $day^{-1}rat^{-1}$; $p< 0.01$, unpaired *Student's t-test*).

Abdominal fat volume and fat fraction: Magnetic resonance studies

Our results showed an increase in abdominal fat over time in both groups (Fig. 1A-D). The quantitative analysis showed significant differences in fat volume between baseline (control: 1.224 ± 0.211; treated: 0.985 ±0.093 mL) and final images (control: 1.904 ± 0.158; treated: 1.633 ±0.142 mL) ($p < 0.05$). However, these differences were related to time, not to treatment with MSG (Fig.1.E).

Regarding fat fraction, both groups showed a time-dependent decrease in the total lipid/water ratio (Fig.1.F), this change being significant in the control group. Fat fraction at the beginning of the experiments was 0.419 ± 0.071 and 0.305 ± 0.081 in control and treated animals respectively; after the six weeks of treatment it was 0.140 ± 0.045 and 0.134 ± 0.018.

Gastrointestinal motor function

General gastrointestinal motility was radiographically evaluated, as shown in representative images in figure 2A. The analysis of these images did not demonstrate any statistically significant differences due to treatment with MSG in any of the gastrointestinal regions evaluated (Fig. 2A'). Regarding colonic motility, in control animals mechanical stimulation produced an increase in the number of contractions and this increase was significantly much more pronounced in the case of animals treated with MSG (Fig. 2B, B').

Cardiovascular function

As expected, the MAP and HR values in control animals corresponded to normotensive rats and they were similar to those obtained in other studies (35,36,37). MSG treatment for 6 weeks did not significantly modify either the MAP values (mmHg) (control group: 83.6 ± 8.1, n=9; MSG-treated group: 93.7 ± 8.5, n=6; $p > 0.05$, Student's t-test with Welch's correction) or the HR (beats per minute) (control group: 323 ± 14, n=9; MSG-treated group: 344 ± 16, n=6;

p>0.05, Student's t-test with Welch's correction). Likewise, basal cardiac function was similar in animals exposed to MSG in drinking water and control rats: neither the left ventricular function (LVDP, mmHg: 105.6 ± 20.4, n=6, in tap water group *vs* 107.9 ± 6.06, n=6, in MSG-treated group; EDP, mmHg: 0.37 ± 4.8, n=6, in tap water group *vs* 1.1 ± 5.3, n=6, in MSG-treated group), nor the coronary tone (CPP, mmHg: 107.3 ± 10.7, n=6, in tap water group *vs* 100.7 ± 9.7, n=6, in MSG-treated group) were affected by this particular taste enhancer.

Regarding vascular functionality, chronic MSG treatment provoked a slight, although not significant, increase in aorta contractile response (Fig. 3A) and a significant reduction in aorta endothelium-dependent relaxation (Fig. 3B), without affecting aorta endothelium-independent relaxation (Fig. 3C). Finally, in the mesenteric bed (resistance vascular territory), no changes in contractile or relaxant function were observed after MSG chronic treatment (Fig. 3A'-C').

Histological analysis

The histologic structure of mucous and serous acini in the submaxilar salivary gland, ileum, colon, aorta, heart and kidney was evaluated. No remarkable qualitative (Fig. 4) or quantitative (data not shown) modification was observed.

DISCUSSION

Dietary MSG effects on different functional and histological parameters were evaluated in adult rats. Most parameters were not modified by MSG exposure in drinking water, suggesting that chronic ingestion of this additive is safe. However, some alterations were

observed, which could be due to local effects of glutamate (increased colonic contractility) or sodium contribution (altered cardiovascular function).

MSG dose

Adult rats were exposed to MSG at 4 gL^{-1}(0.4% wv^{-1}) in drinking water for 6 weeks. This dose corresponds to approximately 0.45 g $kg^{-1}day^{-1}$, well below doses that have shown different adverse and/or toxic effects (2-4 $gkg^{-1}day^{-1}$) when added to drinking water in rats (38,39,40), but still of clinical interest, since it corresponds to 0.073 g $kg^{-1}day^{-1}$ and 5.1 $gday^{-1}$ for a 70 kg man (25), just above the average daily intake estimated for Asian people (3-4 g day^{-1}) (15,16,17).

Effects on fluid intake

MSG added to drinking water increased daily fluid intake approximately by 18%. This might be due to an osmotic effect induced by the proportional increase in sodium intake in MSG-treated rats (sodium constitutes 12% of MSG), compared to control animals (i.e., thirst stimulation) (41). In rats, fluid intake increased about 50% when a solution with 0.9 % NaCl (3.6 gL^{-1} sodium) was offered for drinking instead of water (42). In our case, although the solution offered for drinking had 0.48 g L^{-1}, still increased fluid intake by 18%, not by the expected 6-7% (corresponding to those 7-8 times less sodium). Thus, the increased fluid intake might partly be due to the appetite for the umami taste of glutamate, which occurs also in rats, as shown earlier, when rats given free access to MSG and water showed a high preference (93-97%) for the MSG solution, regardless of the diet they consumed (43).

Effects on food intake, weight and body fat

MSG was suggested to favor the development of metabolic syndrome, obesity and diabetes

(8,16,17), and administered to neonatal rodents it generates pertinent pathological models (18,19,20). However, in adult animals, MSG added to food or drinking water does not alter food ingestion or body weight gain (19,44,45). Moreover, the claimed relationship between endocrine-metabolic diseases and MSG consumption might be merely accidental (22). Interestingly, whereas MSG increases appetite within a meal with its flavor-enhancing effect, it enhances subsequent satiety and delays next meal (46), and could even reduce weight gain and fat mass (43). Here, MSG was added to drinking water, not to food, allowing us to discard any direct effects of MSG on food appetite. Neither food ingestion nor body weight gain or fat parameters evaluated with MRI techniques (which to our knowledge have never before been applied to the study of fat in MSG-treated animals or humans) were significantly modified by MSG exposure. Since oral bioavailability of MSG seems to be so low (21), our results further suggest a lack of systemic effects of this food additive, at least in tissues pertinent to weight control and fat deposition.

Effects on the digestive system

Ingested MSG stimulates the umami taste receptors on the tongue, leading to depolarization, calcium-induced transmitter release and activation of taste nerve fibers (47), and also the glutamate sensors in the stomach and intestine, locally activating the functions of the digestive tract (2,48).

Since MSG acutely administered may increase salivary flow (49,11), it might also alter the histological structure of the salivary glands when chronically consumed. However, serous and mucous acini in the submaxilar salivary gland were not altered. Further research is needed to determine whether saliva composition (50) is affected by daily MSG intake.

Regarding gastric emptying, some reports show no change (51,52) whereas others show accelerated gastric emptying after MSG oral administration, probably due to vagal activation (48). With our radiographic methods, we did not observe any significant alterations in gastric motility due to MSG exposure in drinking water, probably because we evaluated the possible long-lasting effects of MSG (not those occurring immediately after its ingestion). In a recent cine-MRI study MSG accelerated gastric emptying and increased duodenal motility in most, but not all, healthy volunteers (53), and the authors recognized that different factors might influence the final effect of MSG on gastric emptying. MSG might be useful (and safe, due to its reduced bioavailability) as a prokinetic therapy for functional dyspepsia (54). Radiographic and fluoroscopic methods (55) applied immediately after MSG intragastric administration will determine if MSG induces similar effects in rats as those found in humans using cine-MRI (53).

In the intestinal regions no modifications were radiographically detected. However, ingested MSG may increase irritable bowel symptoms (56), and we found a significant increase in the colonic response to mechanical stimulation. Local actions might be involved, since luminal MSG activated the umami taste receptor T1R1/T1R3 in colonic mucosa, leading to peristalsis and increased the velocity of pellet propulsion in distal colon (57). Interestingly, these local actions might be relatively long-lasting and resistant to the anesthetics used in our experiment. Although we did not see a diarrhea-like disorder, nor we observed any histological differences in ileum and colon tissues that could explain our results on intestinal motility, dietary MSG might be beneficial to treat or prevent constipation, but could also induce some bothersome symptoms (colic pain).

Finally, the histological appearance of small intestinal and colonic mucosae was also not

altered by dietary MSG. MSG and other umami tastants may release glucagon-like peptide- 2 (GLP-2) from enteroendocrine cells (24), and GLP-2 is known for its intestine trophic effect, leading to protection and regeneration of damaged gastrointestinal mucosa, but also with the risk for neoplastic proliferation (58). No intestinal neoplastic alteration associated to chronic MSG consumption was observed in our study.

Effects on kidneys and the cardiovascular systems

The extra amount of sodium intake in drinking water (approximately 20.4 ± 0.5 mg day^{-1}, MSG group), added to that in food (0.1% in chow translates into approximately 20.2 ± 0.3 $mgday^{-1}$, control and MSG group), might alter renal and cardiovascular function.

Conventional histologic techniques did not reveal any noticeable modification in kidneys from MSG-treated animals compared to control rats. It seems unlikely that the dose we used could damage the kidneys in longer treatments, but this should be carefully investigated because MSG was nephrotoxic when used at 2-4 $gkg^{-1}day^{-1}$ for up to 9 months (38,39,59).

Chronic ingestion of MSG did not significantly alter blood pressure or heart rate in anaesthetized animals. In addition, no changes in cardiac basal function were observed. Other authors using MSG at 1% w/v (*vs* 0.4% w/v in our study) described similar results regarding blood pressure (43), but bradycardia occurred with doses of 0.5 $gkg^{-1}day^{-1}$ (60) and was aggravated when even higher doses (1.5 $gkg^{-1}day^{-1}$)were used. Most likely, the different experimental conditions between studies, particularly those affecting the route of administration used (drinking water *vs* intravenous), might explain these discrepancies.

In contrast, a significant reduction in endothelium-dependent vasodilatory capacity of aorta, but not resistance vessels (mesenteric bed), was found. Other researchers have described similar results in rabbit aorta (61), but their results in resistance vessels were different. In western countries, MSG is mostly used in the adult age as a flavor-enhancer and, therefore, our results could suggest an incipient vascular dysfunction that should be more systematically evaluated not only in control animals but also in those with frank metabolic syndrome, obesity or diabetes.

The reported low bioavailability of glutamate after MSG ingestion makes it very unlikely that it contributed to these vascular effects, but the increase in sodium intake in MSG rats could be involved. In fact, animal and human studies support an untoward effect of excess dietary salt intake on cardiovascular and renal function and life span. Interestingly, the endothelium reacts to changes in dietary salt intake through a complex series of events that are independent of blood pressure and the renin-angiotensin-aldosterone axis (62). In experimental hypertension studies, 0.6-1% NaCl in food is used as control whereas high-salt diets contain 8-8.9% NaCl (63,64,65). Thus, the total amount of sodium offered to our MSG-treated rats did not exceed the usual amount offered by other researchers in a normal diet. The mechanisms for the vascular effects found here should be clarified in further research.

Concluding remarks

Chronic treatment with MSG may increase thirst sensation and water intake. Although this may increase hydration under certain circumstances (elderly, sportspeople), caution should be taken regarding total sodium intake (particularly in the elderly and cardiovascular patients) due to sodium in MSG. The lack of effect on weight, food ingestion and abdominal fat suggests that regular dietary MSG during adulthood does not induce metabolic syndrome or

obesity. The lack of histological alterations further indicates that regular dietary consumption of MSG is safe in healthy individuals. Nevertheless, the "mild" functional alterations found in cardiovascular and gastrointestinal motor function should not be overlooked as they could be more severe in unhealthy individuals or even could contribute to the development of gastrointestinal and cardiovascular malfunctioning. A better knowledge of the effects of MSG will contribute to achieving a more balanced use of this flavor-enhancer in dietary products in both healthy and pathological situations.

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AUTHOR CONTRIBUTION

- VLM, EH, CG and CB performed and analyzed the experiments on cardiovascular function
- MLSM and MD performed the MRI studies and analyzed the corresponding data
- GV, MMV, ALP and RA performed the gastrointestinal motility evaluation
- JAU performed the histological analysis
- RA, MLSM, VLM, JAU and MD designed the study, obtained financial support and wrote the paper

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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TABLE 1. Diet composition. Modified from Harlan Iberica.

2014 Teklad Global 14% Protein Diet Macronutrient Information	
Crude Protein	14.3%
Fat (ether extract)	4.0%
Crude Fibre	4.1%
Energy Density	2.9 $kcalg^{-1}$ 12.1 kJg^{-1}
Calories from Protein	20%
Calories from Fat	13%
Calories from Carbohydrate	67%
SODIUM	0.1%
GLUTAMIC ACID	0.9 %

FIGURE LEGENDS

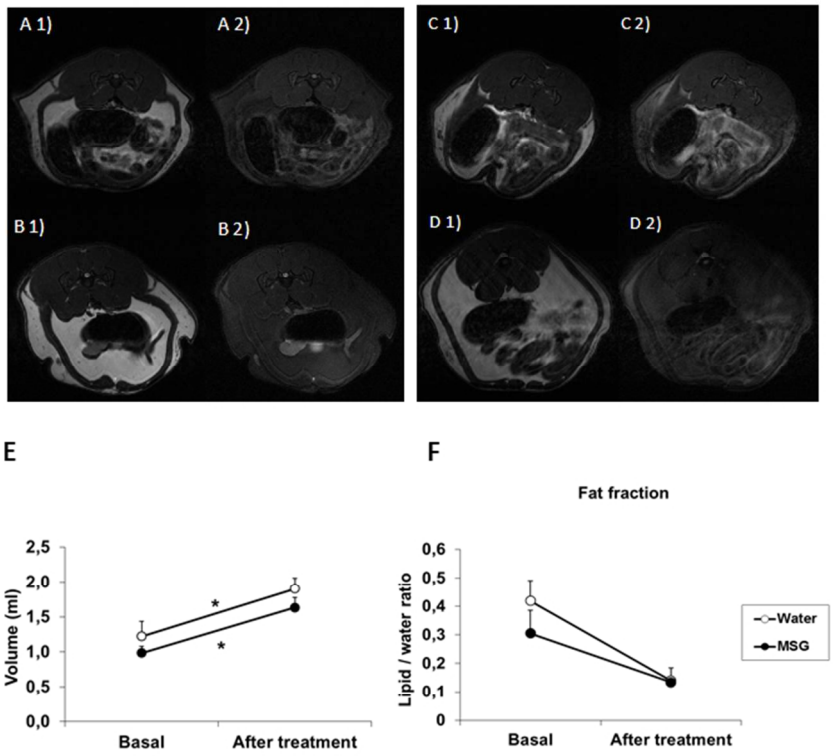
Figure 1. Effect of MSG addition to the diet of the adult rat on abdominal fat. Abdominal fat volume and fat fraction were analyzed by means of magnetic resonance in control rats (WATER, n=5, A-B) and animals treated with MSG (4 g L^{-1} in drinking water, for 6 weeks, n=5, C-D). A and C: basal study. B and D: study after treatment (WATER or MSG). 1: image without fat suppression. 2: image with fat suppression. E and F: fat volume (mL) and fat fraction (waterlipid^{-1} ratio), respectively. Data represent the mean ± SEM. * $p<0.05$ vs basal study (unpaired Student's t test).

Figure 2. Effect of MSG addition to the diet of the adult rat on gastrointestinal motor function. Both general gastrointestinal motor function (A, A', n=5 each group) and colonic motor function (B, B', n=9 each group) were analyzed in control (WATER, white symbols/bars) and treated (MSG, 4 gl^{-1} in drinking water, for 6 weeks, black symbols/bars) rats. Analyses were performed at the end of treatment. A: representative radiographic images obtained at different times (0, 2, 4 y 6 h) after contrast administration (see 2.3 for more details); S = stomach; SI = small intestine; C = caecum; FP = fecal pellets (in colorectum). A': semiquantitative analysis of general gastrointestinal motor function. B: representative recordings of intracolonic pressure in absence (0 mmHg) and presence of mechanical stimulation (30 and 60 mmHg), induced by inflating a balloon inserted into the colon at a depth of 7 cm; small arrows = adjustment of intracolonic pressure after distension responses. B': analysis of the number of contractions produced by the colon in each recording period (10 minperiod^{-1}). Data represents mean ± SEM. * $p<0.05$, *** $p<0.001$ *vs* basal pressure (0 mmHg); ## $p<0.01$ vs WATER (two-way ANOVA followed by Bonferroni *post-hoc* test in A'; paired Student's t-test in B').

Figure 3. Effect of MSG addition to the diet of the adult rat on cardiovascular function. Effect of treatment with MSG (4 g L^{-1} in drinking water) for 6 consecutive weeks. A-C: aorta. A'-C': mesenteric bed. A and A': contractile function. B and B': endothelium-dependent vasorelaxant function. C and C': endothelium-independent vasorelaxant function. Data represents mean ± SEM. * $p<0.05$, ** $p<0.01$ *vs* WATER (two-way ANOVA followed by Bonferroni/Dunn *post-hoc* tests). N=6 each group (4 rings each aorta).

Figure 4. Effect of MSG addition to the diet of the adult rat on the histological structure of different tissues of interest. Representative images of tissues embedded in paraffin and stained with haematoxylin-eosyn obtained from control rats (a, b, c, d, e, f and g) and animals treated with MSG (4 g L^{-1} in drinking water: A, B, C, D, E, F and G) for 6 consecutive weeks. A-B: submaxilar salivary gland, mucous (a and A) and serous acini (b and B). c, C: ileum. d, D: colon. e, E: kidney, with glomerulus and renal tubules. f, F: heart. g, G: aorta. Calibration bar: 100 μm.

Fig. 1



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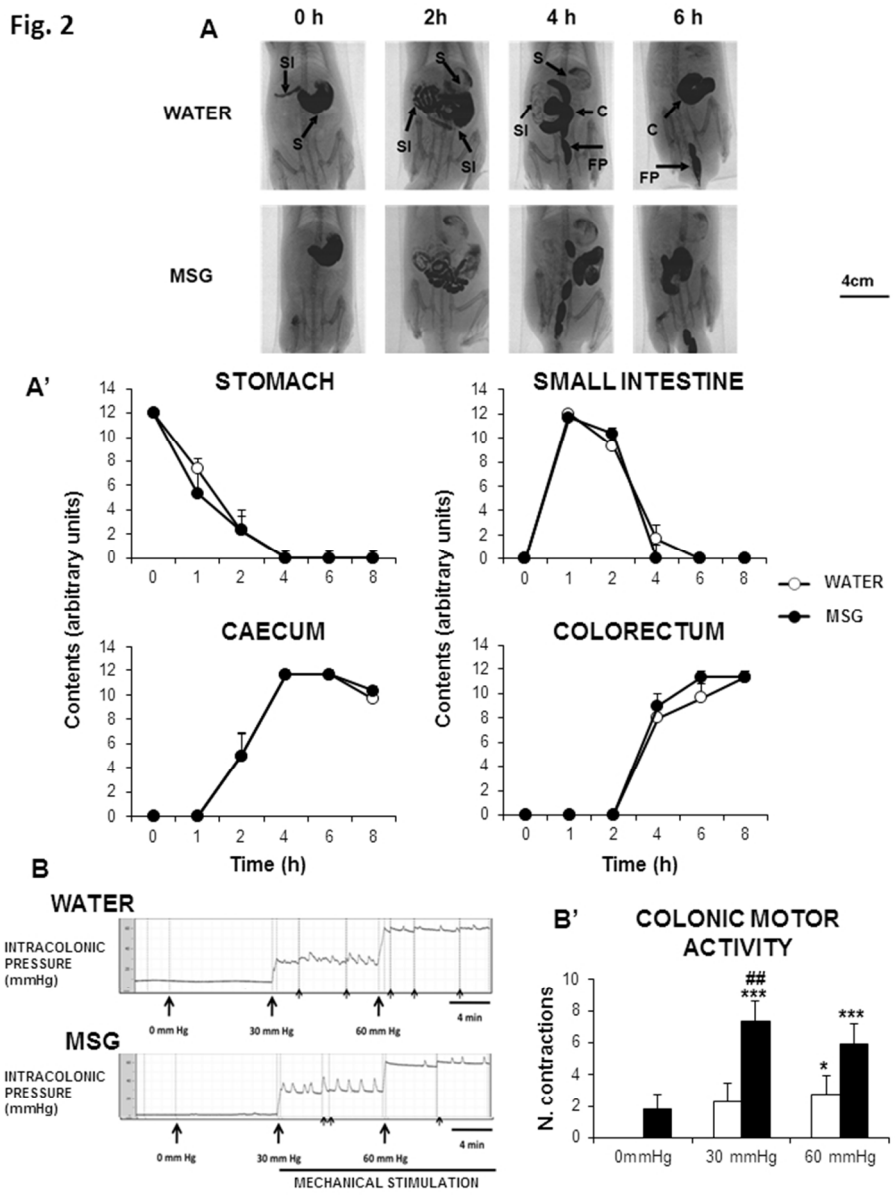

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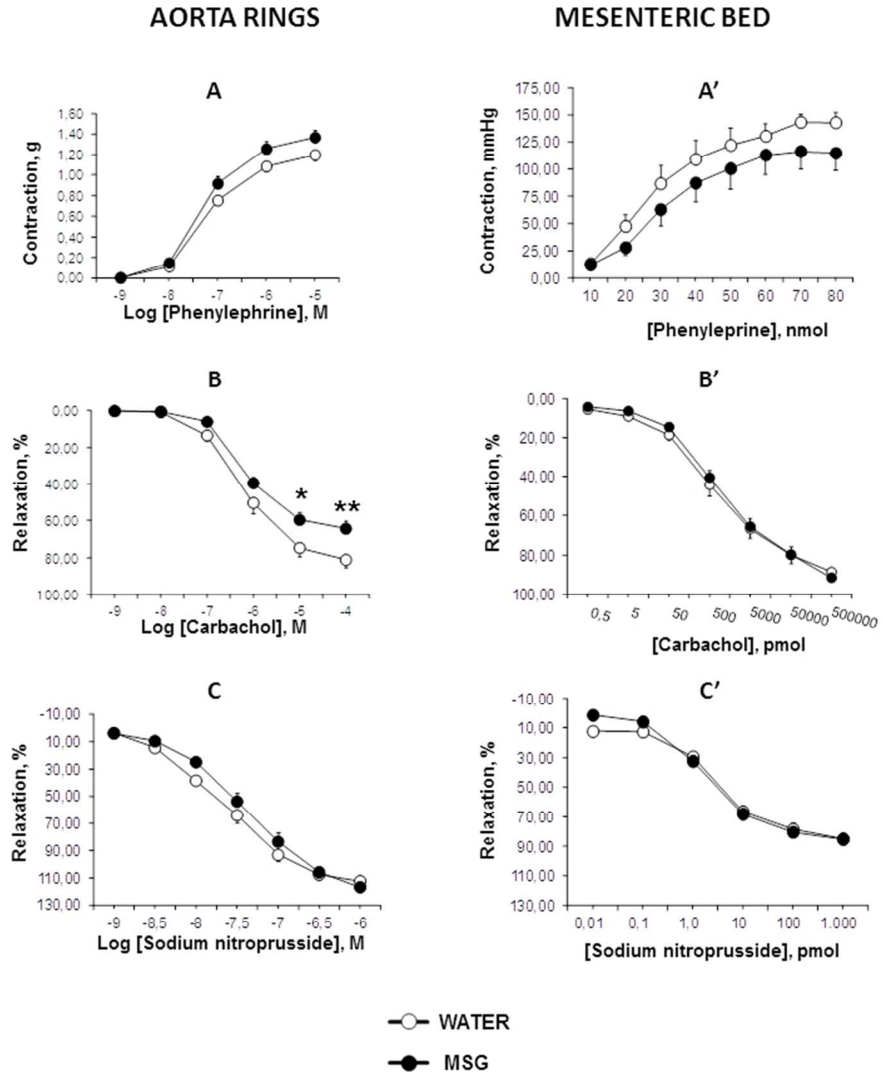

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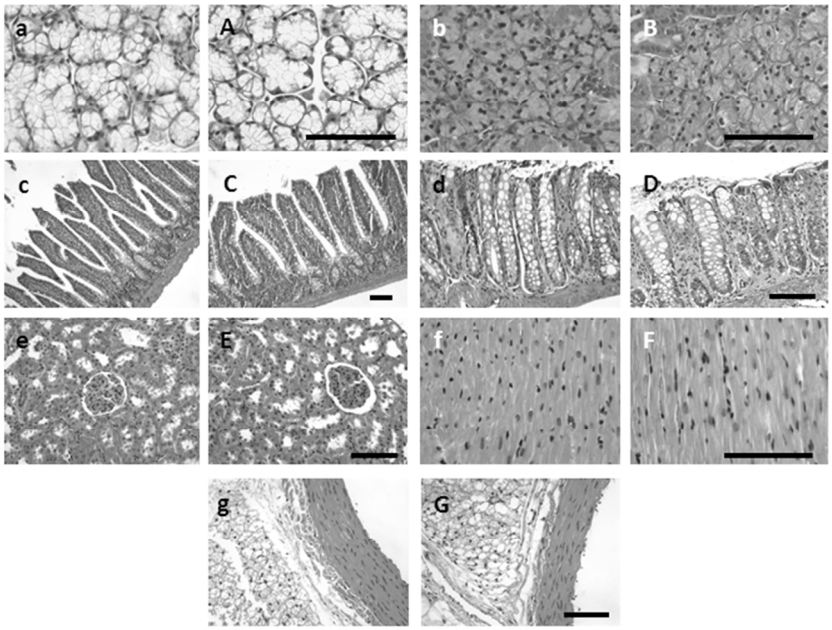

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