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SHORT COMMUNICATION

Oral MSG administration alters hepatic expression of genes for lipid and nitrogen metabolism in suckling piglets

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Abstract This experiment was conducted to investigate the effects of oral administration of monosodium glutamate (MSG) on expression of genes for hepatic lipid and nitrogen metabolism in piglets. A total of 24 newborn pigs were assigned randomly into one of four treatments ($n = 6$/group). The doses of oral MSG administration, given at 8:00 and 18:00 to sow-reared piglets between 0 and 21 days of age, were 0 (control), 0.06 (low dose), 0.5 (intermediate dose), and 1 (high dose) g/kg body weight/day. At the end of the 3-week treatment, serum concentrations of total protein and high-density lipoprotein cholesterol in the intermediate dose group were elevated than those in the control group ($P < 0.05$). Hepatic mRNA levels for fatty acid synthase, acetyl-coA carboxylase, insulin-like growth factor-1, glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase were higher in the middle-dose group ($P < 0.05$), compared with the control group. MSG administration did not affect hepatic mRNA levels for hormone-sensitive lipase or carnitine palmitoyl transferase-1. We conclude that oral MSG administration alters hepatic expression of certain genes for lipid and nitrogen metabolism in suckling piglets.

Keywords Monosodium glutamate · Pig · Liver · Gene expression

Abbreviations

ACC	Acetyl-coA carboxylase
CPT-1	Carnitine palmitoyl transferase-1
FAS	Fatty acid synthase
HDL-C	High-density lipoprotein cholesterol
HSL	Hormone-sensitive lipase
IGF-1	Insulin-like growth factor-1
LDL-C	Low-density lipoprotein cholesterol
MSG	Monosodium glutamate

Introduction

Monosodium glutamate (MSG) is a flavor enhancer generally used in the food industry and is dissociated into glutamate plus sodium in the digestive tract (Kawai et al. 2012; Wu 2013a; Wu et al. 2013a, b; Zhang et al. 2012).

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Glutamate is extensively utilized by the small intestine for the production of ATP and amino acids, including aspartic acid, alanine, proline, ornithine, citrulline and arginine (Ren et al. 2013; Wu et al. 1994a, b, 2012). An appropriate dose of MSG can stimulate food intake by mammals (San Gabriel and Uneyama 2013) and improve the absorption of dietary nutrients by the small intestine (Torii et al. 2013; Zhang et al. 2012, 2013) without causing adverse effects (Nakamura et al. 2013). In addition, Rezaei et al. (2013a) have shown that dietary supplementation with MSG enhances intestinal anti-oxidative capacity and whole body growth, while reducing hepatic lipid concentrations in young pigs. Similar results have been reported for adult rats (Kondoh and Torii 2008; Smriga and Torii 2000; Smriga et al. 2000; Torii et al. 2013). However, the underlying mechanisms are unknown. We hypothesized that MSG may alter hepatic expression of key genes for regulation of fatty acid and amino acid metabolism in animals. The present study was conducted with sow-reared piglets [an animal model for studying human nutrition and metabolism (Chen et al. 2009; Wang et al. 2013; Wu et al. 2011)] to test this hypothesis.

Materials and methods

Preparation of MSG

Monosodium glutamate was purchased from Henan Lianhua Gourmet Powder Co., Ltd (Henan, China). Its purity was 99.9 %.

Animals and diets

Lactating sows had free access to a corn- and soybean meal-based diet (Table 1) and drinking water, and nursed their offspring in an environmentally controlled facility (Wu et al. 2013b, c). Twenty-four newborn piglets (Duroc × Large White × Landrace) with an average body weight of 1.55 ± 0.20 kg were assigned randomly to one of four treatments ($n = 6$/group). The piglets and received oral administration of 0 (control), 0.06 (low dose), 0.5 (intermediate dose), or 1 (high dose) g MSG/kg body weight/day twice daily after birth until 21 days of age. On each day, the first and second MSG administration were given between 08:00 and 09:00 and between 18:00 and 19:00, respectively, after piglets were nursed by their mothers. MSG was dissolved in 2-ml water per kg of body weight before gavage. Piglets in the 0, 0.06, and 0.5 g MSG/kg body weight/day groups received the same amount of sodium in the form of NaCl as piglets in the 1 g MSG/kg body weight/day group, as previously described (Rezaei et al. 2013a, b). The study was conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences.

Table 1 Composition of diets for lactating sows

Diets	%	Nutrition	%
Corn	62	Digestible energy (MJ/kg)	14.23
Soybean meal	15	Crude protein	17.5
Extruded soybean	10	Calcium	0.8
Wheat bran	5	Total phosphorus	0.65
Bean oil	2	Lysine	1.1
Fishmeal	2	NaCl	0.6
Premix	4		

Tissue collection

At 21 days of age, 1 h after gavage at 8:00, 5-ml blood was collected from the anterior vena cava, placed in a centrifuge tube, and centrifuged at 3,000 rpm at 4 °C for 15 min (Tan et al. 2009; Yin et al. 2010). The supernatant fluid (serum) was anaylzed for total protein, urea, triglycerides, and total cholesterol, using an automatic biochemical analyzer (Tan et al. 2012; Wu et al. 2013c). Immediately after blood collection, piglets were euthanized to obtain liver samples (Yao et al. 2008). The tissue was immediately frozen in liquid nitrogen and stored at −80 °C until mRNA analysis (Liu et al. 2012).

Gene expression

Total RNA was extracted from the liver using Trizol Reagent (Invitrogen Corporation) according to the instructions of the manufacturer. The quantity of RNA obtained was checked by measuring the optical density at 260 and 280 nm (He et al. 2013). Before reverse transcription, all RNA samples were treated with DNase I enzyme (amplification grade) to remove any residual DNA, according to the manufacturer's instructions (Invitrogen). RNA (1 μg) sample was combined with 1 μl of 10× reaction buffer, 1 μl of DNase I (1 U/μl), and diethyl pyrocarbonate-treated H_2O up to 10 μl, and incubated at 37 °C for 30 min. Then, 1 μl of 50-mM EDTA was added to stop the reaction by incubation at 65 °C for 10 min. Subsequently, the DNase-treated RNA samples were reverse-transcribed to cDNA using a SuperScript III First-Strand Synthesis System in accordance with the manufacturer's instructions (Invitrogen). Briefly, a solution of RNA (5 μg) and primer mix (1 μl) was added to a DNase-treated sample (12 μl), incubated at 65 °C for 5 min, and then chilled on ice for 1 min. A solution containing 24 μl of

Table 2 Specific primers used for real-time quantitative PCR

Gene	GenBank no.	Primers sequence(5′–3′)	Product size
β-Actin	AFO54837	F:5′-TCTGGCACCACACCTTCTACA-3′	112
		R:5′-ATCTGGGTCATCTTCTCACGG-3′	
IGF-1	NM_214256	F:5′-CTTCAGTTCGTGTGCGGAGAC-3′	225
		R:5′-TACTTCCTTCTGAGCCTTGGG-3′	
GOT	NM_213927	F:5′-TTTGCCAGTCGTGAGGAAGG-3′	228
		R:5′-CCATTGTACCATCGTGCTAAGAA-3′	
GPT	XM_003126995	F:5′-CAATCACCTTCCTCCGACAG-3′	94
		R:5′-CGGGCTCTTTTCTTAGCATCT-3′	
FAS	AY183428	F:5′-CTGCTGAAGCCTAACTCCTCG-3′	207
		R:5′-TTGCTCCTTGGAACCGTCTG-3′	
ACC	EF618729	F:5′-ATGTTTCGGCAGTCCCTGAT-3′	133
		R:5′-TGTGGACCAGCTGACCTTGA-3′	
HSL	AY686758	F:5′-TCAGGTGTCTTTGCGGGTATT-3′	388
		R:5′-AGCCCTTGCGTAGAGTGACAT-3′	
CPT-1	AY181062	F:5′-TATCTGTGTCCGCCTTCTGTC-3′	149
		R:5′-GGCTGTATTCCTCGTCATCC-3′	

The real-time PCR primers are designed by the authors using Primer Premier 5.0

5× reaction buffer, 1 μl Ribolock^{TM} Rnase inhibitor, 2 μl of 10-mM dNTP Mix, 1 μl RevertAid^{TM} M-MuLV reverse transcriptase to a total volume of 20 μl was added to the reaction mixture. After incubation at 25 °C for 5 min, the reaction was incubated at 42 °C for 60 min. To stop the reaction, the reaction mixture was incubated at 70 °C for 5 min and then chilled on ice.

Components of a 25-μl real-time PCR Fig. 1 mixture included 12.5 μl of Maxima SYBR Green/ROX QPCR Master Mix, 0.3-μM forward primer, 0.3-μM reverse primer (Table 1), 500-ng template DNA, and nuclease-free water (a total volume of 10 μl). The PCR conditions used for amplification and quantification were an initial denaturing stage (95 °C for 10 min), 40 cycles of denaturing (95 °C for 15 s) and annealing (60 °C for 30 s), followed by 40 cycles of extension (72 °C for 30 s) and continuous fluorescence measurements (Cui et al. 2007) (Table 2).

Statistical analysis

Values are expressed as mean ± SD. Data were subjected to one-way analysis of variance using the General Linear Model procedures of SAS (SAS 9.1, SAS Institute, Cary, USA). Log transformation of variables was performed when variance of data was not homogenous among treatment groups, as assessed using the Levene's test (Wei et al. 2012). Differences among treatment means were determined by the Duncan multiple comparison test. Differences were considered significant at $P < 0.05$ (Fu et al. 2010).

Results

Effects of MSG on blood biochemical parameters

Compared with the control group, the serum concentration of total protein in pigs fed the intermediate dose of MSG was higher ($P < 0.05$) (Fig. 1). However, oral administration of MSG had no effect on serum concentrations of urea, triglycerides, total cholesterol, or low-density lipoprotein cholesterol (LDL-C) (Fig. 1). In contrast, the serum concentration of high-density lipoprotein (HDL) cholesterol (a good lipid) in pigs fed the intermediate dose of MSG was higher than that in the control group ($P < 0.05$).

Effects of MSG on hepatic mRNA levels for genes in lipid and nitrogen metabolism

Oral administration of the intermediate dose of MSG increased mRNA levels for acetyl-coA carboxylase (ACC) and fatty acid synthase (FAS) by 43.6 and 57.6 %, respectively, compared to the control group ($P < 0.01$). No differences in hepatic expression of these two genes were detected between the control and the high-dose MSG groups. MSG supplementation did not affect the abundance of hormone-sensitive lipase (HSL) or carnitine palmitoyl transferase-1 (CPT-1) mRNA levels.

Supplementation with MSG affected hepatic mRNA levels for insulin-like growth factor-1 (IGF-1), glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase (Fig. 2). The abundance of the IGF-1 mRNA in

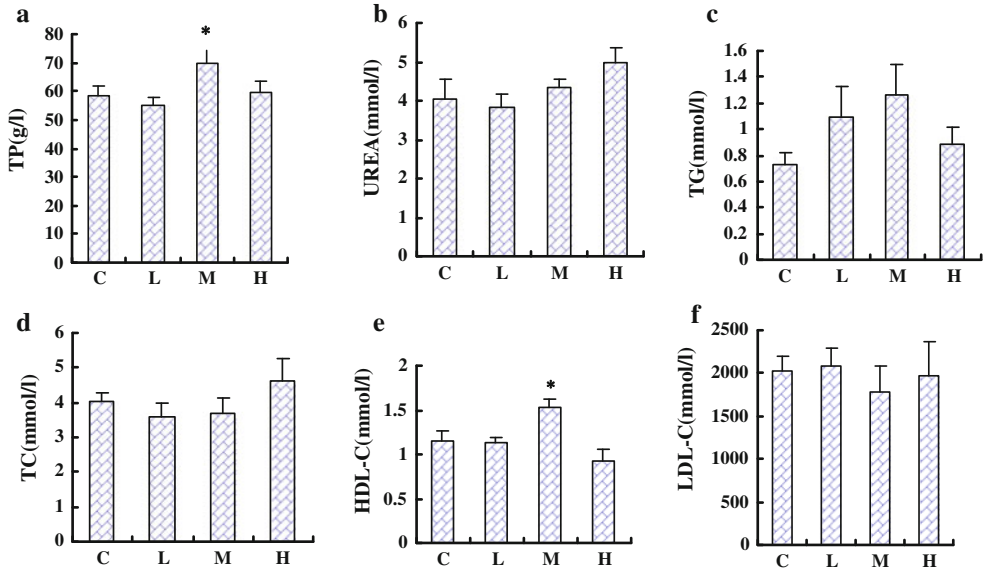

Fig. 1 Effects of oral MSG administration on blood biochemical parameters in suckling pigs. Values are expressed as mean ± SD, $n = 6$. *$P < 0.05$ versus. the control group. *C* control, *L* low-dose MSG, *M* intermediate dose MSG, *H* high-dose MSG

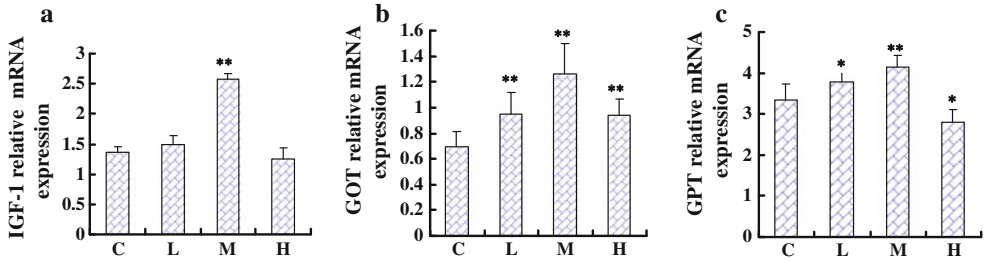

Fig. 2 Effects of oral MSG administration on mRNA levels for IGF-1, glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase in the piglet liver. Data were normalized with *GAPDH* as an internal control. Values are expressed as mean ± SD, $n = 6$. *$P < 0.05$ versus. the control group; **$P < 0.01$ versus. the control group. *C* control, *L* low-dose MSG, *M* intermediate dose MSG, *H* high-dose MSG

pigs fed the intermediate dose of MSG was higher than that in the control group ($P < 0.05$). MSG supplementation also increased mRNA levels for glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase in the liver ($P < 0.05$).

Discussion

Glutamate is a building block for tissue proteins (Wu 2013b). Additionally, as a precursor of glutamine, alanine, aspartate, proline and arginine, MSG plays an important role in amino acid metabolism and whole body function (Brosnan and Brosnan 2013). Furthermore, like other amino acids (Bertrand et al. 2013; Dai et al. 2013; Dillon 2013; Fernstrom 2013; Hou et al. 2013; Satterfield et al. 2013; Suryawan et al. 2013), glutamate can regulate gene expression and nutrient metabolism in multiple tissues (Zhang et al. 2013). Thus, glutamate has been recognized as a functional amino acid in animal nutrition (Rezaei et al. 2013b; Wu 2013a).

Total protein in the serum is a useful indicator of protein nutritional status in animals (Wu 2013b). A novel and important finding of this study is that an appropriate dosage of MSG increased the total protein concentration in the piglet serum, indicating an overall increase in the synthesis of hepatic proteins. Elevated concentrations of protein may contribute to a higher level of HDL in the serum of piglets receiving MSG supplementation than the control group (Fig. 1). HDL is mainly synthesized in the liver and is responsible for transporting cholesterol from the peripheral tissue to the liver for disposal of its cholesterol moiety. The concentration of HDL in the serum is positively correlated with the body's ability to remove cholesterol (Jobgen et al. 2006). Thus, oral administration of MSG is not toxic to neonates and has a beneficial role in regulating lipid

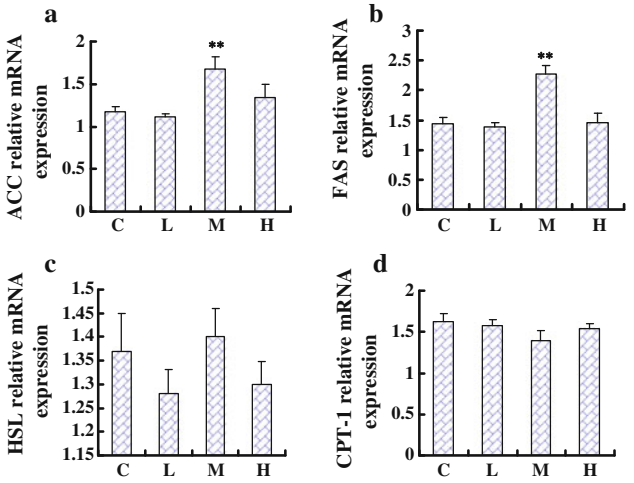

Fig. 3 Effects of oral MSG administration mRNA levels for ACC, FAS, HSL, and CPT-1 in the piglet liver. Data were normalized with *GAPDH* as an internal control. Values are expressed as mean ± SD, $n = 6$. $^{**}P < 0.01$ versus. the control group. *C* control, *L* low-dose MSG, *M* intermediate dose MSG, *H* high-dose MSG

metabolism in the body (Kondoh and Torii 2008; Smriga and Torii 2000; Smriga et al. 2000).

Fat is mainly deposited in the body as triglycerides of fatty acids. In swine, de novo synthesis of fatty acids occurs primarily in white adipose tissue (Jobgen et al. 2006). Interestingly, we found that supplementation with intermediate dose MSG enhances ACC and FAS mRNA levels in the liver of suckling piglets (Fig. 3). These results reveal an ability of glutamate in the regulation of hepatic gene expression. However, because fatty acid synthesis from acetyl-CoA is absent from the pig liver (Jobgen et al. 2006), these findings should not be interpreted to indicate an effect of glutamate in enhancing lipogenesis in the body. An increase in fatty acid oxidation in the liver and skeletal muscle likely plays a major role in augmenting HDL in blood (Fig. 1).

IGF-1 has been reported to promote protein synthesis and inhibit protein degradation in animals (Deldicque et al. 2005). Thus, IGF-1 promotes protein anabolism (Russell-Jones et al. 1994). Other studies have also shown that IGF-1 plays a role in promoting protein synthesis in myocardial and muscle cells (Fuller et al. 1992). Of note, results of our study demonstrated, for the first time, that oral administration of an appropriate dose of MSG increased hepatic IGF-1 gene expression in animals (Fig. 2). This may aid in our understanding of an important role for MSG in enhancing protein synthesis in suckling piglets. Further studies are warranted to determine whole body and tissue-specific protein turnover in animals.

In conclusion, oral administration of MSG increased the concentrations of total protein and HDL in the piglet serum and hepatic mRNA levels for ACC, FAS, IGF-1, glutamate–oxaloacetate transaminase, and glutamate–pyruvate transaminase in the piglet liver. These findings are novel and important for understanding a regulatory role of glutamate in hepatic lipid and nitrogen metabolism. The underlying mechanisms need to be elucidated in future studies.

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Conflict of interest The authors declare no conflict of interest.

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