

Effect of Fasting and Refeeding on Duodenal Alkaline Phosphatase Activity in Monosodium Glutamate Obese Rats

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Summary

In the present work the effects of fasting and refeeding on fat pad weight and alkaline phosphatase activity in the brush border of individual duodenal enterocytes have been evaluated in male Wistar rats with obesity induced by monosodium glutamate (MSG) treatment during the early postnatal period. Neonatal rats were treated subcutaneously with MSG (2 mg/g b.w.) or saline (controls) for 4 days after birth. At 4 months of age, two types of experiments were performed. In the first experiment rats, were submitted to 3 or 6 days lasting food deprivation. In the second experiment the rats were refed for 3 or 6 days *ad libitum* or restrictedly (60 % of pre-fasting intake) after a 6 day-fasting period. Fasting and refeeding influenced the body fat and function of the duodenum in MSG-treated rats differently as compared to the controls. However, alkaline phosphatase activity and the weight of epididymal and retroperitoneal fat depots were significantly increased in MSG obese rats ($P < 0.001$) during all the periods examined. While 3 days of food deprivation resulted in both groups in a similar loss of adipose tissue weight and alkaline phosphatase activity, the decrements of these parameters after 6 days of fasting were lower in obese rats suggesting that their capacity to spare body fat stores was enhanced. After 3 days of *ad libitum* refeeding, a more marked adaptational increase of food consumption and also a significantly increased alkaline phosphatase activity above the pre-fasting level ($P < 0.01$) was observed in the MSG-treated rats. Consequently, a more rapid body fat restoration was demonstrated in these animals. Refeeding of rats at 60 % of the pre-fasting intake level resulted in a significant increase of alkaline phosphatase activity in both the MSG and control group; moreover, as food restriction continued, MSG-treated rats tended to further increase the enzyme activity. Our results revealed that MSG treatment of neonatal rats may significantly change the intestinal functions. Permanently increased alkaline phosphatase activity observed in MSG obese rats during all investigated periods suggests that this functional alteration is probably not a consequence of actual nutritional variation but could be a component of regulatory mechanisms maintaining their obesity at critical values.

Key words

Fasting/refeeding • Duodenal alkaline phosphatase • Monosodium glutamate • Obese rats

Introduction

Monosodium glutamate (MSG) treatment of neonatal rats results in a syndrome characterized by neuroendocrine disturbances, arrested skeletal growth and

obesity in adulthood (Olney 1969, Bakke *et al.* 1978, Abe *et al.* 1990, Sun *et al.* 1991, Zhang *et al.* 1994). Obesity of MSG-treated rats is accompanied with increased plasma levels of insulin, triglycerides (Abe *et al.* 1990) and corticosterone (Škultétyová *et al.* 1998, Macho *et al.*

1999a,b), a decreased volume and number of hypothalamic neurons (Sun *et al.* 1991), loss of neuromodulators in the mediobasal hypothalamus (Dawson *et al.* 1989, Abe *et al.* 1990) and damage of the leptin receptors in the hypothalamic arcuate nucleus (Dawson *et al.* 1997). It is a specific feature of MSG-induced obesity that increased adiposity occurs in the absence of hyperphagia or even with hypophagia (Zhang *et al.* 1994, Dawson *et al.* 1997, Stricker-Krongrad *et al.* 1998). The mechanisms by which MSG induces obesity without overeating are not fully understood. It is generally accepted that disturbances in the regulation of adiposity, i.e. reduced lipolytic activity in the adipocytes (Cheung *et al.* 1988), increased adipose tissue lipoprotein lipase activity (Nascimento Curi *et al.* 1991), and enhanced sensitivity of adipose tissue to insulin (Marmo *et al.* 1993) finally caused obesity. In contrast, impaired glucose tolerance and signals of insulin resistance in fat tissues of MSG-treated rats have been recently described (Hirata *et al.* 1997, Zórad *et al.* 1997, Macho *et al.* 2000). Moreover, permanently increased alkaline phosphatase (AP) activity in the brush border of duodenal enterocytes in adult rats postnatally treated with MSG has also been demonstrated (Možeš *et al.* 2000, Martinková *et al.* 2000) suggesting that, besides neurohormonal and metabolic disturbances, the altered intestinal function may be a further key factor contributing to the development of the MSG syndrome.

Among the enzymes of the small intestine, alkaline phosphatase is a representative brush border enzyme functionally involved in nutrient absorption and transport of long-chain fatty acids in the intestinal mucosa (Takase and Goda 1990, Bernard *et al.* 1992, Kaur *et al.* 1996). In mature non-obese rats, the alkaline phosphatase activity in the small intestine displays circadian fluctuations closely related to their food intake (Ishikawa *et al.* 1983) and their activity markedly decreases after a short period (3-4 days) of food deprivation (Ishikawa *et al.* 1983, Holt and Kotler 1987). On the other hand, when food-deprived rats were refed, the activity of duodenal alkaline phosphatase (Holt and Kotler 1987) and the expression of the 3.0 kb of intestinal AP mRNA species increased and within 48 h their values returned to the level found prior to fasting (Hodin *et al.* 1994). These results suggest a close relationship between the feeding status and alkaline phosphatase activity and also indicate that eating after food deprivation elicits rapid functional changes in the small intestine. However, little is known about the effects of fasting and refeeding

in MSG-treated rats whose physiology clearly differs from that of lean rats. Such fundamental data for alkaline phosphatase activity would be useful for better understanding of the relationship between actual nutrition and function of the small intestine in MSG obese rats.

The activity of duodenal alkaline phosphatase was therefore studied in individual enterocytes of rats neonatally treated with MSG or saline (control rats) subjected to 6 days of fasting followed by 3 or 6 days refeeding either *ad libitum* or at 60 % level of *ad libitum* food intake.

Material and Methods

Animals

Neonatal Wistar male and female rats housed from birth to weaning in litters of nine pups were divided into two groups. The first group (MSG-treated) and the second group of animals (saline-treated) received a s.c. injection of 2 mg/g b.w. monosodium salt of L-glutamic acid (Sigma, St. Louis, MO) or an equivalent volume of saline for 4 days after birth. MSG was dissolved in distilled water and the drug concentration adjusted so that the pups (male and female) received 50 µl of solution per gram of body weight. After weaning (on day 30), the female rats were excluded from the experiment and the male rats fed a standard rat chow (Velaz/Altromin 1520 DOS 2b) in a temperature-controlled environment of 22 °C with a 12L:12D regime (light from 07.00 to 19.00 h).

At 4 months of age, the MSG-treated and control rats were individually housed in wire cages with food and water *ad libitum*. At least one week prior to the experiments their body weight and food consumption were recorded each day. The food intake was measured at 24-h intervals as the difference between the amount offered and remnants left in the cups.

A group of MSG-treated and a group of saline-treated rats in experiment 1 were exposed to 3 days and the other two groups of rats to 6 days of fasting (food except water was removed at 01:00 h) while the control group of rats continued to receive food *ad libitum* throughout the 6 days' lasting study. In experiment 2, all rats fasted for 6 days. After this food deprivation, one group of rats was refed for 3 days and the second group received food for 6 days either *ad libitum* or in restricted amounts (60 % of the mean intake consumed before fasting, one third given in the morning, two thirds in the

evening at 19:00 h). The control groups of rats were fed *ad libitum* throughout the experiment (for 12 days).

The epididymal and retroperitoneal white adipose depots were quickly excised and weighed after the animals had been sacrificed.

Separation of enterocytes

After decapitation of the rats (at 01:00 h), small (0.5 cm) segments of the proximal duodenum were removed, everted and frozen in hexane (to -70°C) immediately after washing in distilled water.

Epithelial cells were collected by the procedure of Weiser (1973) modified by us (Možeš *et al.* 1998). The frozen duodenal segments were incubated for 5 min at room temperature (20°C) in a sodium citrate buffer (1.5 mM KCl, 96 mM NaCl, 8 mM KH_2PO_4 , 5.6 mM Na_2HPO_4 , 27 mM sodium citrate, pH 7.3). After this initial incubation, the tissue was transferred into plastic tubes containing 0.15 M phosphate buffer (pH 5.3) and incubated for 1, 5 and 10 min. At the end of each incubation period the tissue segment was spun briefly to dissociate the cells, then transferred into another tube containing a phosphate buffer, and the incubation continued. The epithelial cells released after 10 min incubation (central part of the villus) were centrifuged for 3 min at $800 \times g$, and the pellet was mixed with distilled water. A small droplet of the cell suspension was transferred onto a glass slide, covered by a thin plastic film and squashed by pressing with another glass slide. After removing the film, the cells were air-dried.

Enzyme assay

Demonstration of alkaline phosphatase activity was performed using the modified simultaneous azo-coupling method (Lojda *et al.* 1979). The incubation medium contained 0.4 mM naphthol AS-BI phosphate (Sigma, Deisenhofen, Germany), 0.8 mM Hexazotized New Fuchsin (Serva, Heidelberg, Germany), 20 mM N,N-dimethylformamide and 0.05 M veronal acetate buffer (pH 9.2). Incubation was performed at 37°C in the presence of 13 % w/v polyvinyl alcohol (PVA, Sigma). Enzyme activity was cytophotometrically analyzed with a Wickers M85a microdensitometer. The measurements were performed using a $\times 40$ objective, an effective scanning area of $28.3 \mu\text{m}^2$ and a scanning spot of $0.5 \mu\text{m}$. The integrated absorbance was measured at a wavelength of 520 nm (Frederiks *et al.* 1987). AP activity was

calculated as the absorbance values recorded by the instrument in $\text{min}/\mu\text{m}^3$ brush border \pm S.E.M. Thirty cells were always measured in each animal and their mean values referred to one animal.

Statistical analysis

Statistical evaluation of the results was carried out by one-way analysis of variance (ANOVA). Significance of the differences between the means was calculated by Tukey's test.

Results

Experiment 1

Table 1 shows the values of body weights, epididymal plus retroperitoneal adipose tissue weight and duodenal alkaline phosphatase activity before and after 3 or 6 days of food deprivation in the control and MSG-treated groups. In fed animals, the body weight as well as the values of body fat and AP activity were significantly higher in obese rats than in the lean controls. Three days of fasting decreased these parameters in both groups. When compared to non-fasted animals, a 10 % vs 11 % decrease of body weight, a 21 % vs 25 % decrease of fat depots and a 15 % vs 12 % decrease of AP activity were observed in the control and MSG obese rats, respectively. After 6 days of fasting, the body weight, adipose tissue weight and enzyme activity further decreased in both groups, but the decrements were more pronounced in lean than in obese rats. The body weight declined by about 20 % vs 16 %, the body fat by about 80 % vs 40 % and AP activity by about 45 % vs 20 % in control and MSG-treated rats, respectively (Table 1).

Experiment 2

At the beginning of the experiment (before 6 days of fasting) a significantly higher food consumption was observed in control than in MSG-treated rats (Table 2). The quantity of food intake was significantly increased in both groups of animals during the first 3 days of *ad libitum* refeeding and this eliminated the weight losses induced by 6 days of fasting. The overeating was more pronounced in obese than in lean rats. As compared to the initial (pre-fasting) values food consumption increased by about 32 % and 20 % in MSG-treated and control rats, respectively.

Table 1. Body weight, body fat and alkaline phosphatase (AP) activity in MSG-treated and control rats fed *ad libitum* or fasted for 3 and 6 days.

	Body weight (g)	Weight loss	Body fat (% b.w.)	AP activity
<i>Ad libitum fed</i>				
Control	465±3		1.9±0.1	13.2±0.1
MSG	403±13 [‡]		3.5±0.5 [‡]	15.2±0.1 [‡]
<i>Fasted 3 days</i>				
Control	463±7	10.1±0.7	1.5±0.1	11.5±0.2 ^c
MSG	421±4 [†]	10.9±0.7	2.7±0.1 [*]	13.5±0.3 ^{†c}
<i>Fasted 6 days</i>				
Control	403±3	19.8±0.7	0.3±0.1 ^c	7.5±0.2 ^c
MSG	372±2 [*]	16.0±0.4 [†]	1.9±0.2 ^{†c}	11.8±0.1 ^{†c}

The data are means ± S.E.M. of 6 rats in each group. AP activity is given as the integrated absorbance in $\text{min}/\mu\text{m}^3$ brush border of individual duodenal enterocytes at a wavelength of 520 nm. Weight changes are expressed as the percentage of differences from the initial (prefasting) weight. Body fat (%) represents epididymal plus retroperitoneal pads. Significantly different from control rats: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$. Significantly different from *ad libitum fed* rats: c $p < 0.001$ (Tukey's test).

Table 2. Changes in food intake, body weight and body fat in MSG-treated and control rats after 3 and 6 days of *ad libitum* or restricted refeeding.

Refeeding methods	Food intake (g/b.w.)		Body weight changes		
	Initial	After refeeding	Fasting Weight loss	Refeeding Weight gain	Body fat (% b.w.)
<i>Ad libitum 3 days</i>					
Control	7.4±0.2	8.9±0.1 ^a	17.7±0.9	13.9±0.6	0.8±0.1
MSG	6.1±0.3 [*]	8.1±0.5 ^c	14.7±0.5 [†]	12.7±0.6	2.7±0.3 [‡]
<i>Ad libitum 6 days</i>					
Control	7.5±0.1	8.6±0.1 ^c	18.5±0.9	19.8±1.6	1.7±0.2
MSG	6.6±0.1 [†]	7.1±0.2 [‡]	15.6±0.4 [*]	11.3±0.6 [‡]	3.4±0.4 [†]
<i>Restriction 3 days</i>					
Control	6.4±0.1	3.7±0.2 ^c	18.3±0.7	6.5±0.4 ^c	1.0±0.1
MSG	5.7±0.3 [*]	3.3±0.2 ^c	15.6±0.4 [†]	5.1±0.5 ^c	2.4±0.2 [†]
<i>Restriction 6 days</i>					
Control	6.7±0.2	3.9±0.2 ^c	17.0±0.4	5.7±0.6 ^c	0.8±0.2
MSG	5.6±0.3 [*]	3.3±0.2 ^c	14.1±0.5 [†]	5.4±0.8 ^c	2.2±0.2 [†]

The data are means ± S.E.M. of 6 rats in each group. Weight changes are expressed as the percentage of differences from the initial (pre-fasting) weight. Body fat (%) represents epididymal plus retroperitoneal pads. Significantly different from control rats: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$. Significantly different from initial values: a $P < 0.05$, c $P < 0.001$ (Tukey's test).

During *ad libitum* refeeding, the recovery of fat in the control and MSG-treated rats followed a different pattern (Table 2). After 3 days of refeeding in MSG-treated rats, the adipose tissue weight of the latter did not differ significantly from that observed in the *ad libitum* fed groups (2.7 ± 0.3 % b.w. vs. 3.5 ± 0.5 % b.w. in refed and *ad libitum* fed MSG-treated rats, respectively). On the other hand, in control rats such body fat recovery is achieved only on day 6 (1.7 ± 0.2 % b.w. vs. 1.9 ± 0.1 % b.w. in refed and *ad libitum* fed controls, respectively). In contrast, after 3 or 6 days of refeeding at a 60 % level of mean baseline intake, the body fat weight of MSG-treated and control rats did not attain the values observed in the *ad libitum* fed groups (Tables 1 and 2).

Table 3. Alkaline phosphatase (AP) activity in individual duodenal epithelial cells of MSG-treated and control rats fed *ad libitum*, fasted for 6 days or refed *ad libitum* or restrictedly for 3 or 6 days.

Refeeding methods	Control	MSG
<i>Ad libitum fed</i>	13.5 \pm 0.2	15.4 \pm 0.1 [†]
<i>Fasted for 6 days</i>	7.5 \pm 0.2 ^c	11.8 \pm 0.1 ^{†c}
<i>Ad libitum refed</i>		
Day 3	14.1 \pm 0.2	16.7 \pm 0.1 ^{†c}
Day 6	14.7 \pm 0.3	15.0 \pm 0.6
<i>Restrictedly refed</i>		
Day 3	16.2 \pm 0.2 ^c	17.3 \pm 0.2 ^{†c}
Day 6	16.2 \pm 0.2 ^c	18.2 \pm 0.2 ^{†c}

The data are means \pm S.E.M. of 6 rats in each group. AP activity is given as the integrated absorbance in $\text{min}/\mu\text{m}^3$ brush border of individual duodenal enterocytes at a wavelength of 520 nm. Significantly different from control rats: [†] $P < 0.001$. Significantly different from *ad libitum* fed rats: ^c $P < 0.001$ (Tukey's test).

The alkaline phosphatase activity in the brush border of individual duodenal enterocytes of MSG-treated rats was significantly increased in comparison with their controls (except for the 6 days of *ad libitum* refeeding) throughout all the periods followed (Table 3). The enzyme activity increased in MSG-treated and control rats during *ad libitum* refeeding, but the increase in obese rats was more pronounced and significantly

exceeded the pre-fasting levels at 3 days. In contrast, AP activity in the control rats rose slightly above the values observed in *ad libitum* fed rats. After restricted refeeding a more marked increase of enzyme activity was found in both groups. Moreover, as the food restriction continued, a further increase of enzyme activity occurred in the obese rats. In both groups of rats the increase of AP activity was closely related to refeeding, but not to the amount of food consumed. This was apparent in restrictedly refed rats, in which, despite sustained hypophagia, the mean AP activity in the enterocytes was higher on all days examined than in the hyperphagic *ad libitum* refed animals (Table 3).

Discussion

The significantly decreased body weight, body fat and alkaline phosphatase activity in fasted rats observed in our experiments are in agreement with earlier studies (Ishikawa *et al.* 1983, Hill *et al.* 1988). Moreover, our results revealed a different pattern of the response to food deprivation in obese and lean rats. This was apparent in 6-days' fasted rats where the decrements of weight, body fat and AP activity were lower in MSG-treated than in control rats. This coincides with previous reports about the remarkable ability to maintain both body protein and fat pad content of MSG obese rats during fasting (Ribeiro *et al.* 1989, 1997). The higher capacity to spare body fat stores in obese rats is attributed to some metabolic and hormonal disturbances as a consequence of neonatal MSG administration (Arndt *et al.* 1993). In comparison with the lean controls, alkaline phosphatase activity was significantly higher in MSG obese rats during food deprivation. Whether this increase in MSG-treated rats, observed at the time when intraluminal stimulation of food gradually disappeared, is due to a higher synthetic rate or to a decreased rate of enzyme breakdown, remains to be established. Moreover, the present results, together with our previous work (Martinková *et al.* 2000), have shown that increased AP activity also occurred in *ad libitum* fed MSG-treated rats despite their lower food intake in comparison with the lean controls. From this point of view, the permanent increase of AP activity in MSG obese rats is probably not a consequence of actual nutritional variations, but could rather be apart of a more general effect of MSG characterized by neurohormonal and metabolic alterations.

While food deprivation is associated with lowered nutrient absorption and decreased intestinal function, the adaptation that occurs during refeeding stimulates replenishment of the depleted body fat stores and is related to increased food intake and the enhanced activity of many absorptive enzymes (Buts *et al.* 1990, Henry *et al.* 1997). Prior to food deprivation, the mean food intake of MSG-treated rats was by 14 % lower than that of the controls. In contrast, there was an 80 % increase in their fat pad weight. During *ad libitum* refeeding, a different time course of adaptational changes and a more marked compensatory overeating was observed in obese rats as compared to the controls. With 3 days of refeeding, the overshoot of food intake represents about 32 % and 20 % of baseline food intake in MSG-treated and control rats, respectively. Due to these differences, the body fat in MSG-treated rats did not significantly differ from the values observed prior to fasting, while in the controls such body fat normalization occurred only on day 6. This somewhat longer time needed for fat restoration in the controls is similar to that reported earlier when the epididymal fat weight after 3-5 days of refeeding remained below the prefasting level in rats submitted to 3 or 7 days of food deprivation (Björntorp *et al.* 1980, Fried *et al.* 1983).

The resumption of food intake after fasting induced increased AP activity in both MSG-treated and control rats. However, the enzyme activity in MSG obese rats rose significantly above the control values after 3 days and returned to the initial levels after 6 days of refeeding. In contrast, in lean rats, the AP activity on day 3 and 6 of refeeding only moderately exceeded the values of AP activity observed prior to food deprivation despite their permanent hyperphagia. It is therefore probable that this dynamic enzyme adaptation was associated with the rate of body fat restoration during refeeding. Such a possibility is suggested by increased AP activity observed in rats refed with a limited amount of food. In these rats, the lowered fat depot was associated with a substantial increase of duodenal alkaline phosphatase activity which exceeded on day 3 the values observed in *ad libitum* refed rats. Furthermore, as food restriction continued, the increase of AP activity in MSG-treated rats showed a

tendency to a further increase, while the enzyme activity in the controls remained unchanged. The exact mechanism inducing these differences between obese and lean rats has not been fully elucidated. It has been well established that long-term food restriction in non-obese animals leads to enhanced intestinal absorption and increased activity of the brush border enzymes (Kelly *et al.* 1991, Holt *et al.* 1991, Nunez *et al.* 1996, Casirola *et al.* 1996). The same phenomenon of increased AP activity in the small intestine of animals receiving only 60 % of their normal daily food rates during refeeding has been confirmed by the present experiment. It can therefore be suggested that the decreased food intake in consequence of neonatal MSG treatment may elicit functionally different adaptation and enhancement of enzyme activity in obese rats. Such a relation seems to be in agreement with our earlier experiment where lowered food intake and higher duodenal AP activity were observed in intact non-fasting MSG-treated rats while an increased food intake and decreased AP activity were seen in the controls (Martinková *et al.* 2000).

The present results extend our knowledge on the intestinal function in MSG-treated rats and allow better understanding of the adaptive mechanisms that may limit or prevent the efficacy of fasting or severe food restriction in the treatment of obesity. It could be speculated whether the effectiveness of nutrient absorption, higher enzyme activity or both may be of importance for enhanced body fat accumulation in neonatally MSG-treated rats. The sustained increase of duodenal alkaline phosphatase activity and body fat content observed in our experimental rats suggest that functional adaptation of the small intestine may be a part of the regulatory mechanisms to maintain their obesity at critical values. With regard to the presented data, however, further research is required before definite conclusions concerning the causal relationship between increased alkaline phosphatase activity and development of obesity in MSG-treated rats can be drawn.

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