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Effect of central enterostatin on fat intake in neonatal chicks

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HIGHLIGHTS

- Enterostatin derives from procolipase in the upper intestine.
- Enterostatin specifically suppresses fat intake in rodents.
- Low-fat food intake does not alter by ICV administration of enterostatin in chicks.
- Central enterostatin inhibits high-fat diet intake in overnight fasted chicks.
- ICV enterostatin stimulates high-fat intake in chicks fasted 3 h.

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ABSTRACT

Enterostatin, a gut-brain pentapeptide cleaved from procolipase has been shown to inhibit fat intake in rodents after both peripheral and central administration. In this study, the effect of intracerebroventricular (ICV) injection of enterostatin on fat intake was investigated in neonatal chicks. In Experiment 1, 3-h-fasted chicks fed a low-fat diet were injected with the various doses of enterostatin. Experiment 2 was similar to experiment 1 except that the birds were fasted overnight. In Experiment 3, the 3-h-fasted and in Experiment 4, the overnight fasted chicks adapted to a high-fat diet received different doses of enterostatin. ICV injection of enterostatin caused a dose-dependent increase in high-fat diet intake in 3-h-fasted chicks whereas a decrease in high-fat intake was observed in chicks that were fasted overnight. However, low-fat diet intake was not affected by enterostatin in either 3-h or overnight fasted chicks. These results suggest that enterostatin acts within the brain of chicks to influence fat intake. It appears that in chicks, the eating effect of enterostatin has a biphasic nature similar to those seen in rodents.

Enterostatin is an intestinal pentapeptide derived from the N-terminal end of pancreatic procolipase, in the presence of trypsin [7]. The residual protein called pancreatic colipase is a cofactor for hydrolysis of fat within the duodenum [7]. There are three forms of enterostatin, Ala-Pro-Gly-Pro-Arg (APGPR), Val-Pro-Asp-Pro-Arg (VPDPR) and Val-Pro-Gly-Pro-Arg (VPGPR) [7,42]. Evidence for the existence of procolipase in chickens has been reported by Bosc-Bierne et al. [4,5]. Also the presence of enterostatin binding protein has been shown in chicken and rat serum [42]. Several investigations have revealed that the β-subunit of F1-ATPase acts as a receptor for enterostatin [2,3,24,33]. This receptor is found in the plasma membranes of liver cells as well as amygdala [33].

Significant evidence has demonstrated the role of enterostatin as a specifically regulator of fat intake. In rats, Erlanson-Albertsson and Larson found for the first time that intraperitoneal (IP) injection of enterostatin decreases food intake [8]. These authors conducted their studies on the effect of enterostatin on fat intake since both enterostatin and colipase are released more specifically during lipid assimilation in the intestinal phase of the integrated response to a meal [8]. They demonstrated that either central or peripheral administration of enterostatin specially inhibits the intake of high-fat diet as opposed to low-fat diet in rats [10]. In another study, Okada et al. reported that IP injection of enterostatin inhibits fat intake in rats, when the food was given as three-choice diet between fat, protein and carbohydrate [30]. Taken to gather, it was suggested that enterostatin acts as a negative feedback regulator during lipid assimilation [9] and intact vagus afferent innervation was important for this function [27,28]. In addition to feeding, it has also been reported that enterostatin increases energy expenditure through both central and peripheral pathways [2,19,22,35].

It is important to investigate the regulating mechanisms of food and fat intake in commercial lines of poultry. For example, overconsumption in broiler breeder strains, selected for rapid growth and high meat production, can lead to excessive accumulation of fat tissue, an undesirable body weight, and a series of health-related complications such as leg problems, reduced reproductive efficiency, etc. [34]. Although there are numerous reports to support the effect of enterostatin on feeding in rat and mouse, none is

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Table 1
Composition of the experimental high-fat diets (based on Ref. [29]).

	Fat %		
Ingredient	4 (% of diet)	7	11
Soybean meal	41.8	42.7	43.6
Corn	53.5	48.6	43.3
Soybean oil	0.8	4.7	9.1
$CaCO_3$	1.7	1.8	1.8
Dicalcium phosphate	1.4	1.4	1.4
Methionine	0.2	0.2	0.2
Sodium chloride	0.4	0.4	0.4
Vitamin and mineral mix	0.2	0.2	0.2

known about the behavioral effect of enterostatin in avian. In this regard, we conducted the current study to investigate the enterostatin function in broiler chicks due to control of fat intake. The objective was to determine whether centrally administered enterostatin could inhibit fat intake in neonatal chicks.

Day-old Ross broiler chicks from both genders were purchased from a local hatchery (Mahan Chicken Meat Production Complex, Kerman, Iran). Chicks were housed in the temperature (30 ± 1 °C) and humidity (40–50%)-controlled electrical heating batteries and kept at 24 h lighting. They were maintained as flock, and then transferred to individual cages at least 1 day prior to injections. A commercial low-fat diet (23% crude protein and 2850 kcal/kg metabolizeable energy) and water were provided *ad libitum*. In Experiments 3 and 4, chicks were switched to feed multilevel fat diets 3 days before injections. The switching was started with a 4% fat diet and then continued to a 11% fat diet at 24 h time intervals in order to adapt the chicks to high-fat diet. Each part of fat diets separately purveyed and then admixed according to Table 1 [29]. Chicks at the age of 6–8 days were used for intracerebroventricular (ICV) injections which allowed time for chicks for yolk absorbion.

Enterostatin (APGPR) was purchased from Tocris Bioscience Co. (Bristol, UK) and was dissolved in a 0.85% NaCl solution containing 0.1% Evans Blue. This solution was also used as control. Solutions were injected ICV in a volume of 10 μl as described by Davis et al. [6] and Furuse et al. [11]. In brief, the head of the chick was held with an acrylic device that holds the bill of the chicks at a 45° angle and calvarium was parallel to the surface of the table as described previously [40]. A hole was made in a plate. This plate was placed over the skull immediately over the right lateral ventricle. Then a microsyringe was inserted into the ventricle through the hole in the plate and the test solution was injected. The tip of the needle penetrated only 4 mm below the skin of skull. This procedure dose not induces physiological stress in neonatal chicks [37]. Ten to twelve chicks received injections for each group.

Four experiments were conducted on each of the four treatment groups. In Experiments 1 and 2, low-fat intake and in Experiments 3 and 4, high-fat intake was monitored. The birds were deprived of food for either 3 h (Experiments 1 and 3) or overnight (Experiments 2 and 4) before ICV injections, but given free access to water. It does not necessarily expect that 3-h-fasting lead to negative energy balance in chicks.

After injection, feed was given freely and cumulative food consumption was measured at 15 through 180 min postinjection. Food intake was expressed as a percent of body weight to adjust differences between body weights.

At the end of the experiments, chicks were euthanatized using an intra cardiac sodium thiopental injection. Then, the brain was removed and the presence of Evans Blue dye in the lateral ventricle validated the proper injection. Data of birds that the dye was present in their lateral ventricle were used in the analysis. Sex was also determined by dissection.

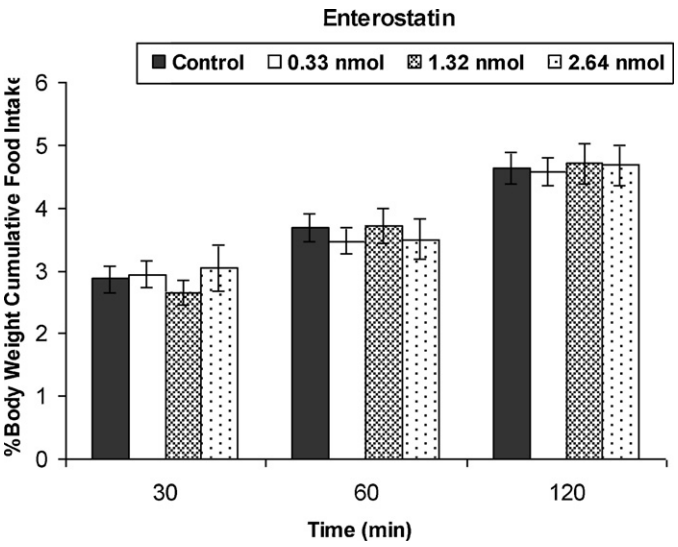

Fig. 1. Effect of intracerebroventricular administration of various doses of enterostatin on food intake (g/100 g BW) in 3 h fasted neonatal chicks fed with a low-fat diet. Values represent the Mean ± SEM.

Food intake was analyzed using a two-way analysis of variance (ANOVA) with repeated measure. The model was included sex and enterostatin as the between subject factors and time as within subject factor for repeated measures. A one-way ANOVA and post hoc Duncan's multiple range or Bonferroni tests were used for multiple comparisons between treatment groups at each time period. In all cases, significance was accepted at $P < 0.05$.

The data analysis of the current study showed that ICV injection of various doses of enterostatin has not any significant effect on low-fat intake of either 3 h or overnight fasted chicks (Figs. 1 and 2). In contrast, when the chicks were fasted for 3 h, high-fat diet intake was increased significantly in a dose dependent manner ($P < 0.01$) in all measured periods with the most effective dose of 1.32 nmol (Fig. 3). However, when the chicks underwent overnight fasting, high-fat intake tended to decrease through 120 min and was inhibited significantly at 180 min postinjection (Fig. 4). The repeated measure analysis revealed that in Experiment 3, the

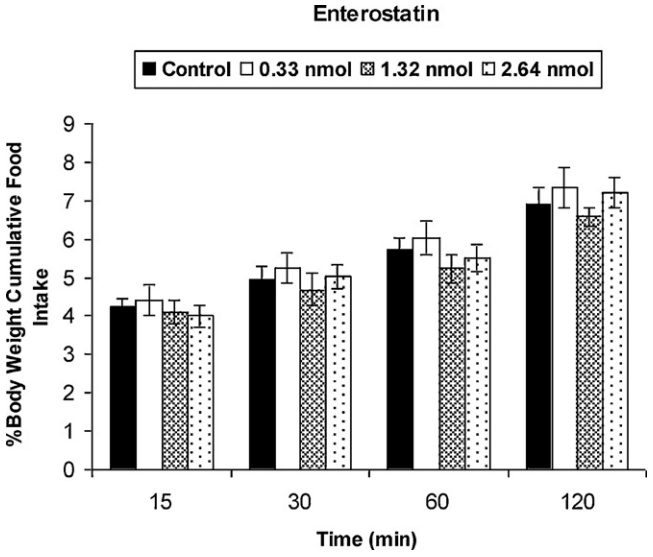

Fig. 2. Effect of intracerebroventricular administration of various doses of enterostatin on food intake (g/100 g BW) in overnight fasted neonatal chicks fed with a low-fat diet. Values represent the Mean ± SEM.