# Anti-Obese Effects of GLP-1 Related Peptides on MC4R Deficient Mice and Nauseous Experiment Systems

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## Introduction

Melanocortin 4 receptor (MC4R) is expressed in hypothalamus and controls feeding behavior and energy homeostasis. Recently, glucagon-like peptide-1 (GLP-1) related peptides were investigated as anti-obesity agents. GLP-1 receptor is also present in the central nervous system, but it is unclear whether GLP-1 related peptides are effective in people with MC4R-POMC pathway deficiency. Therefore, we evaluated anti-obese effects of clinical stage GLP-1 related peptides, semaglutide, tirzepatide, and retatrutide using MC4R KO mice.
Moreover, according to clinical study of these peptides, their main side effects were nausea and gastrointestinal disorders such as diarrhea, constipation and stomachache. In this study, we also evaluated these side effects of GLP-1 related peptides using conditioned taste aversion (CTA) test, kaolin intake test, and amylase secretion tests using rodent models.

## Method

【Efficacy study】
Thirty-five-week-old male MC4R KO and C57BL/6J mice were kept on a normal diet. Then, Saline, 0.1 mg/kg semaglutide, 0.05 mg/kg tirzepatide or 0.05 mg/kg retatrutide administered subcutaneously to mice daily for 3 weeks. Body weight, food intake, body composition, and energy expenditure were measured.

【Salivary amylase activity test】
Six-week-old Wistar rats had cotton balls placed in their mouths for one minute to collect saliva. The test articles were then administered, and saliva was collected again 11, 4, and 24 hours after administration. Salivary amylase activity was measured using L-Type amylase (Fujifilm Wako).

【Conditioned taste aversion test (CTA test)】
Nine-week-old C57BL/6J mice were housed under restricted water access (3 hours per day) for 7 days. On Day 8 and Day 10, taste aversion conditioning was performed by administering the test articles simultaneously with water containing 0.1% saccharin. On Day 12, mice were offered normal water and water containing 0.1% saccharin, and the percentage of saccharin water intake was calculated using the following formula.
Saccharin preference ratio (%) = 100× (saccharin water intake(g)/ total water intake(g))

【Gastric emptying test】
Ten-week-old C57BL/6J mice were fasted overnight, followed by one hour of restricted feeding. The test articles were then administered, and food was removed again. Gastric samples were collected 2 and 4 hours after administration, and the volume of gastric contents was quantified. The gastric emptying rate was subsequently calculated using the following formula.
Gastric emptying ratio (%) = 100× (1- (gastric contents(g)/food intake(g))

【Kaolin intake test】
Six- to seven-week-old SD rats were supplied with kaolin pellets ad libitum. The test articles were administered four days after the start of kaolin pellet supplementation. Kaolin intake was measured for one day following test articles administration.

**We possess a variety of expertise and technologies for obesity research.**
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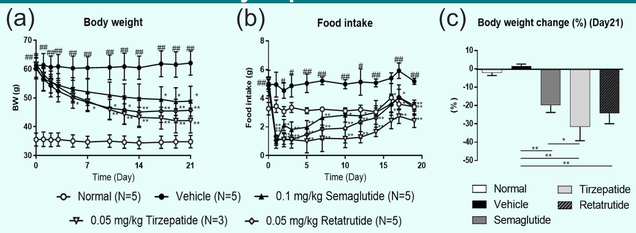
**COI disclosure information:**
We have no financial relationship to disclose for our presentation contents.

## Results

### GLP-1 related peptides showed significant body weight reduction as a clinical efficacy dependent manner

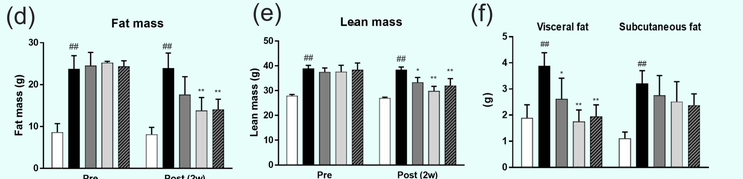




**These results mean MC4R knockout mice reflect the clinical effects of anti-obesity agents.**

EchoMRI-900
(Hitachi Aloka Medical, Ltd.)

Latheta LCT-200
(Hitachi Aloka Medical, Ltd.)

### GLP-1 related peptides reduced fat mass as well as lean mass




Oxymax system
(Columbus Instructions)

### All GLP-1 related peptides reduced energy expenditure

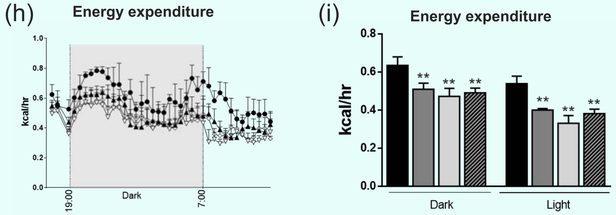
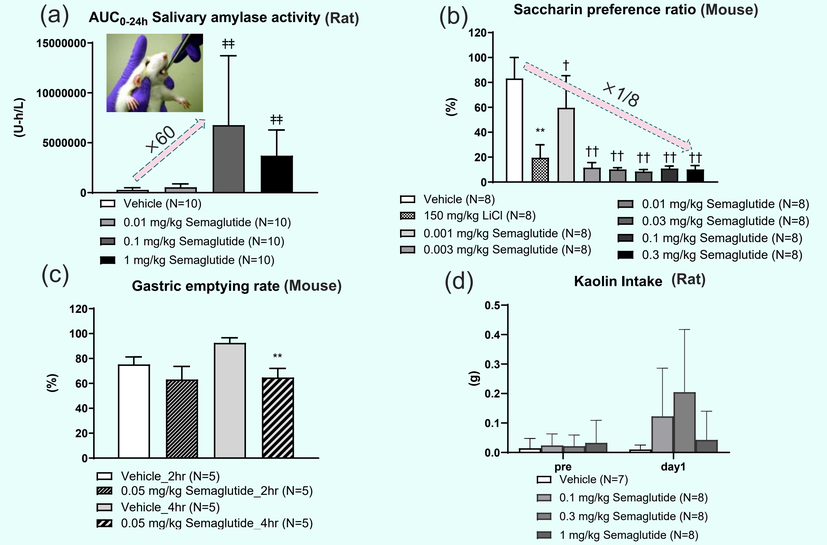



**Fig. 1 Effects of GLP-1 analogs on body weight and food intake in MC4R KO mice**
(a) Body weight. (b) Food intake. (c) Percentage change in body weight over the 21-day treatment period. (d) Fat mass. (e) Lean mass. (f) Visceral & subcutaneous fat. (g) X-ray CT image. (h)Time course of energy expenditure. (i) Mean of energy expenditure in dark and light phase. Data are represented as the mean ± SD. # p<0.05, ## p<0.01, vs Normal group by Student's *t*-test, * p<0.05, ** p<0.01, vs Vehicle or Semaglutide group by Tukey test

### Various tests reflect nausea-inducing properties of GLP-1-related peptides.

| Assay | Concordance with nausea mechanism | Limitation |
|---|---|---|
| Salivary Amylase | Stimulation of the sympathetic nervous system increases amylase secretion. | These responses may also occur in reactions other than nausea-inducing stress. |
| Conditioned Taste Aversion (CTA) | By conditioning sweetness with aversion, mice are induced to avoid sweetness. | |
| Gastric Emptying Delay | The retention of stomach contents is a contributing factor to nausea and vomiting. | |
| Kaolin Intake | Stress causes rodents to exhibit behaviors involving ingestion of non-food items (pica behavior). | |




**Amylase and CTA may have high sensitivity in assessing the clinical risk of nausea.**

**Fig. 2 Evaluation of responsiveness of Semaglutide in rodent models of nausea**
(a) Salivary amylase activity. (b) Saccharin preference ratio (CTA test). (c) Gastric emptying rate. (d) Kaolin intake. Data are represented as the mean ± SD. ** p<0.01, vs Vehicle group by Student's *t*-test, † p<0.05, †† p<0.01, vs Vehicle group by Williams' test, ‡‡ p<0.01, vs Vehicle group by Shirley-Williams' test,

## Conclusion & Next step

- All GLP-1 related peptides demonstrated superior anti-obesity effects in MC4R knockout mice.
  ⇒ These data suggest efficacy of GLP-1-related peptides in patients with MC4R-POMC pathway deficiency.
  ⇒The MC4R knockout mouse is a model that reflects the clinical efficacy of anti-obesity agents.
- Semaglutide responsiveness was confirmed in various rodent nausea models, reflecting the side effects of GLP-1-related peptides.
  ⇒These models may prove useful for developing more beneficial drugs

We aim to contribute to the discovery of GLP-1-related peptides and combination therapies without nausea side effects using these evaluation systems.