Comparison of anti-obese effects on GLP-1 analogue peptides, Semaglutide, Tirzepatide and Retatrutide using MC4R deficient obesity model mice

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Introduction

POMC-MC4R pathway in hypothalamus controls food intake and body weight[1]. Patients with POMC mutation causes severe early phase obesity, increase of food intake[2]. The aim of this study was to evaluate the efficacy of GLP-1 analogs in obesity caused by MC4R-POMC pathway deficiency. We also evaluated and compared the efficacy of Semaglutide, Tirzepatide and Retatrutide, recently developed as anti-obesity agents.

Method

【Animals】
35-week-old male MC4R KO[3] 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.

【EchoMRI & X-ray CT】
Body components of fat and lean mass were measured using the Echo-MRI system at 1 day before dosing and 14 days after dosing.
Abdominal visceral and subcutaneous fat mass, and muscle mass were measured using an in vivo X-ray CT system (Latheta LCT-200; Hitachi Aloka Medical) 19 days after dosing.
CT images were acquired using the following parameters: 48 mm axial field of view, and 40×96×2016 μm voxel size to analyze abdominal visceral and subcutaneous fat mass. Ten cross-sectional images taken from the sacral to lumbar spine were analyzed to quantify abdominal visceral and subcutaneous fat.

【Oxymax】
At day 15 after dosing, the mice were housed individually in the metabolic chamber of the Oxymax system (Columbus Instructions) according to the manufacturer's instructions. At 5:00 pm, mice were administered with the vehicle (saline), semaglutide (0.1 mg/kg, s.c.), tirzepatide (0.05 mg/kg, s.c.), and retatrutide (0.05 mg/kg, s.c.). The metabolic rate and RQ were measured from 7 pm to 1 pm (19:00-7:00, dark phase; 7:00- 13:00, light phase)

【Statistics】
Data involving more than two groups were assessed using one-way analysis of variance, followed by Tukey's multiple comparisons test. Differences between two groups were assessed using Student's t-test. Bonferroni's correction was applied for comparative analysis at multiple time points.
The results of the statistical analysis are shown below.
# p<0.05, ## p<0.01, vs Normal (Student's t-test)
* p<0.05, ** p<0.01, vs Vehicle (Tukey's test)

Results

All GLP-1 analogs showed significant food intake and body weight reduction

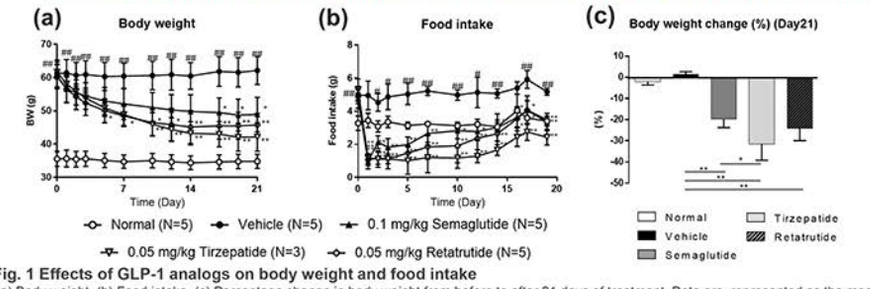


Fig. 1 Effects of GLP-1 analogs on body weight and food intake
(a) Body weight. (b) Food intake. (c) Percentage change in body weight from before to after 21 days of treatment. Data are represented as the mean ± SD.

All GLP-1 analogs improved plasma parameters and lipid synthesis related gene expression

Table1 Levels of plasma biochemical parameters
Data are represented as the mean ± SD

	Normal (N=5)	Vehicle (N=5)	Semaglutide (N=5)	Tirzepatide (N=3)	Retatrutide (N=5)
ALT (U/L)	26.8 ± 12.5	143.2 ± 94.4#	33.5 ± 7.7*	37 ± 16.6	29.7 ± 23.5*
Total choresterol (mg/dL)	69.9 ± 13.3	152.7 ± 62.3#	96.2 ± 17.6	69.8 ± 14.3*	79.4 ± 9.2*
Insulin (ng/mL)	0.94 ± 0.41	11.92 ± 6.31##	0.92 ± 0.52**	0.94 ± 0.96**	2.04 ± 1.37**
HOMA-IR	14.82 ± 5.23	178.38 ± 93.14##	13.30 ± 7.92**	7.47 ± 6.28**	24.87 ± 19.48**

Table2 Hepatic mRNA expression levels of lipid synthesis and inflammation marker
Rplp0 was used as the endogenous control. Data are represented as the mean ± SD

	Normal (N=5)	Vehicle (N=5)	Semaglutide (N=5)	Tirzepatide (N=3)	Retatrutide (N=5)
Scd1	1.00 ± 0.15	2.46 ± 0.65##	0.96 ± 0.53**	0.47 ± 0.37**	0.82 ± 0.27**
Fasn	1.00 ± 0.41	2.83 ± 1.08##	1.86 ± 0.41	0.87 ± 0.53*	1.53 ± 0.72
Tnf	1.00 ± 0.62	3.12 ± 1.70#	3.97 ± 1.21	2.41 ± 0.93	4.04 ± 2.87
Il1b	1.00 ± 0.18	2.57 ± 1.04#	2.31 ± 0.72	3.44 ± 1.50	2.93 ± 1.94

Ameliorative effects on liver and heart hypertrophy were observed

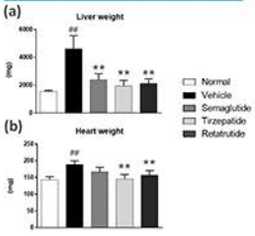
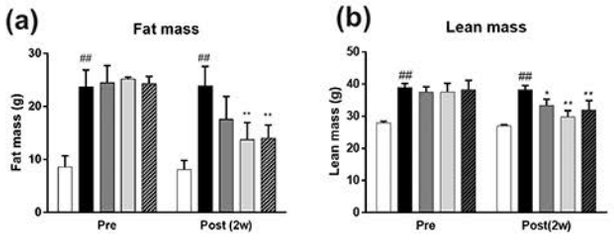
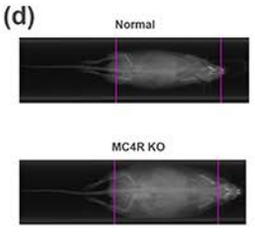
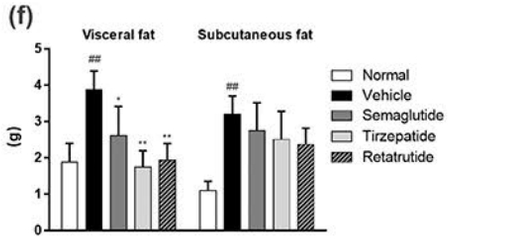


Fig. 2 Effects on Liver and heart weight
(a)Liver weight, (b)heart weight. Data are represented as the mean ± SD.

GLP-1 analogs reduced fat mass as well as lean mass







(c)

	Vehicle	Semaglutide	Tirzepatide	Retatrutide
Soleus muscle (mg)	13.36 ± 0.43	11.70 ± 0.94	11.91 ± 1.91	11.10 ± 1.15*
Gastrocnemius muscle (mg)	173.50 ± 8.18	154.68 ± 17.11	152.67 ± 17.41	157.76 ± 13.08
Tibialis anterior muscle (mg)	59.78 ± 4.18	55.23 ± 3.71	55.62 ± 5.50	55.42 ± 5.58
Extensor digitorum longus (mg)	12.02 ± 4.44	12.01 ± 2.84	12.15 ± 1.43	12.98 ± 2.37
Total (mg)	258.67 ± 12.16	233.62 ± 22.60	232.35 ± 25.80	237.26 ± 21.59

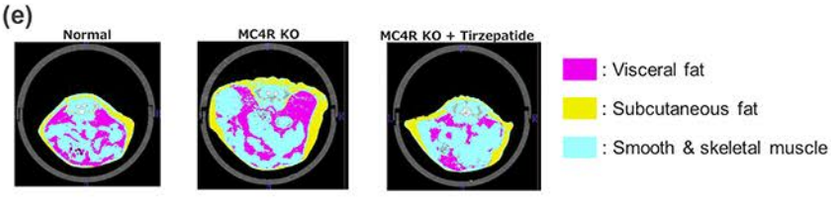
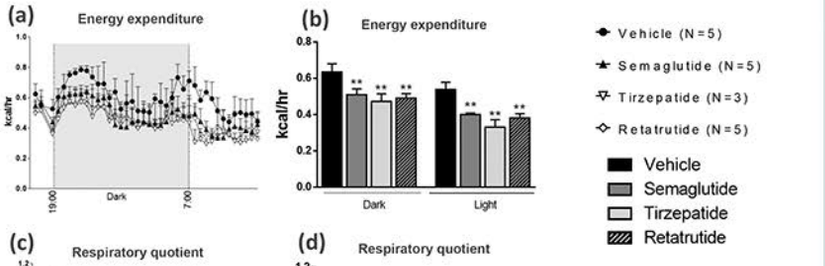


Fig. 3 Evaluation of the effects of GLP-1 analogs on body composition
(a,b) Fat and lean mass before and after treatment. (c) Various muscle weights of the right thigh. (d) Scout image of mice whole body scanned by X-ray CT. (e) Cross-sectional image of the peri-abdominal area imaged by X-ray CT. (f) Quantitative evaluation value of visceral and subcutaneous fat. Data are represented as the mean ± SD.

All GLP-1 analogs reduced energy expenditure



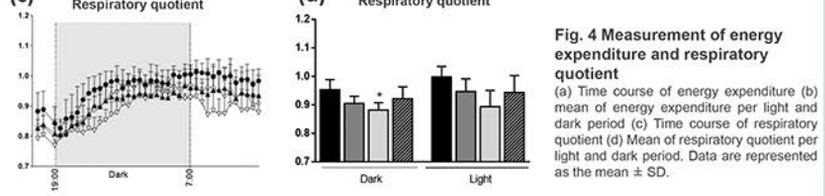


Fig. 4 Measurement of energy expenditure and respiratory quotient
(a) Time course of energy expenditure (b) mean of energy expenditure per light and dark period (c) Time course of respiratory quotient (d) Mean of respiratory quotient per light and dark period. Data are represented as the mean ± SD.

Conclusion

The present study confirms that all GLP-1 analogs have significant anti-obesity effects in MC4R KO mice. Comparison of the effects of weight loss and suppression of food intake showed that Tirzepatide had a stronger effect than Semaglutide. On the other hand, all GLP-1 analogs showed to decrease lean mass and energy expenditure, suggesting decreased lean mass may contribute to decreased energy expenditure. The muscle loss effect of GLP-1 analogs has been reported in clinical trials[4] and may be an issue in the development of future obesity drugs.

These results mimic the previously reported clinical effects of GLP-1 analogs thus indicate that MC4R KO mice are a suitable animal model of obesity for preclinical evaluation of anti-obesity drugs. The study also confirmed the efficacy of GLP-1 analogs in the MC4R-deficient condition, suggesting that GLP-1 analogs may be a good therapeutic option for patients with MC4R-POMC pathway deficiency.

1) M J Krashes, *et al.*, *Nat Neurosci.* 2016 Feb;19(2):206-19.
2) H Krude, *et al.*, *Nat Genet.* 1998 Jun;19(2):155-7.
3) M Matsumoto, *et al.*, *PLoS One.* 2020 Jan 28;15(1).
4) I J Neeland, *et al.*, *Diabetes Obes Metab.* 2024 Sep:26 Suppl 4:16-27.

We possess a variety of devices and technologies for obesity.
And, we have deep knowledge and experience in drug discovery research on obesity!
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