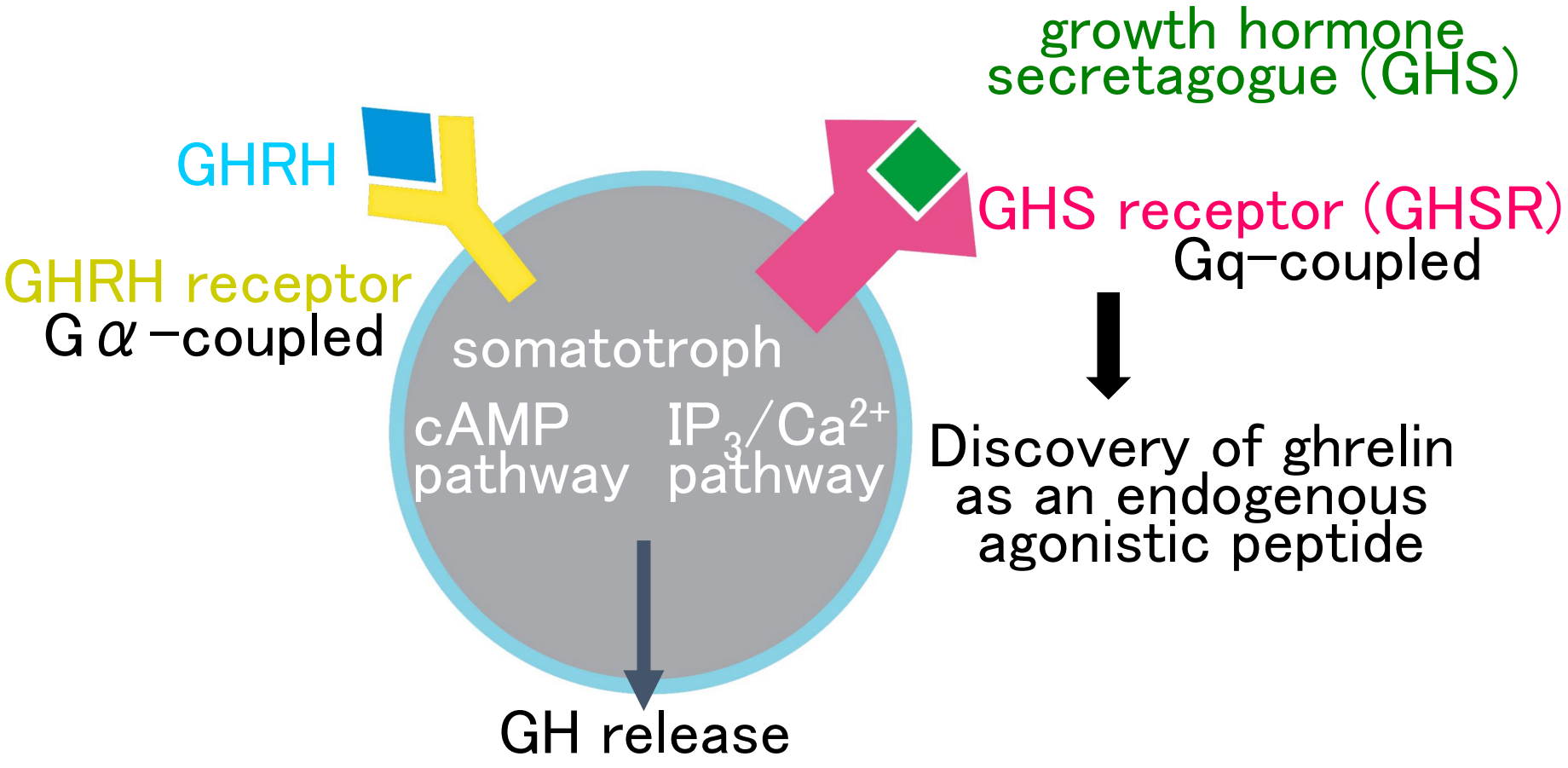
From Discovery to Clinical Insights: Ghrelin, LEAP2, and the Stomach-Liver Axis in Appetite Control

Masamitsu Nakazato

Osaka University

Ghrelin stimulates growth hormone release

Kojima M, Nakazato M, Kangawa K *et al*, Nature 1999, 402, 656-660



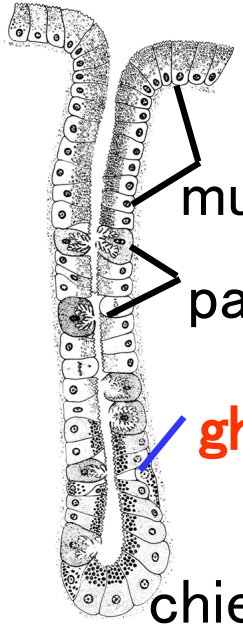
Ghrelin: Kojima M, *et al*, Nature 1999, Nakazato M, *et al*, Nature 2001

hybrid of amino acid and fatty acid

Oxyntic gland in the stomach

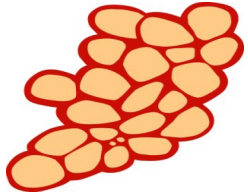
Ghrelin–*O*–acyltransferase (GOAT)

octanoyl acid contained in the diet (middle chain fatty acid)

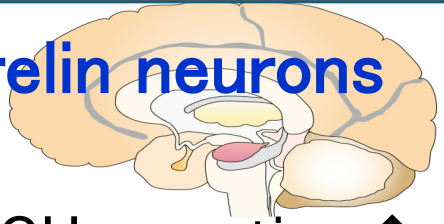


Desacyl ghrelin lacks fatty acid addition

Ghrelin has multifaceted roles

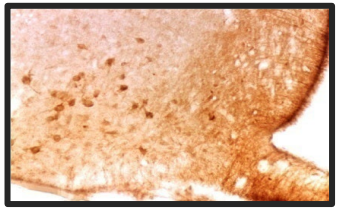


fat accumulation

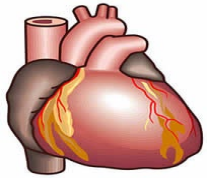


ghrelin neurons

GH secretion ⬆



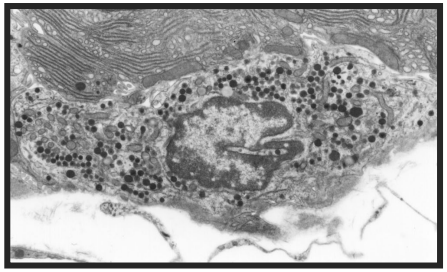
appetite ⬆



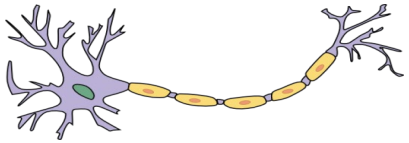
cardiovascular protection

gut motility ⬆

gastric acid secretion ⬆



vasodilation

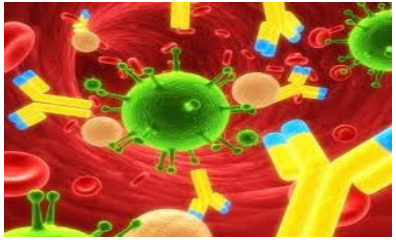


neuroprotection

bone growth

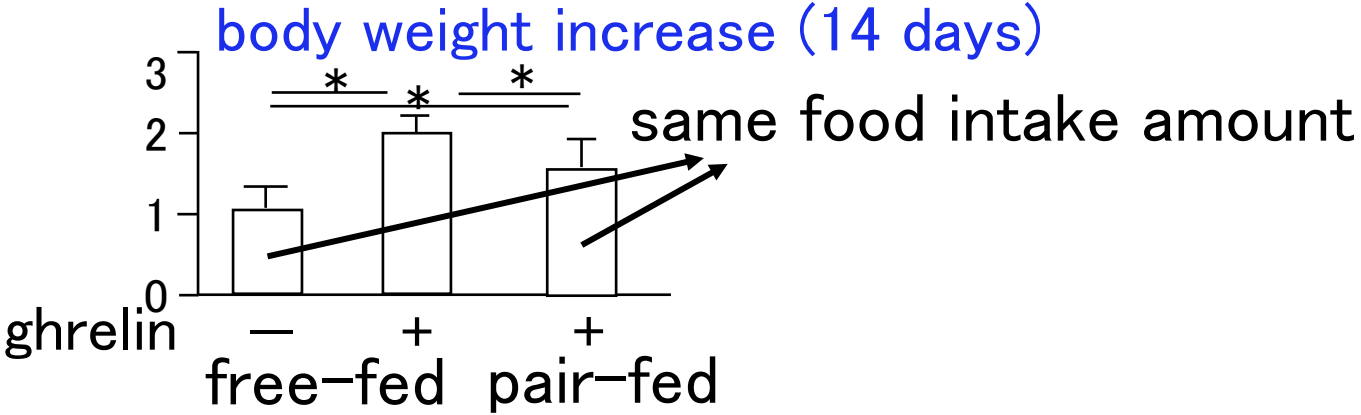
ghrelin-producing cells

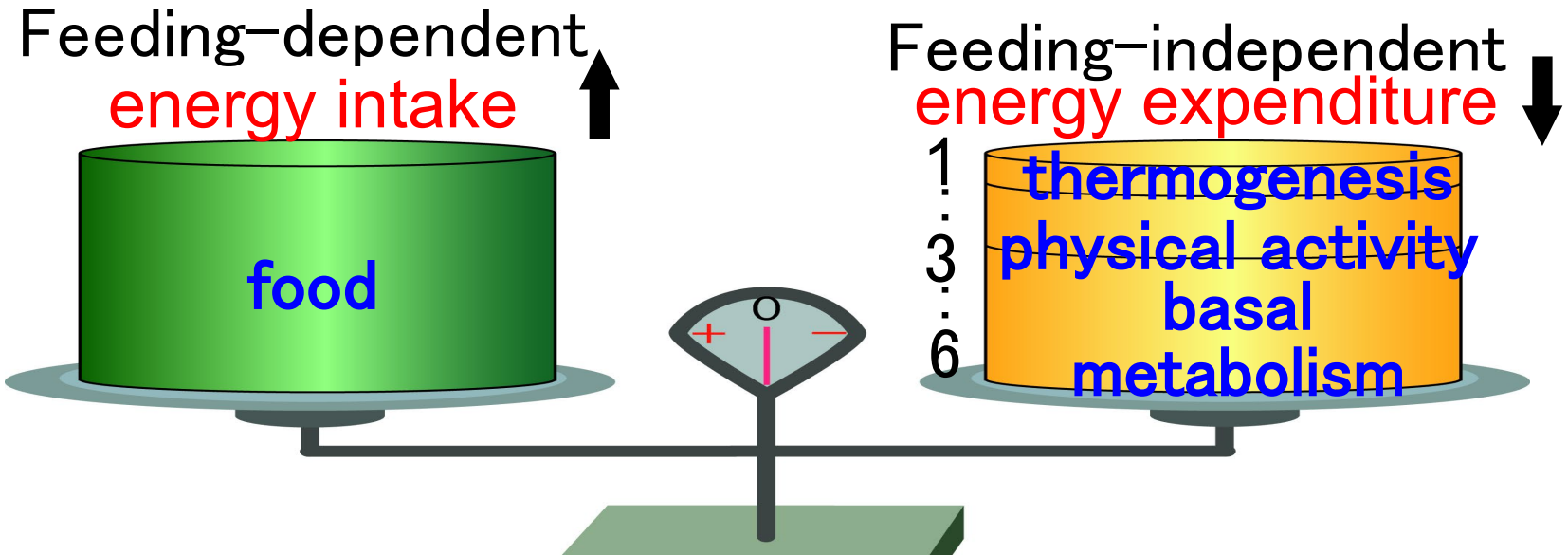
body temperature ⬇



inflammation ⬇

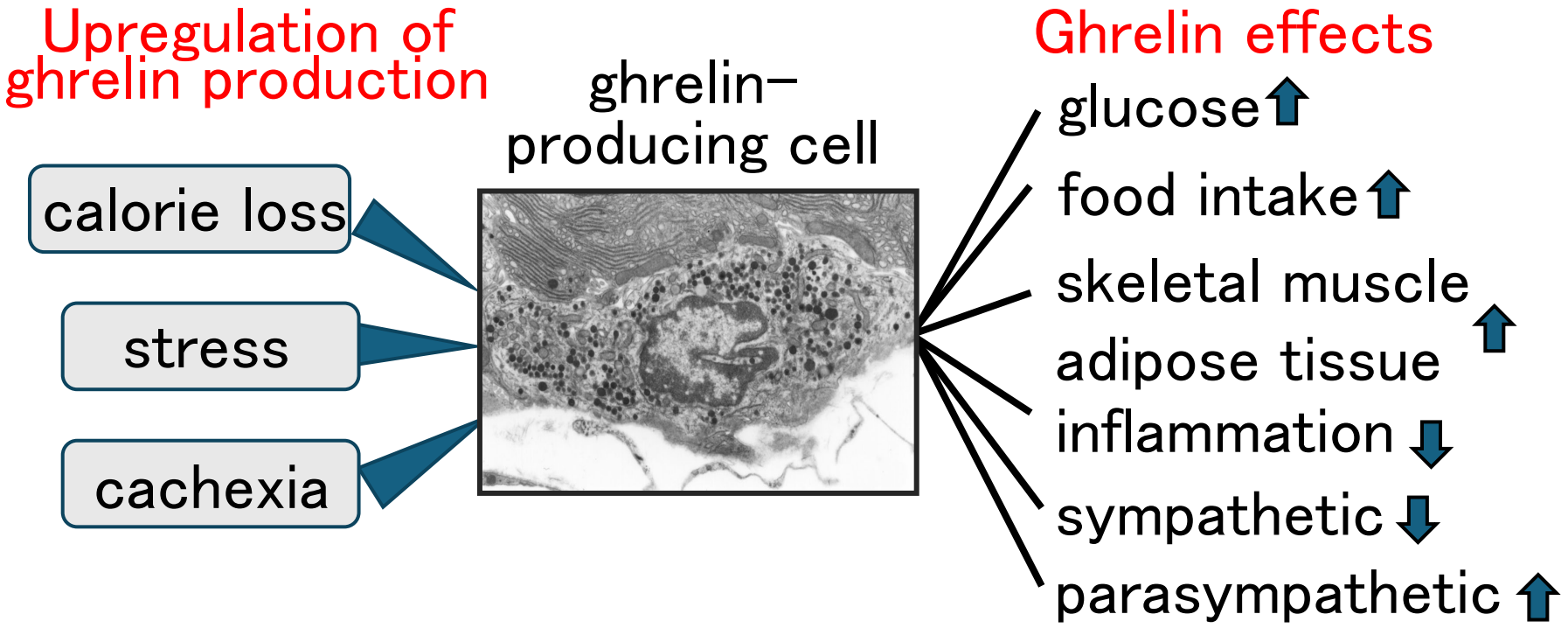
Ghrelin increases body weight by the two mechanisms





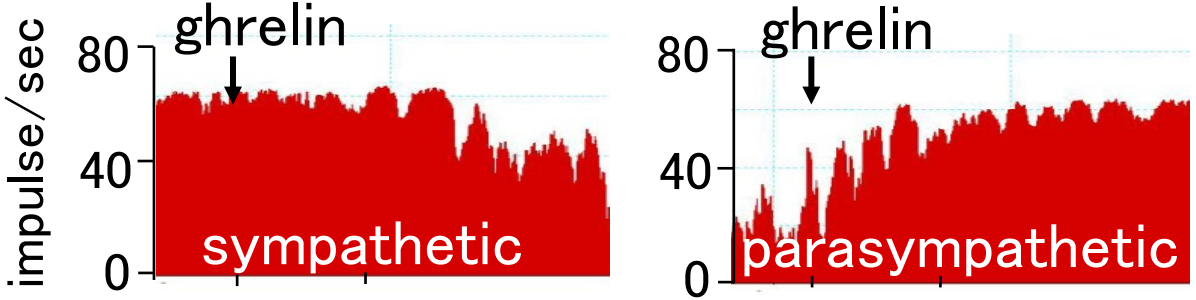
Ghrelin functions as a vital peptide

The ratio of ghrelin to desacyl ghrelin in the human blood 1 : 10
(ghrelin: 10–20 fmol/mL, desacyl ghrelin: 100–150 fmol/mL)



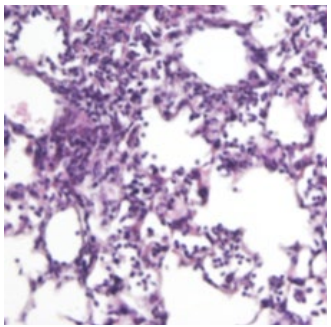
Yanagi S, Nakazato M *et al*. Cell Metab, 2018. 27: 786–804

Ghrelin suppresses sympathetic efferent and activates parasympathetic efferent

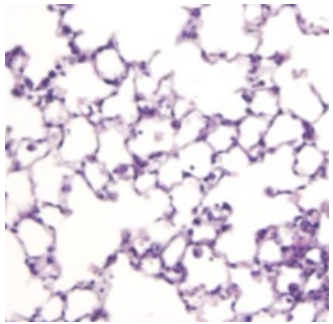


Ghrelin curbs lung inflammation via the vagal nerve (rat)

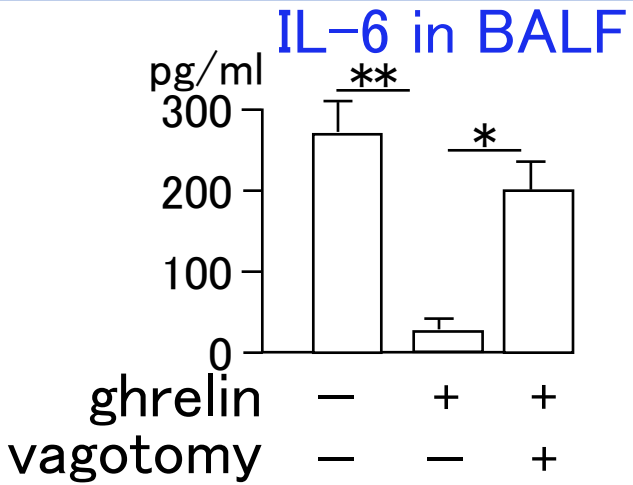
ghrelin (−)



ghrelin (＋)

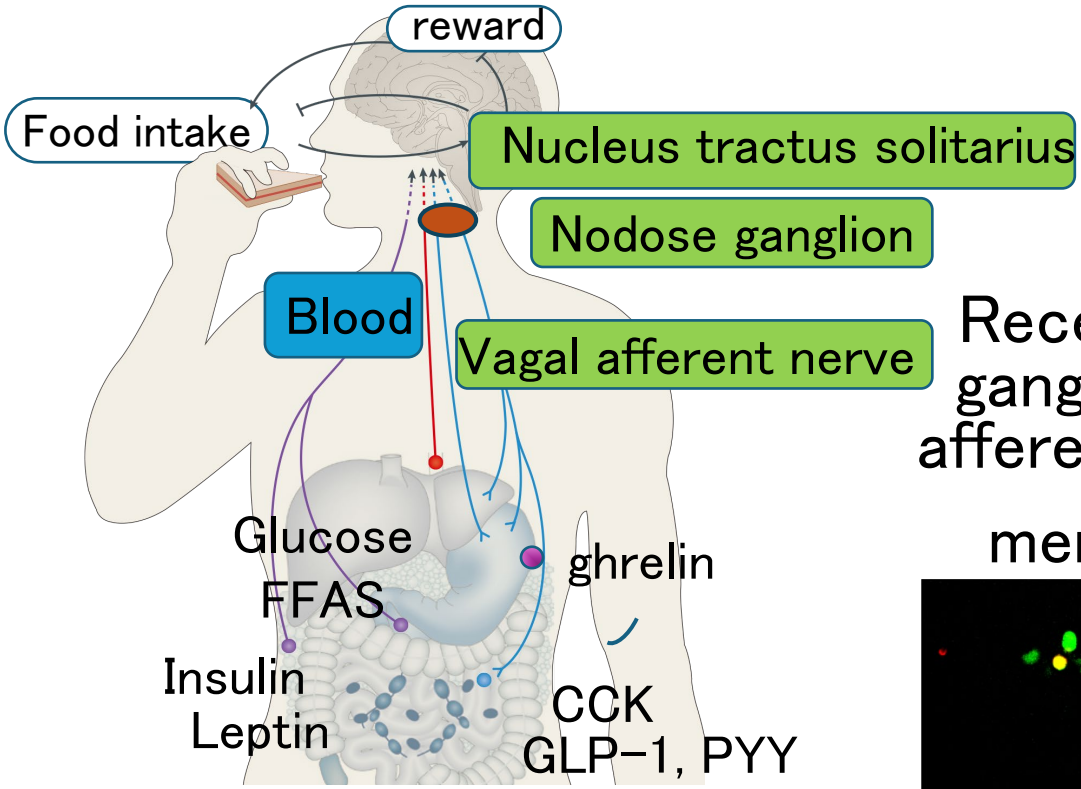


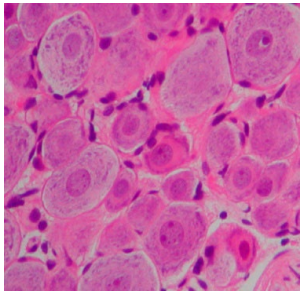
Ghrelin suppressed lung inflammation in a rat model of bleomycin-induced lung injury



Matsumoto N, *et al.* Endocrine J. 2017

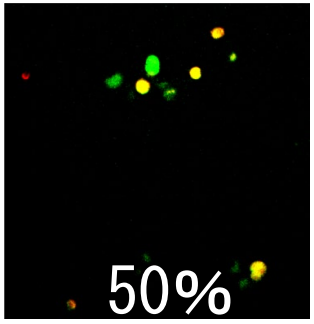
Metabolic information from the periphery to the brain in feeding regulation

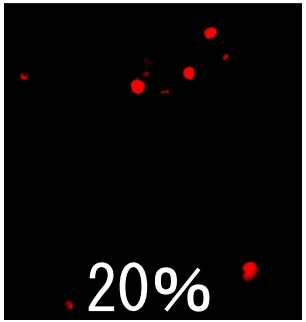


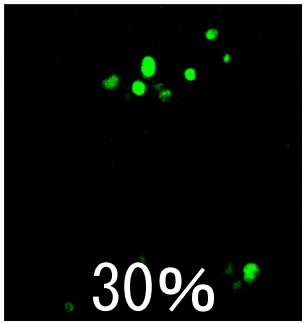


nodose ganglion neurons

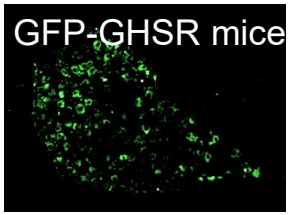
Receptor expression in nodose ganglion neurons which receive afferent signals from the stomach



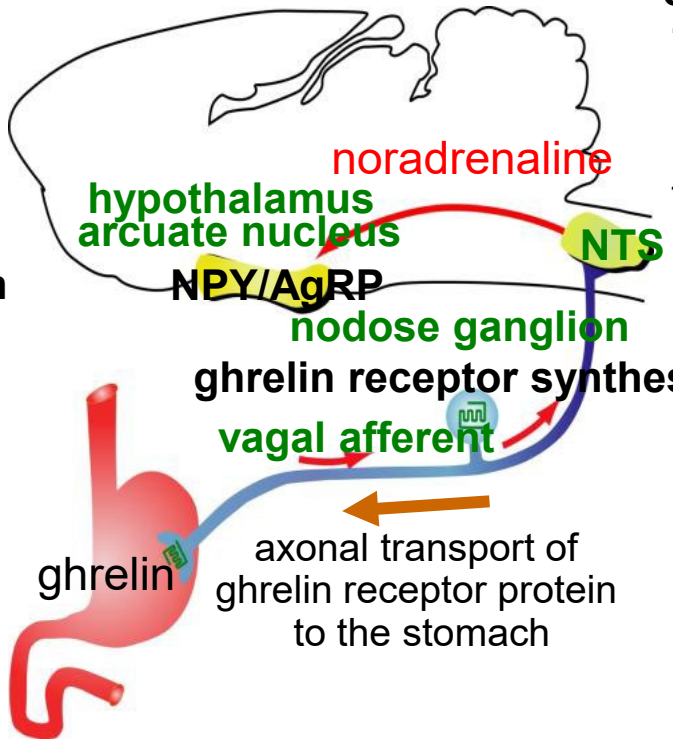




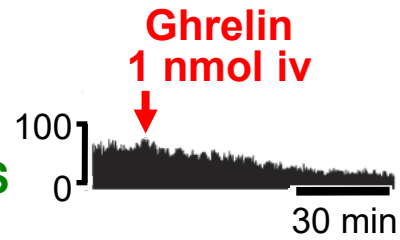
Stomach-derived ghrelin signals (feeding and GH secretion) are relayed to the hypothalamus via the vagal afferent nerve



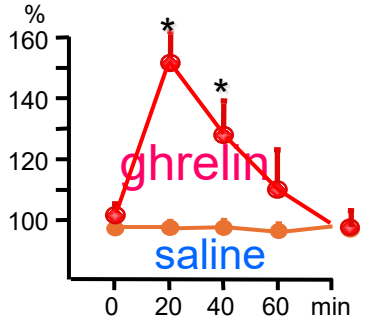
Ghrelin receptor-expressing neurons in the nodose ganglion

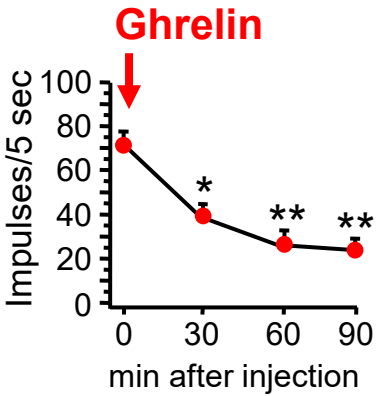


Ghrelin suppressed electric activity of the vagal afferent



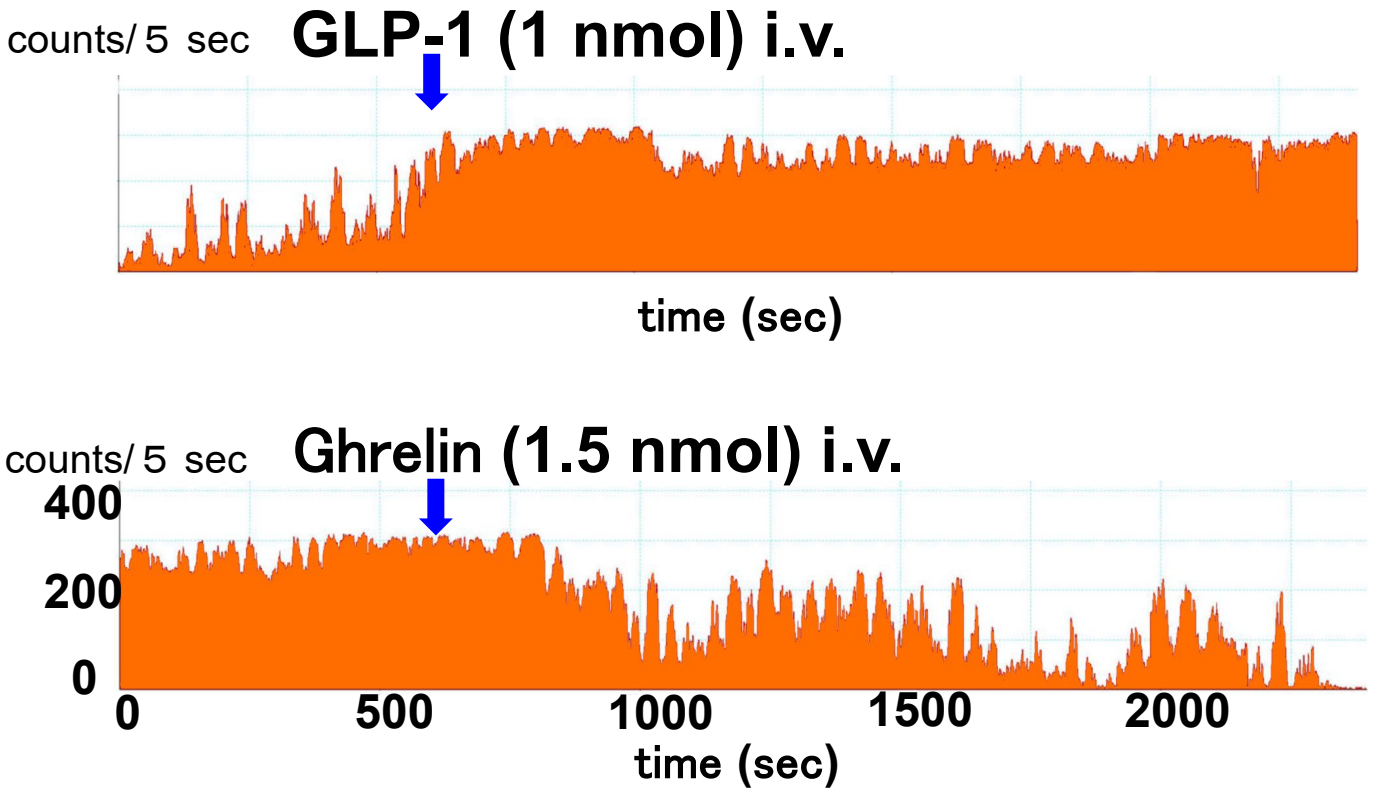
Ghrelin increased noradrenaline release in the arcuate nucleus



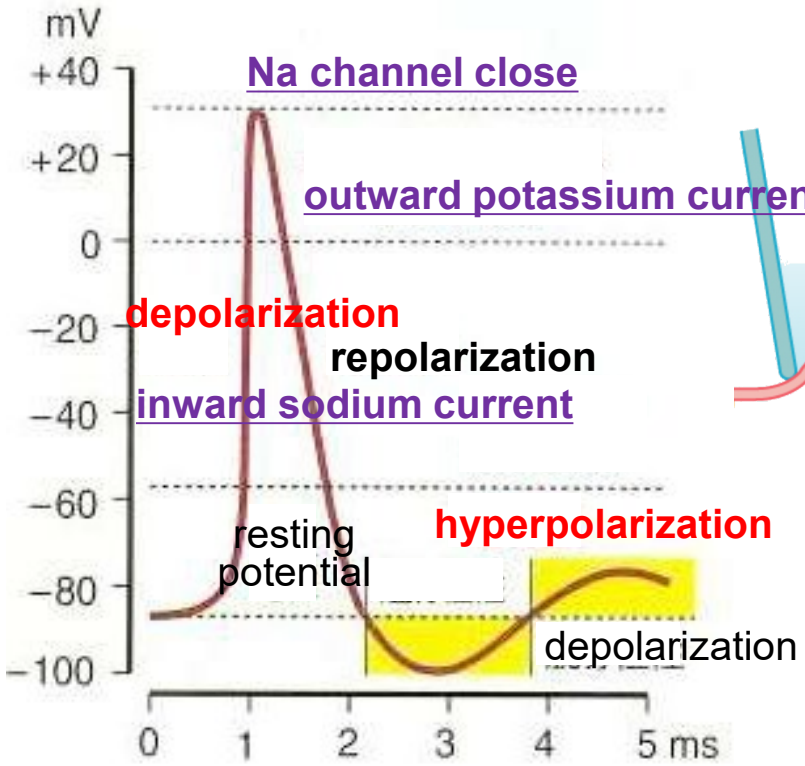


Nakazato M. *et al.* Cell Metab 2006, 4, 323-331

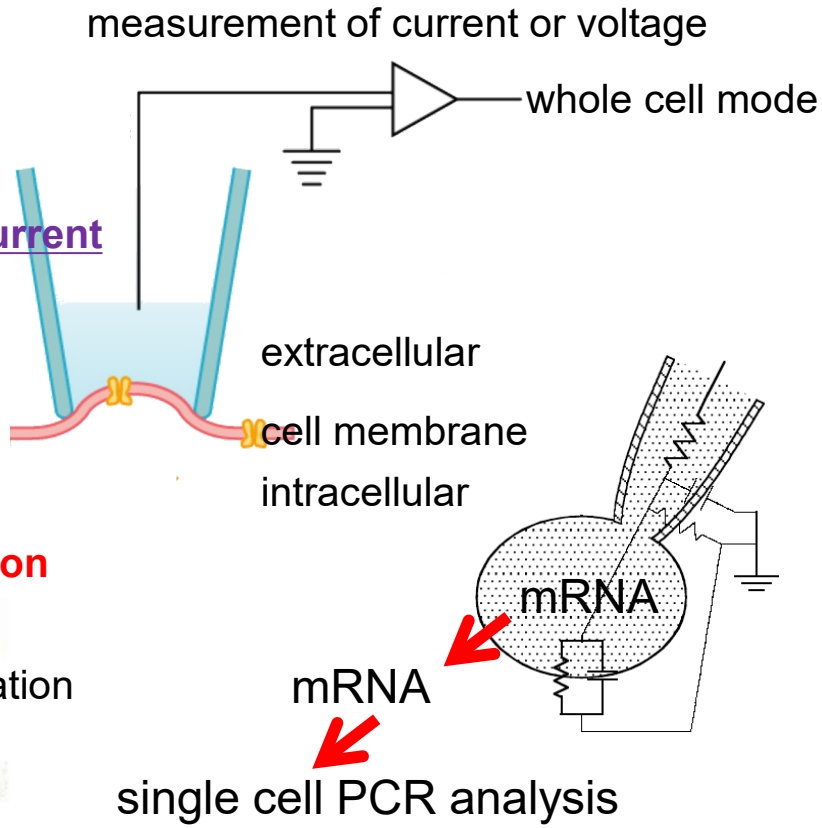
GLP-1 potentiates vagal afferent electrical activity, whereas ghrelin attenuates



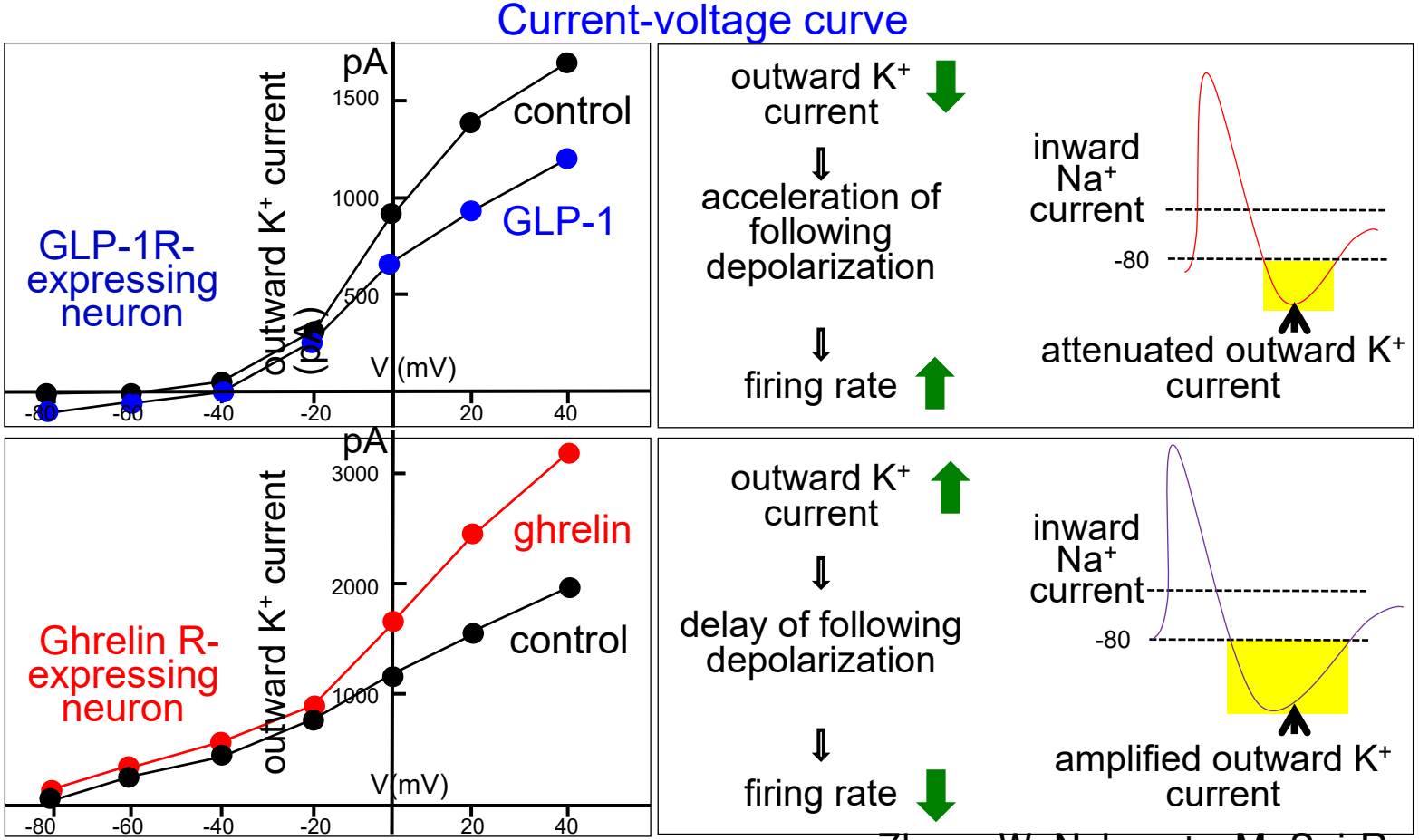
Action potential



Whole-cell patch clamp

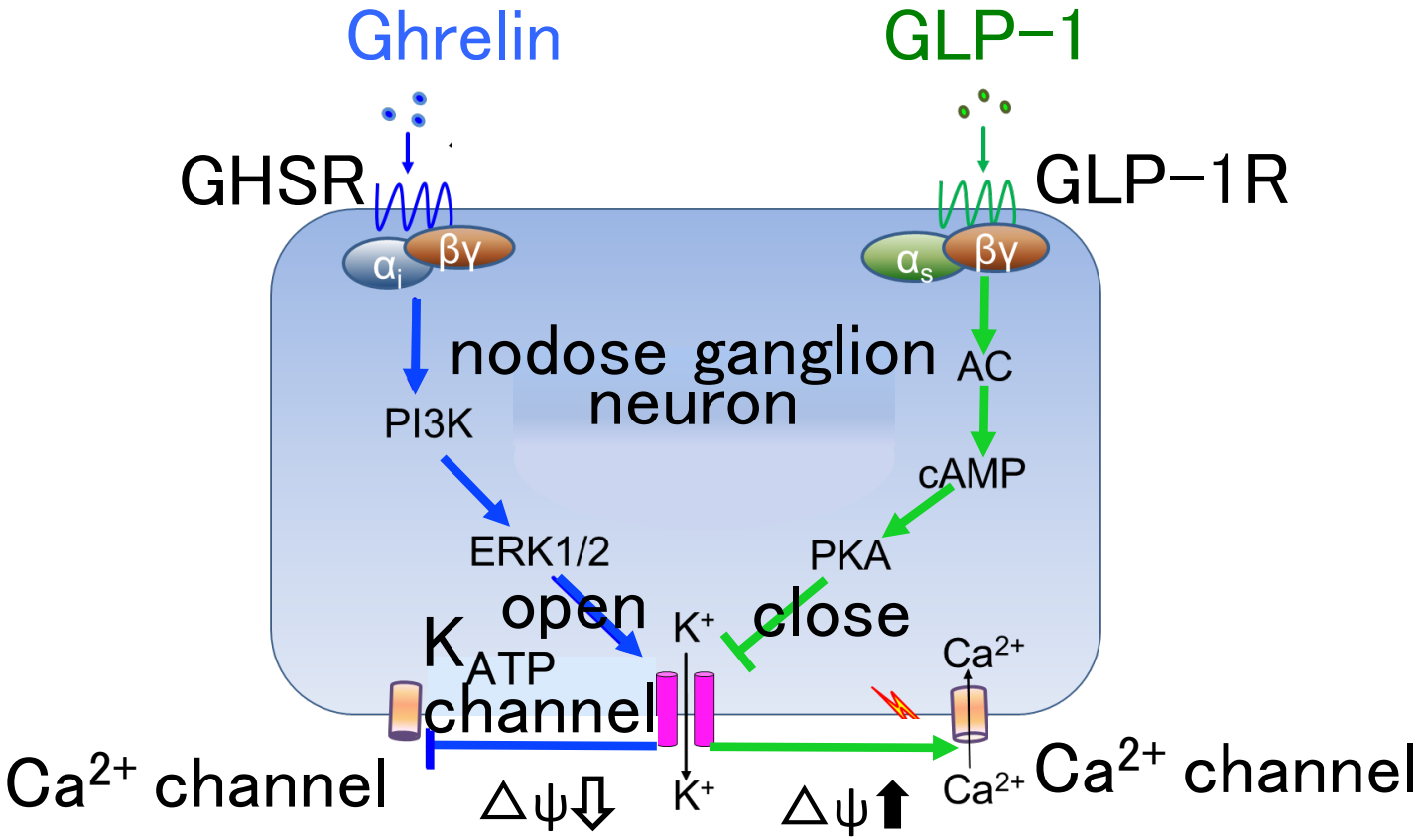


Electrophysiological properties of vagal ganglion neurons in response to ghrelin and GLP-1



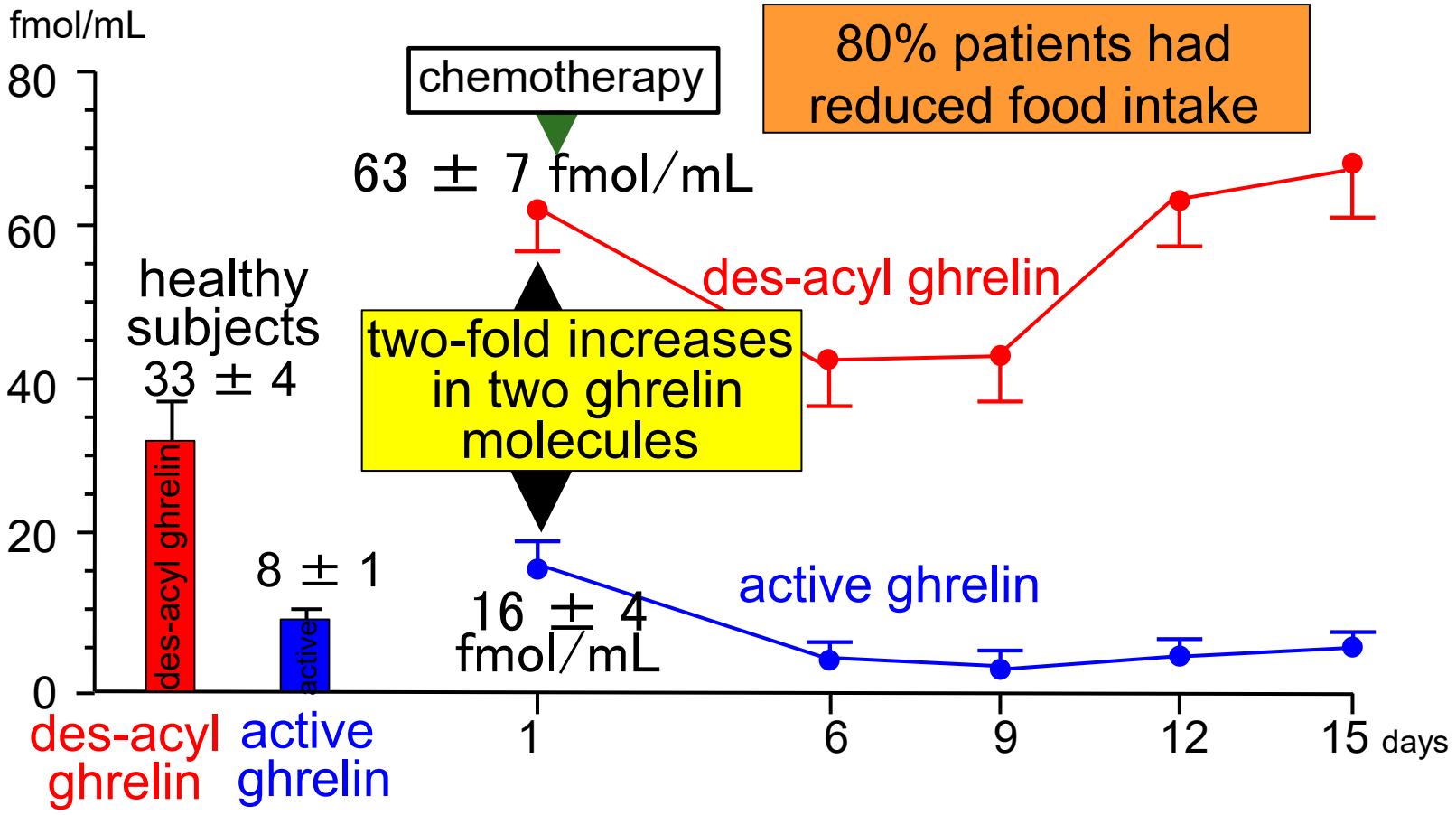
Zhang W, Nakazato M, Sci Rep 2022

Ghrelin and GLP−1 act on the K_{ATP} channel−Ca^{2+} channel axis to regulate nodose ganglion neuron in the feeding regulation

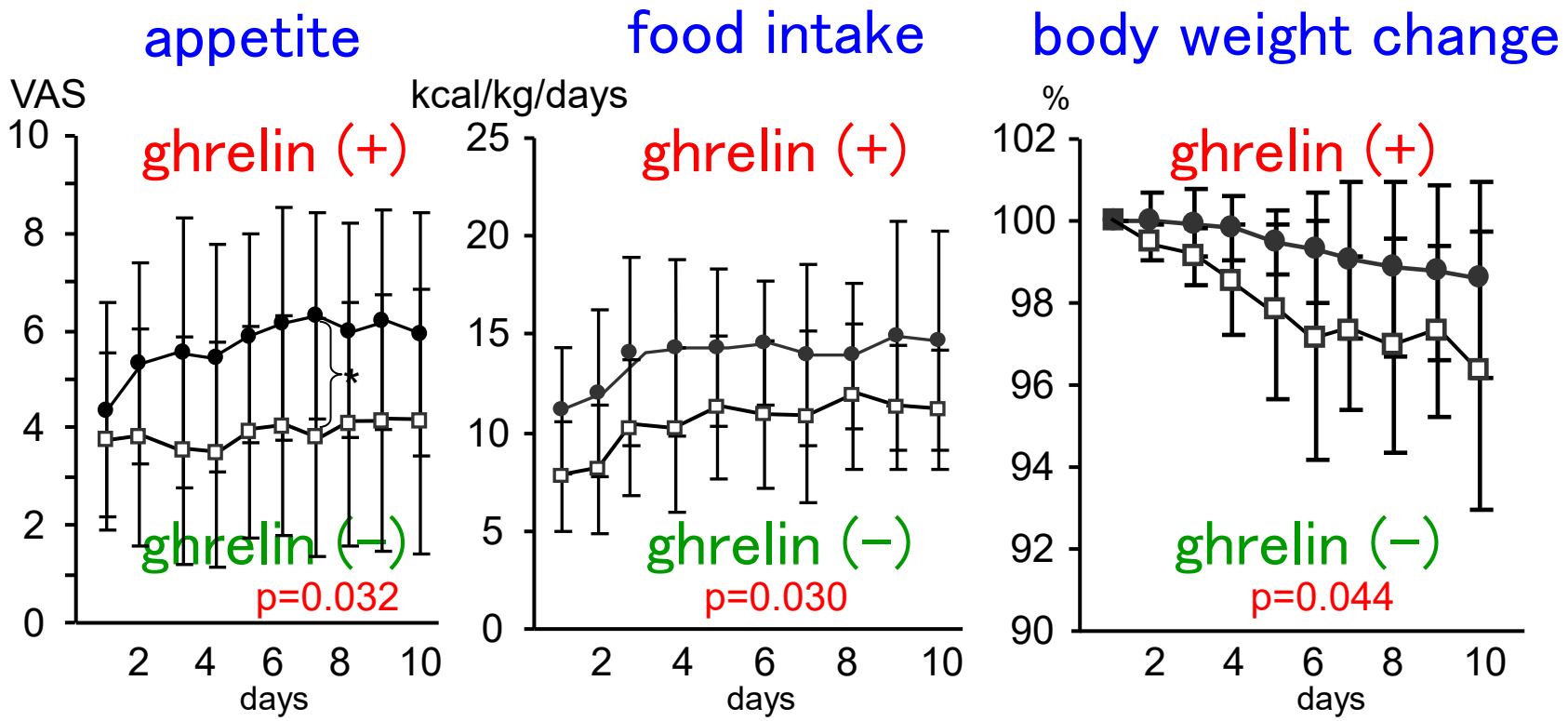


Zhang W, Nakazato M, Sci Rep 2022

Ghrelin production is upregulated in lung cancer, but decreased by chemotherapy



Daily ghrelin administration increased food intake and body weight in patients undergone gastrectomy



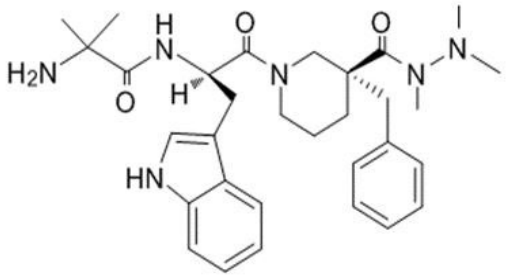
Adachi S, et al. Gastroenterology, 2010

Clinical trials of ghrelin in Japan (252 cases since 2006)

category	number
A. Respiratory	
lung cancer	10
COPD	33
chronic respiratory failure	42
lower tract infection	14
B. Digestive	
gastric cancer	20
esophageal cancer	100
hepatic cancer	7
functional dyspepsia	7
C. Circulatory	
chronic cardiac failure	7
D. Anorexia nervosa	5
E. Diabetic polyneuropathy	7

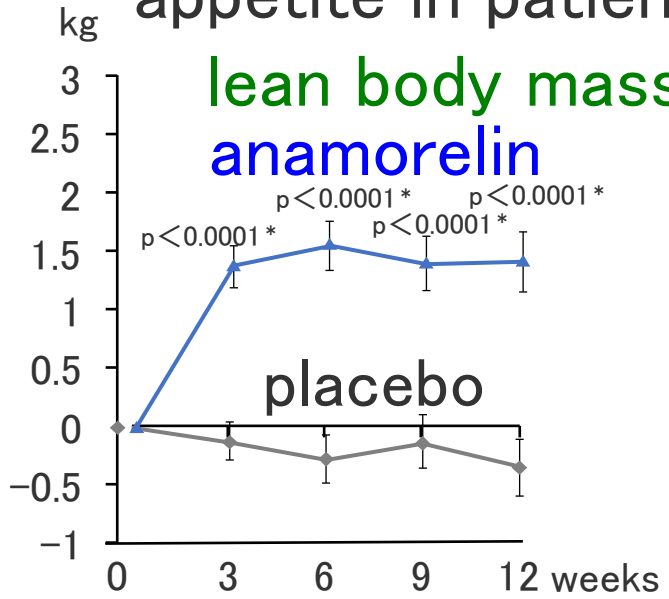
Yanagi S, Sato M, Kangawa K, Nakazato M. Cell Metab. 2018. 27: 786–804

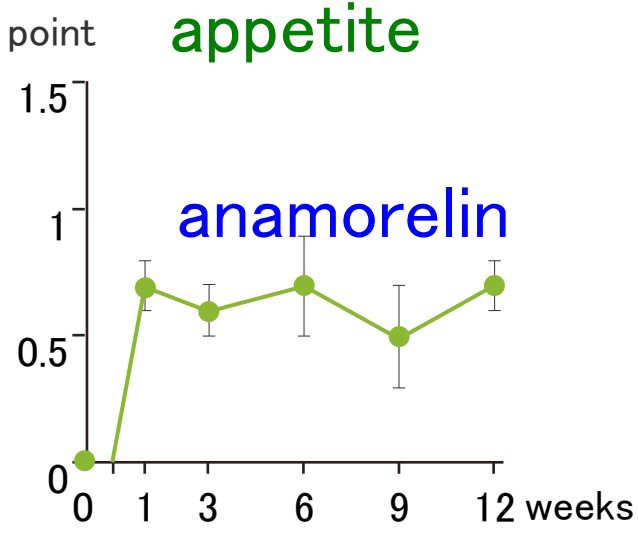
Anamorelin, a GHSR agonist



Anamorelin, a ghrelin-based chemical compound, was launched in Japan in 2021 for the treatment of cancer cachexia

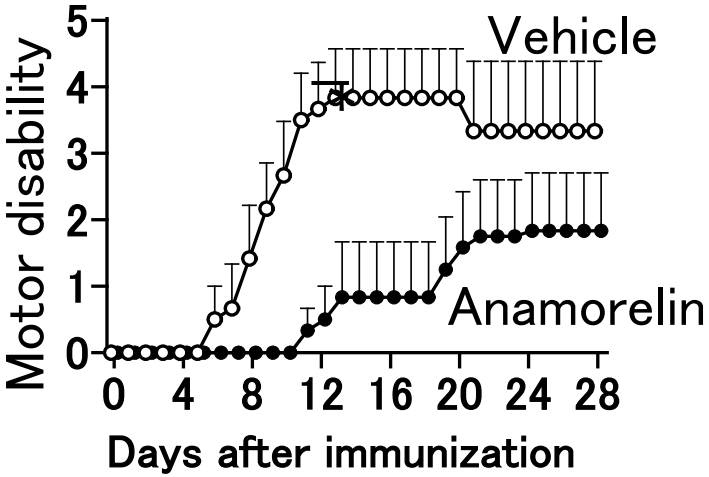
Daily anamorelin increased lean body mass and appetite in patients with cancer cachexia



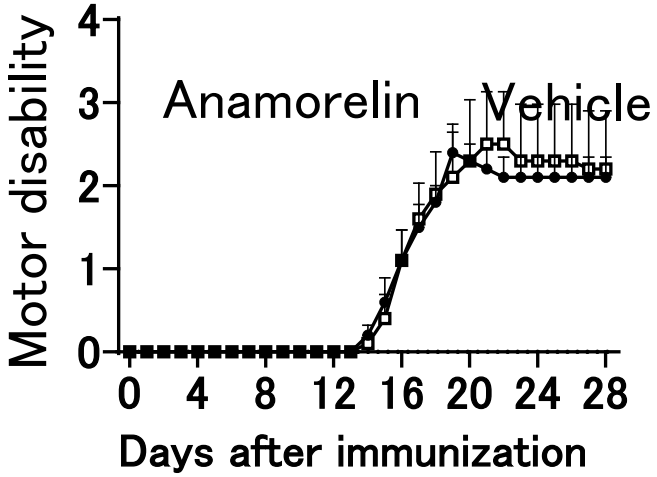


Anamorelin, aGHSR agonist alleviates neurological symptoms in multiple sclerosis

Wild type mouse



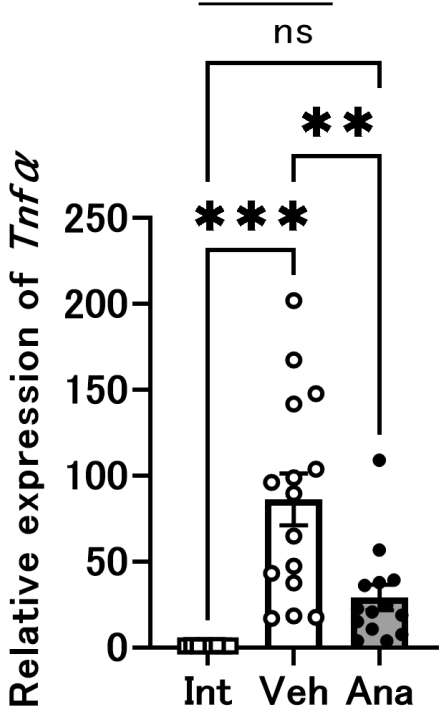
GHSR deficient mouse



Anamorelin（－）

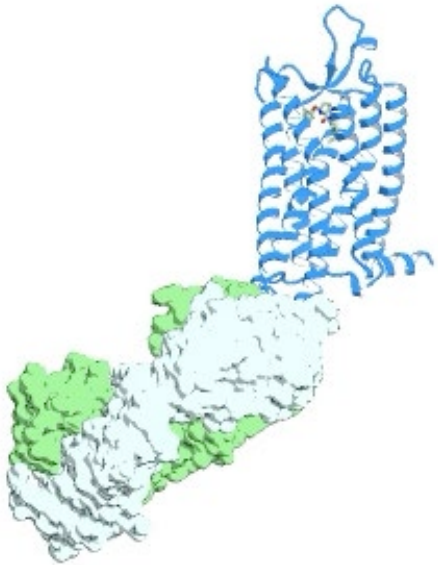
Anamorelin （＋）

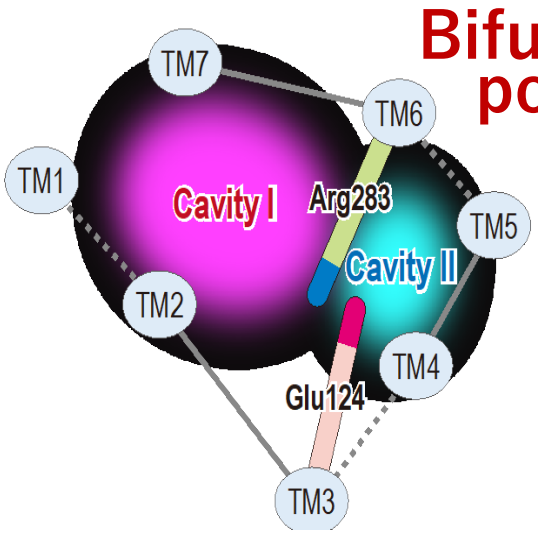
Tnfα



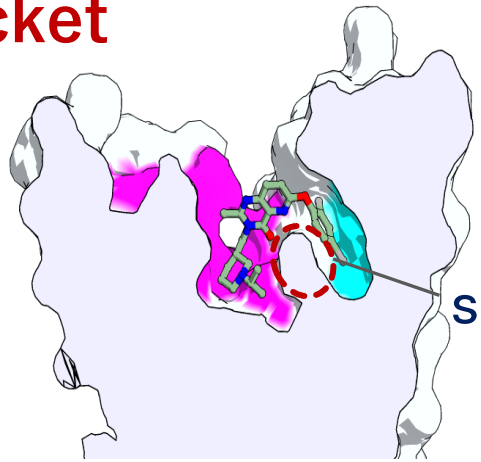
in submission

Structure of ghrelin receptor–Fab complex





(top view)

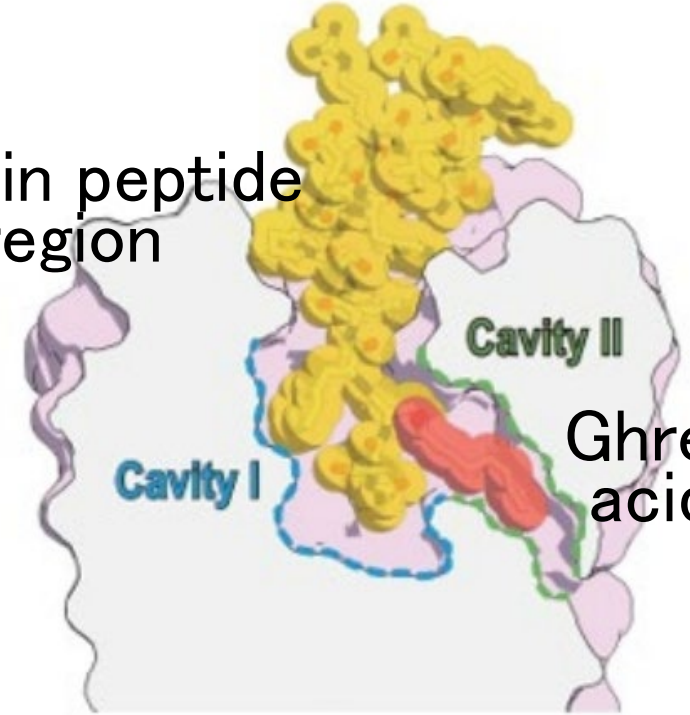


(side view)

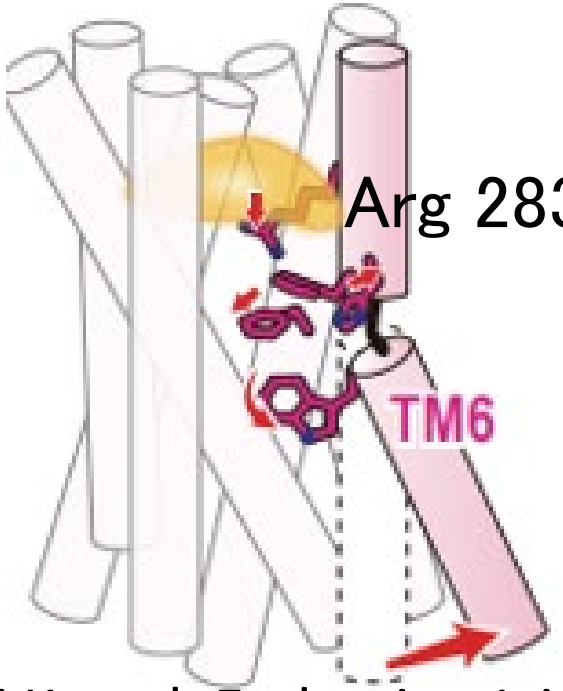
GHSR has a characteristic bifurcated pocket structure that has not been found in any GPCRs.

The pocket is divided into two cavities by a salt bridge between glutamate 124 of transmembrane helix 3 and arginine 283 of TM6.

Ghrelin pushes downward Arg 283 that forms a salt bridge, shifting TM6 outward. The intracellular region of GHSR more opens, allowing G protein access and activating intracellular signaling.

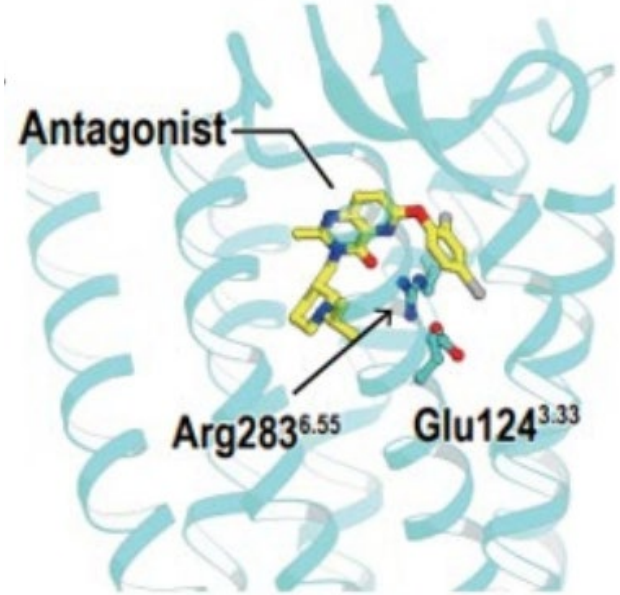


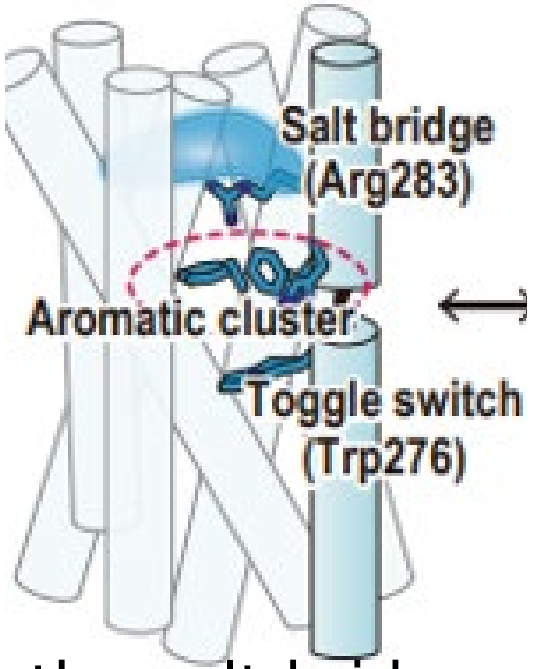
Ghrelin–bound GHSR



Sakai K et al. Endocrine J, in press

Antagonist–bound GHSR structure

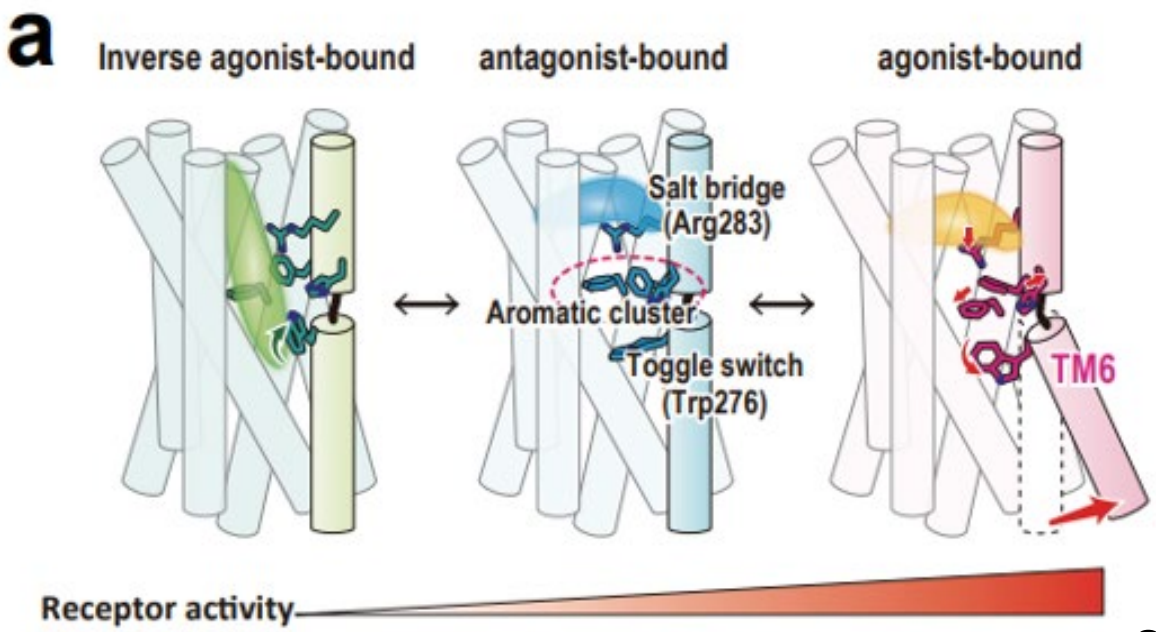


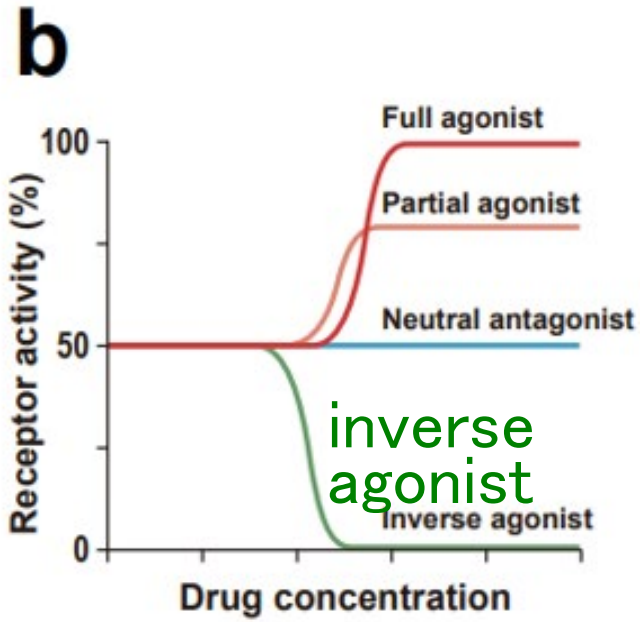


The antagonist in yellow straddles the salt bridge and fills the two cavities. Tryptophan 276 reorientated and TM6 moves inward to block G protein access.

Sakai K et al. Endocrine J, in press

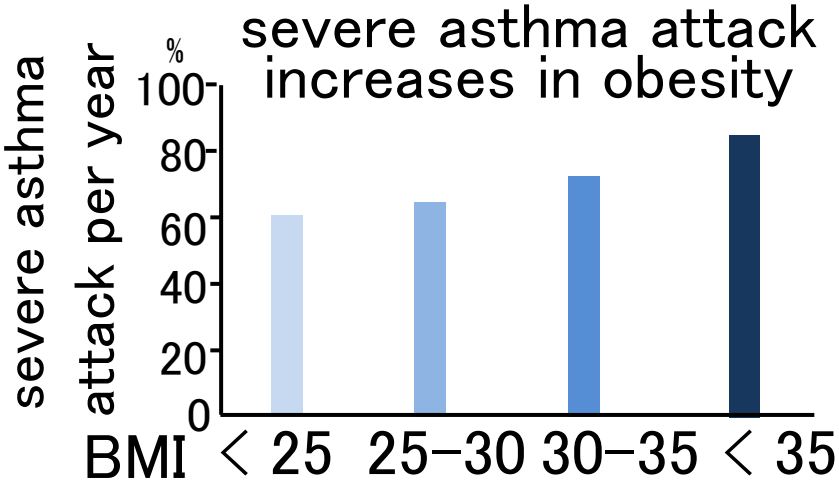
GHSR has a high constitutive activity (50% signaling activity in the basal state).
A GHSR inverse agonist coordinates TM6 vertically.
This prevents tryptophan 276 from shifting to its active form, thereby diminishing receptor activity.

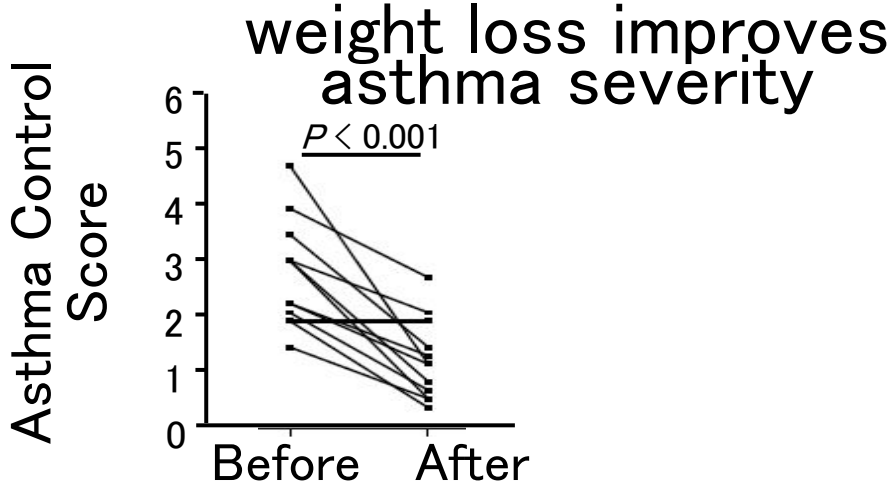




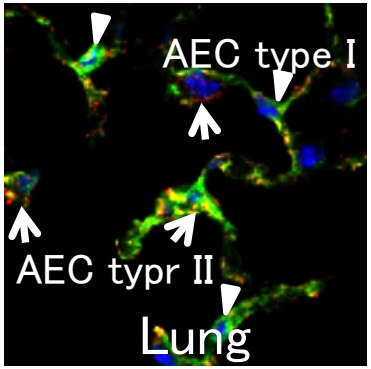
Sakai K et al. Endocrine J, in pres

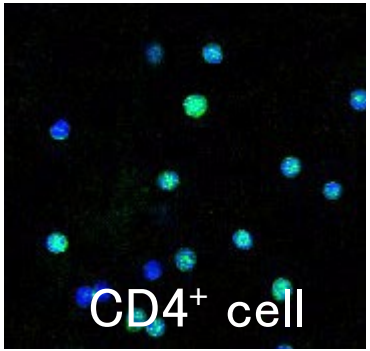
Obesity is the risk of asthma attack





GHSR expression in lung and $CD4^+$ cells in GHSR–GFP Tg mice

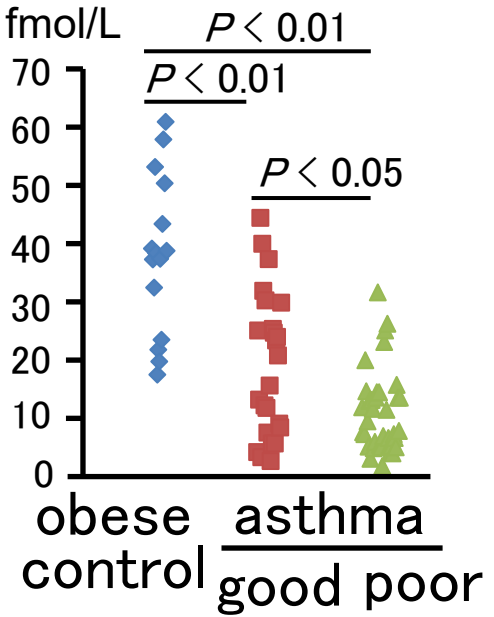




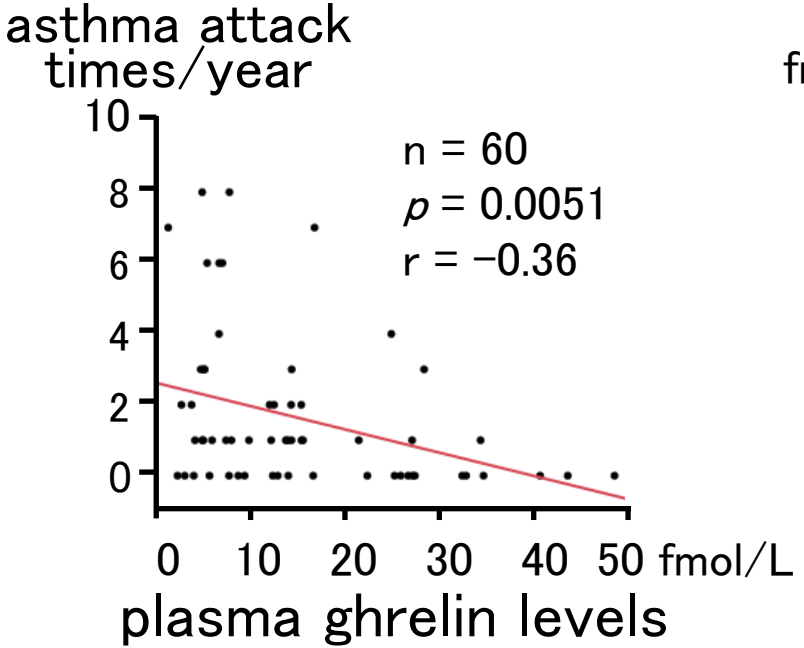
green, GFP blue, DAPI

Plasma ghrelin in asthma patients with obesity (BMI > 25)

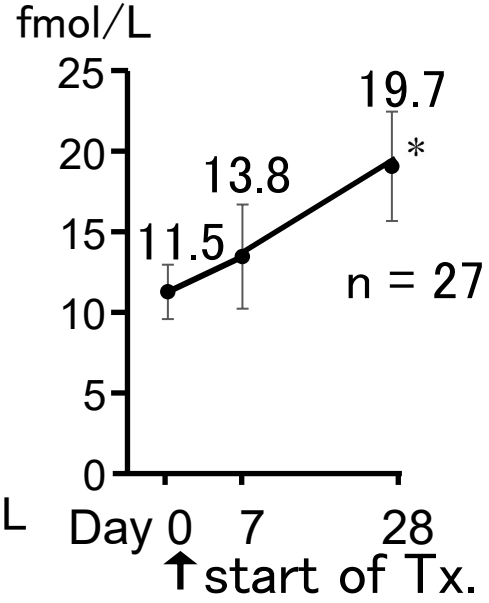
plasma ghrelin



plasma ghrelin and asthma attacks



plasma ghrelin changes after asthma treatment



Ghrelin has an anti-inflammatory role to prevent asthma attack of patients with obesity and lower plasma ghrelin could be a prediction marker for asthma attack.

Ghrelin reduction is involved in obesity-related asthma

Asthma pathogenesis

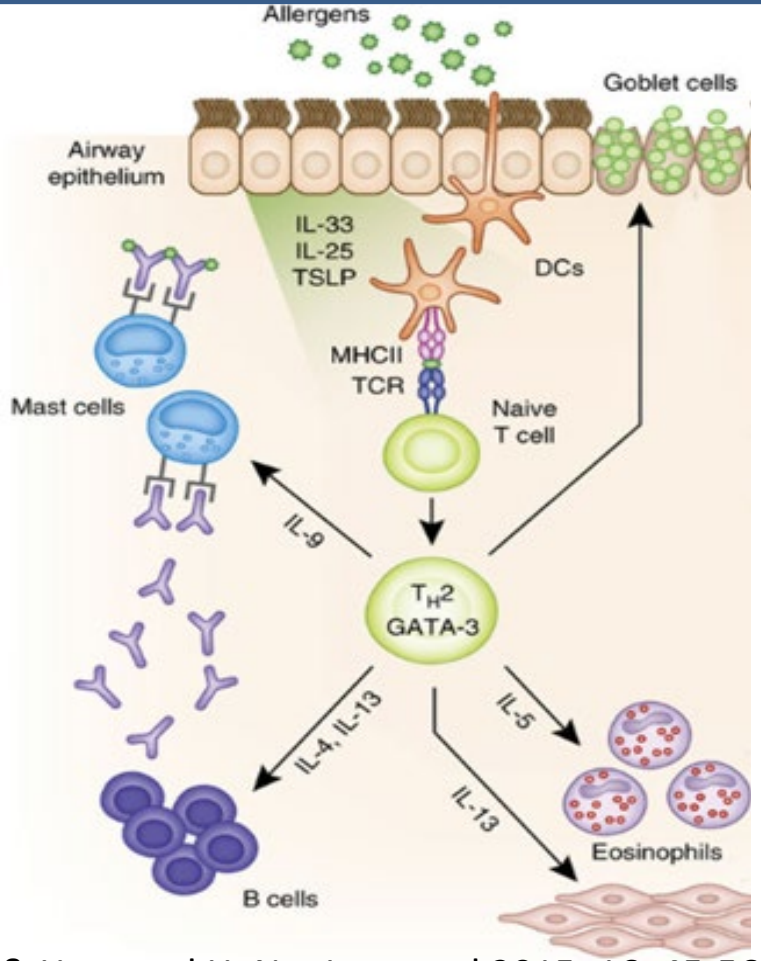
Obesity

Ghrelin

Airway epithelial injury

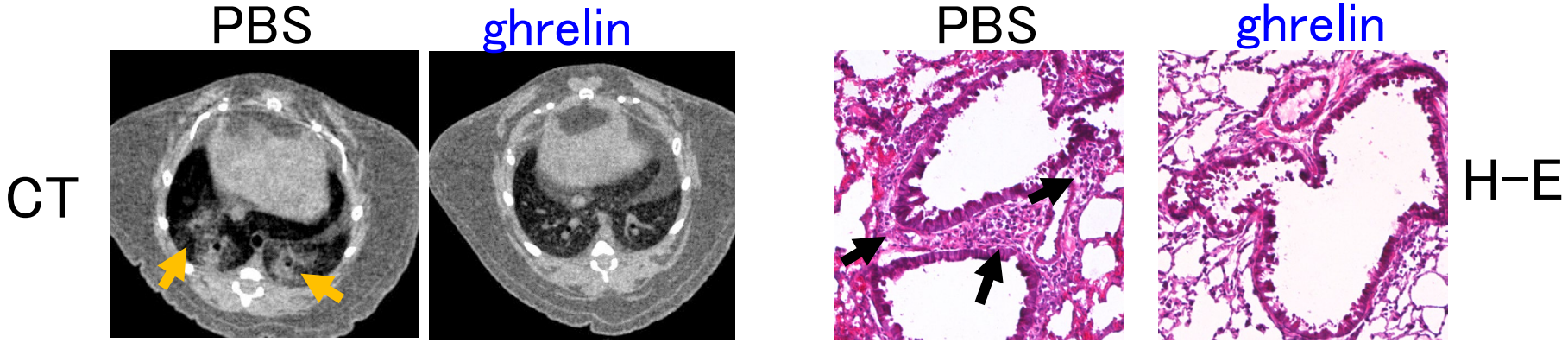
Airway eosinophilic inflammation

Airway remodeling

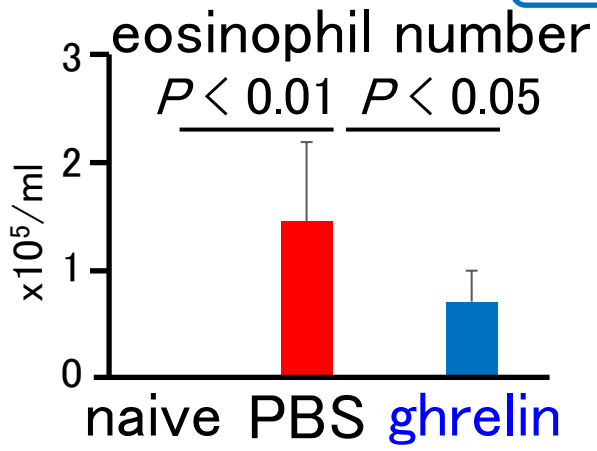


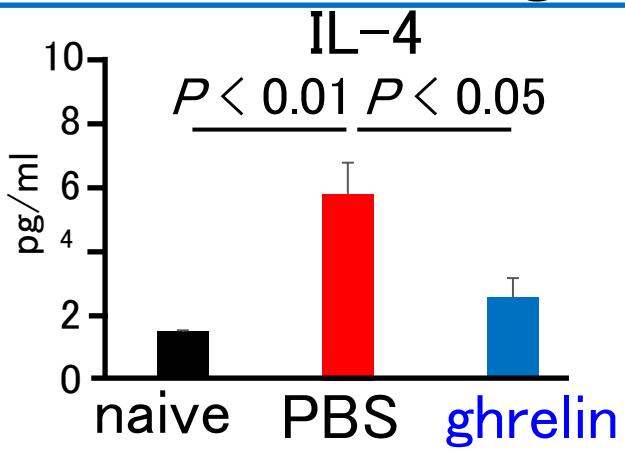
Lambrecht BN & Hammad H. Nat Immunol 2015, 16, 45-56

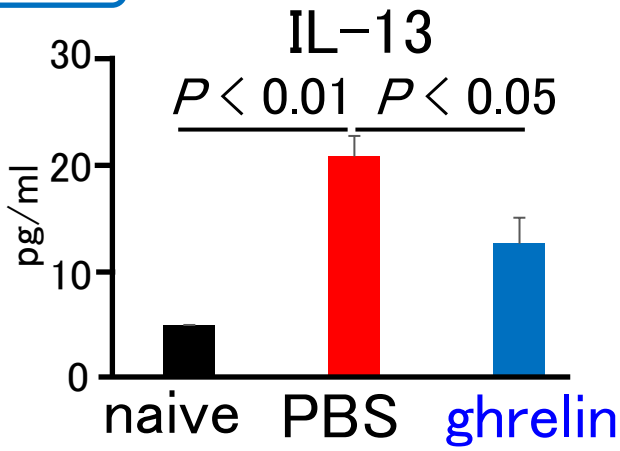
Ghrelin suppresses eosinophilic airway inflammation in HFD/ovalbumin-induced mouse model of asthma and obesity



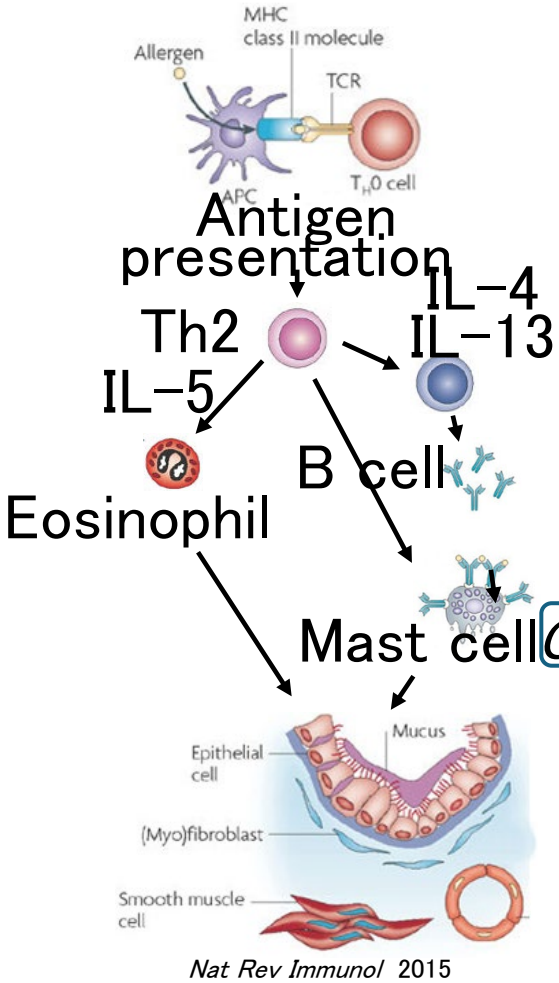
Bronchoalveolar lavage fluid





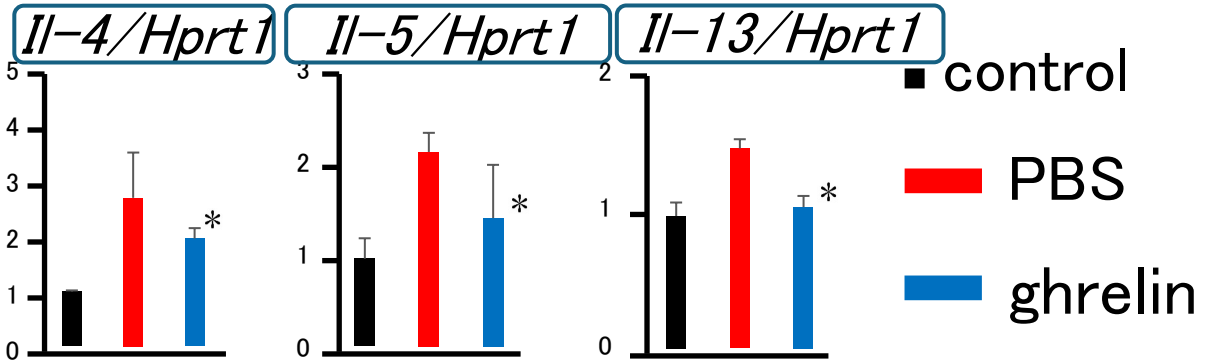


Ghrelin suppresses Th2-related cytokines in HFD-OVA asthma mice

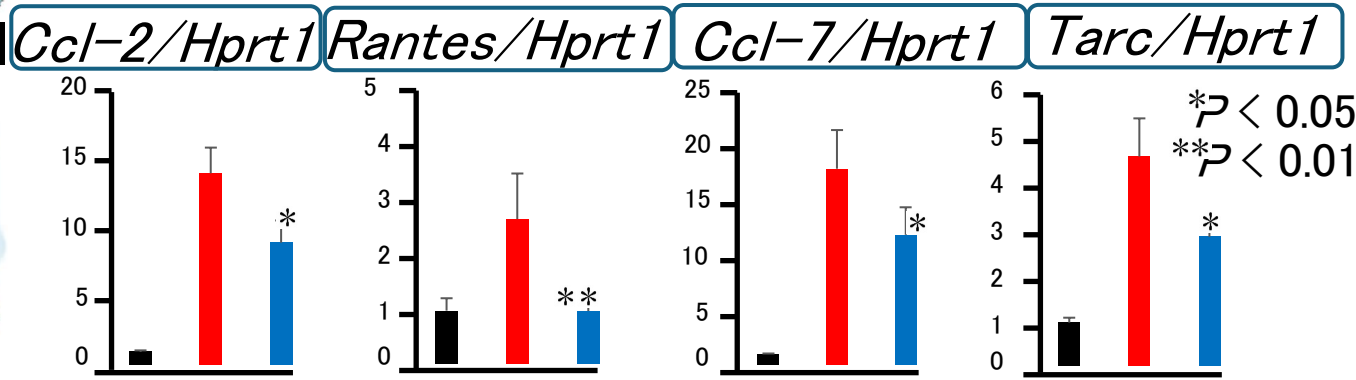


Nat Rev Immunol 2015

Th2-related cytokines

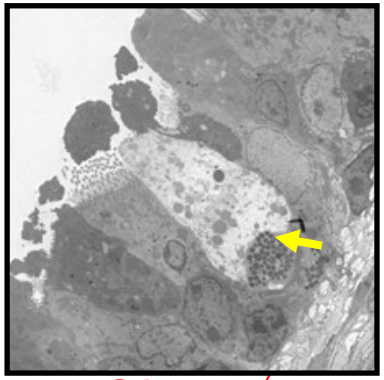


histamine release, recruitment of T cells and eosinophils

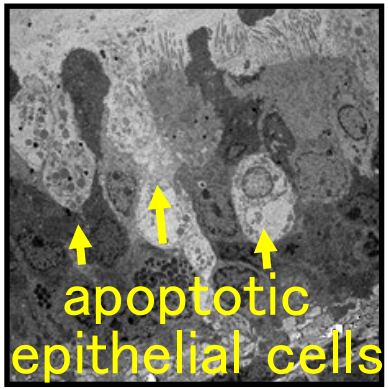


Ghsr$^{-/-}$ HFD–OVA asthma mice show severer asthma pathology

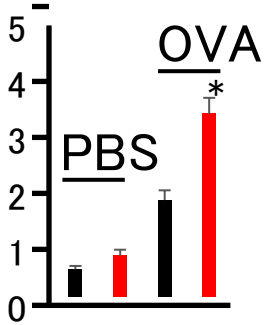
Wild type



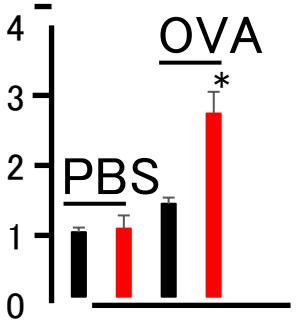
Ghsr$^{-/-}$



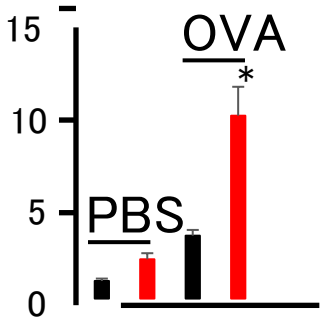
Il-4/Hprt1



Il-5/Hprt1



Il-13/Hprt1

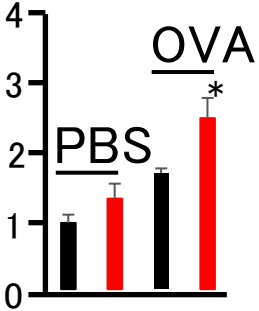


Wild type

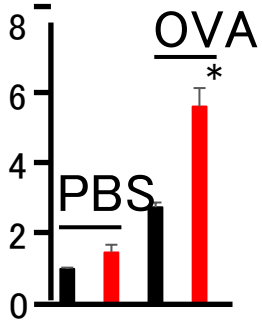
Ghsr$^{-/-}$

$*P < 0.05$

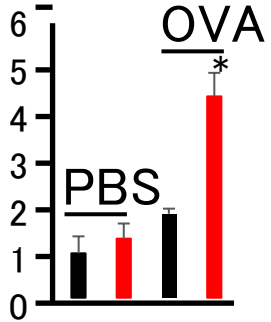
Ccl2/Hprt1



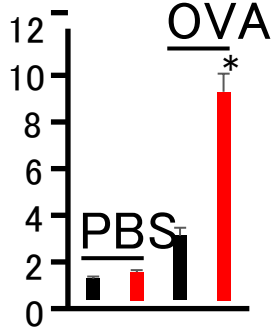
Ccl7/Hprt1



Eotaxin1/Hprt1



Tarc/Hprt1



Ghrelin functions in the homeostatic feeding regulation to respond to energy insufficiency. None of ghrelin-targeting agents like GHSR antagonists, GHSR inverse agonists, nor ghrelin *O*-acyltransferase inhibitors have yet progressed to late-stage clinical trials for obesity or diabetes treatment.

The keys to successful weight management

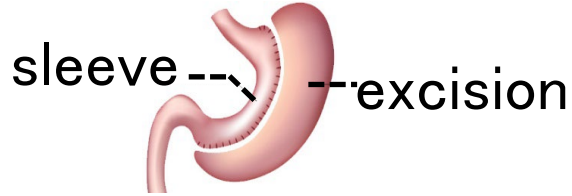
- It's not about lowering ghrelin.
- It's not a battle against hunger.
- Feeling full.

The reason why incretin-based pharmacotherapy has succeeded.

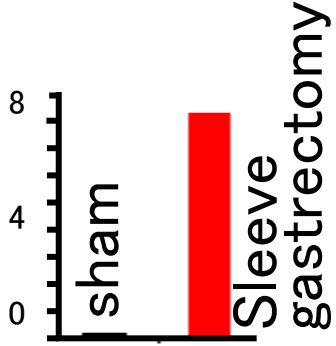
Liver-Expressed Antimicrobial Peptide 2 (LEAP-2) is an endogenous antagonist of the ghrelin receptor

Ge X *et al.* Cell Metab. 2018, 27, 461-469

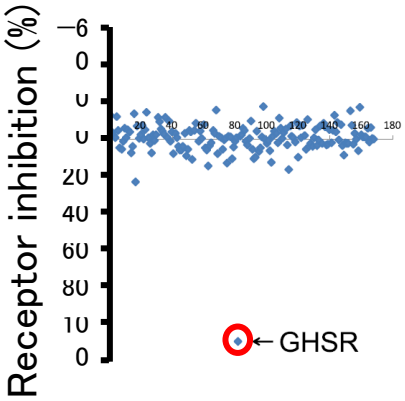
sleeve gastrectomy



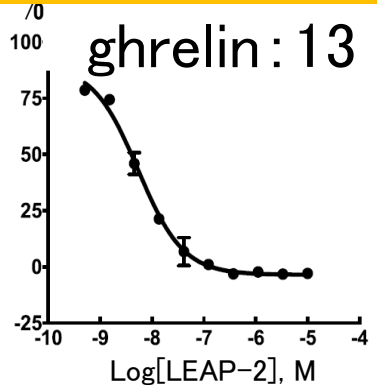
Leap-2 mRNA stomach



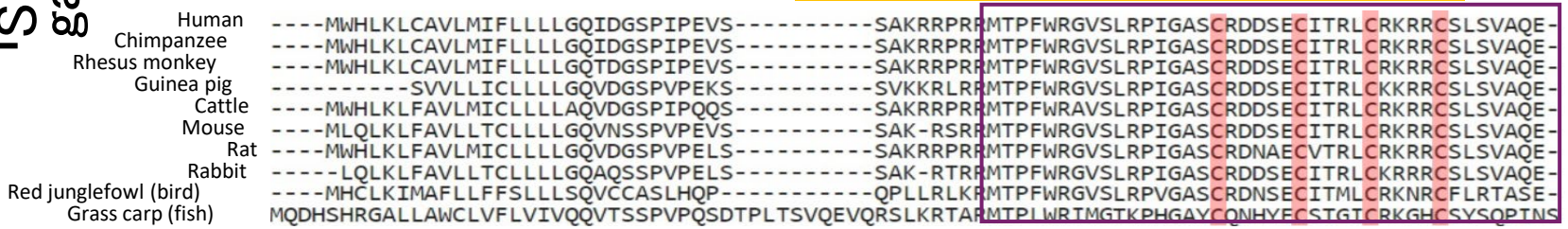
Activity of LEAP-2 against 168 GPCRs



Concentration-response curve of LEAP-2 on GHSRz



LEAP-2 (40 amino acids)



LEAP2 (Liver-expressed antimicrobial peptide 2) was identified in human blood as an antimicrobial peptide produced in the liver

LEAP2 (NW 4,581)

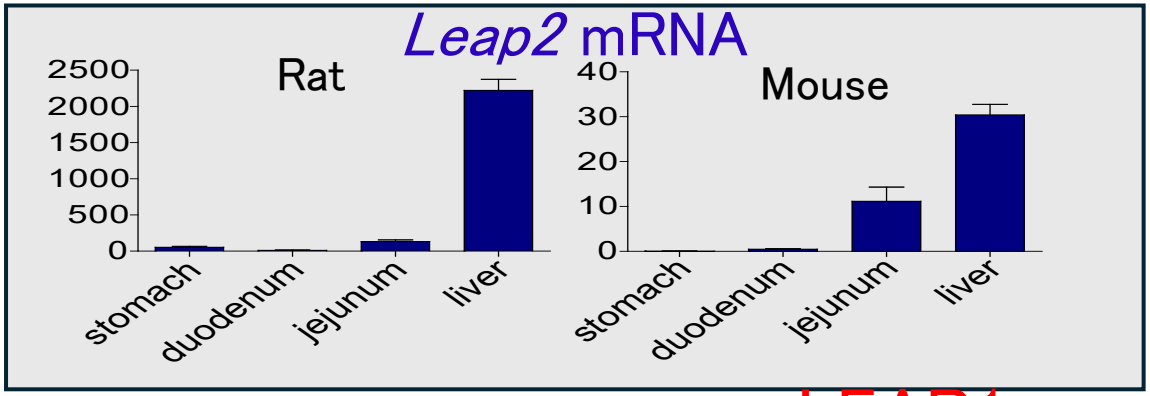
Krause *et al*. Protein Sci. 2003, 12: 143-152

1 14 40

MTPFWRGVSLRPIGASCRDDSECITRLCRKRRCSLSVAQE

Binding region to ghrelin receptor

blue: basic amino acids common in antimicrobial peptides



ghrelin

LEAP1

S-S

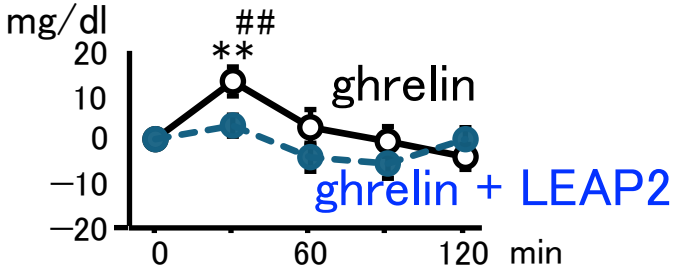
1 25

DTHFPICFCCGCCHRSKCGMCCKT

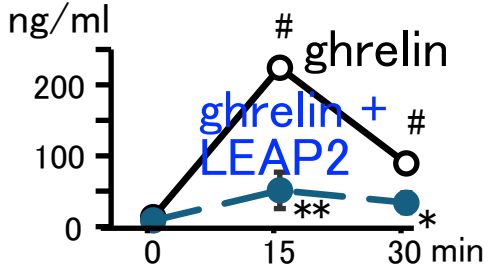
now identified as “hepcidin” to serve in iron metabolism

LEAP-2 abolished ghrelin-induced changes in blood glucose, GH secretion, body temperature and food intake (rats)

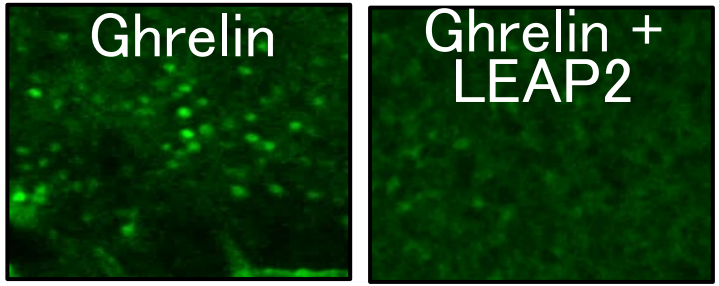
blood glucose



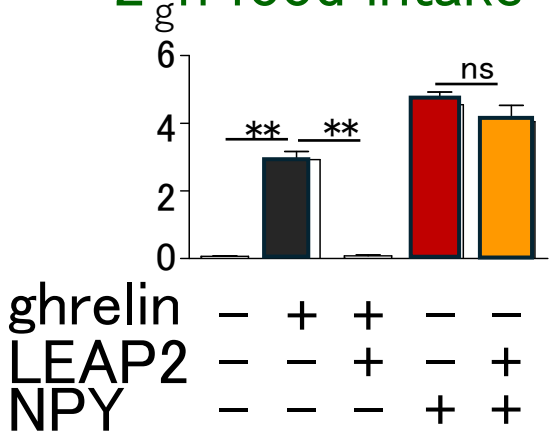
plasma GH



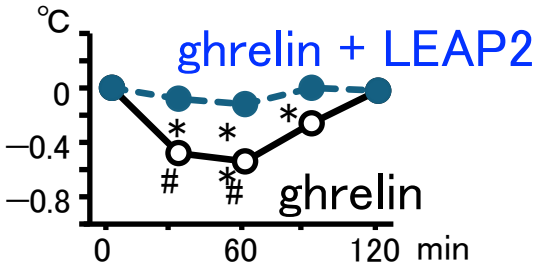
Fos expression (hypothalamus)



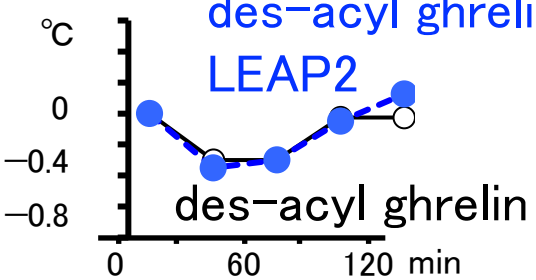
2-h food intake



body temperature



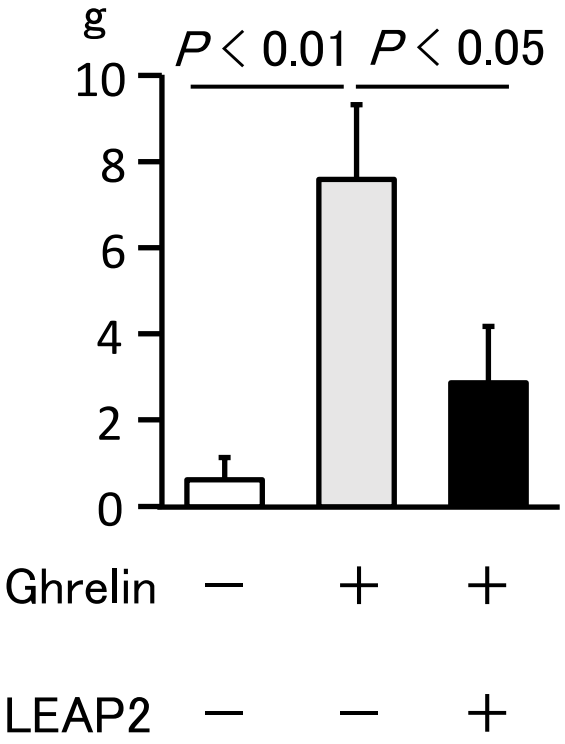
body temperature



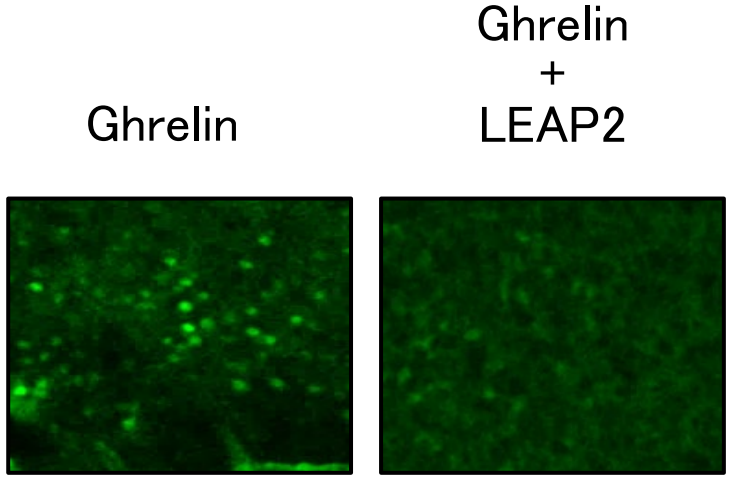
Islam MN, Nakazato M, *et al.*, J Endocrinol, 2020, 244: 13-23

ICV LEAP2 suppressed ghrelin-induced feeding (rats)

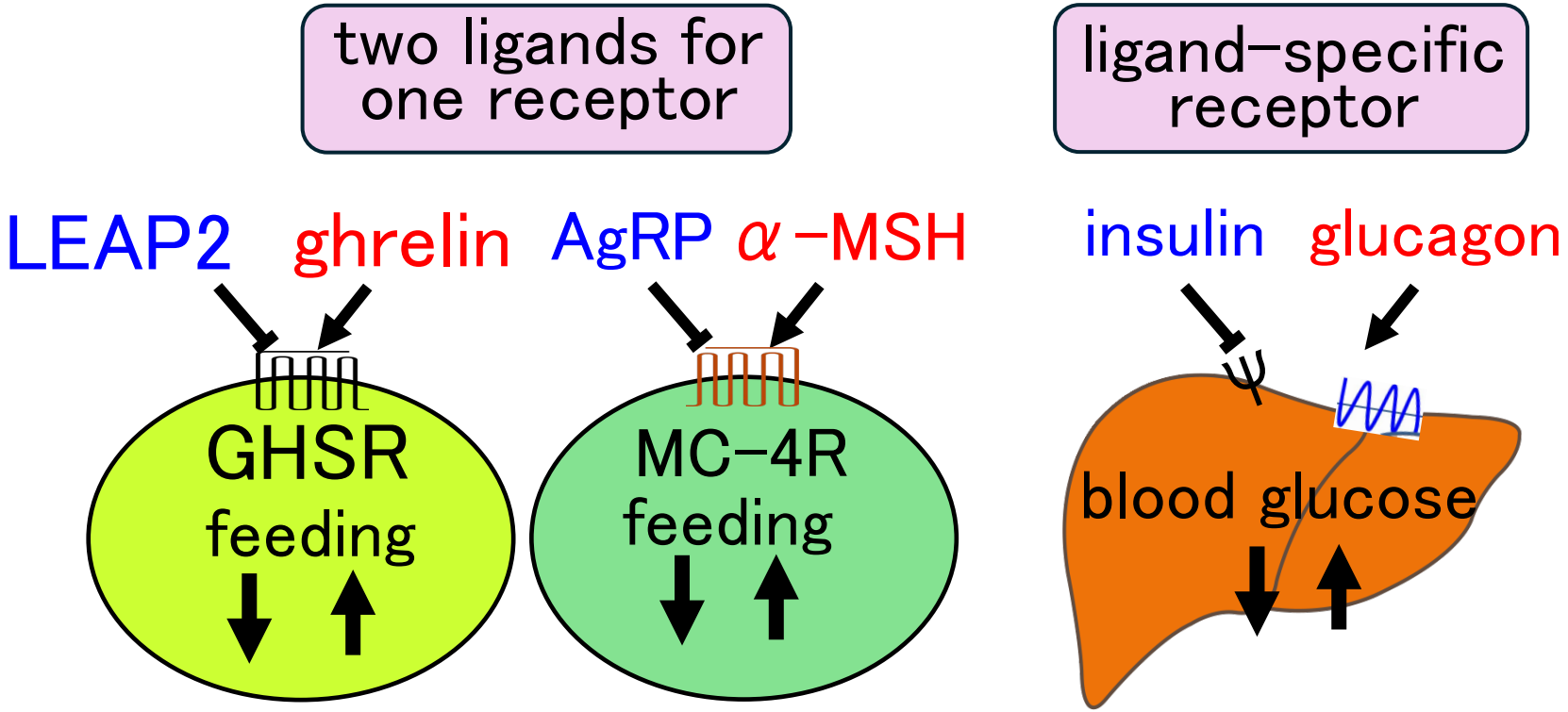
feeding



Fos in ARC

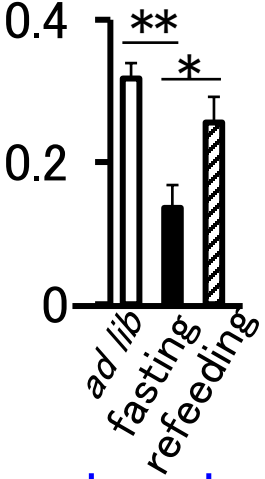


Unusual dual regulation of receptor signaling by two ligands with opposing actions



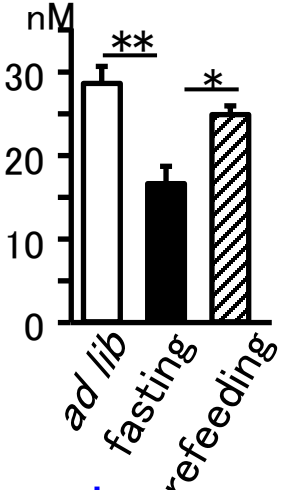
LEAP2 decreased under fast and ghrelin reduced *Leap2* mRNA in the liver

Leap2 mRNA mouse liver

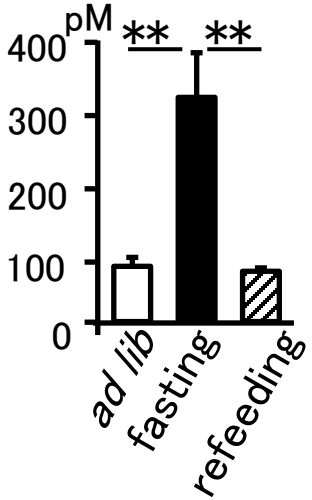


Plasma

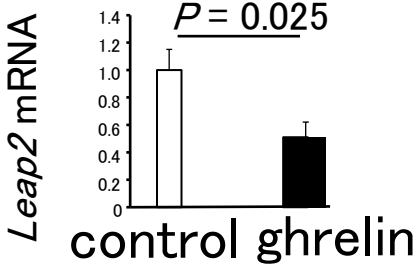
LEAP2



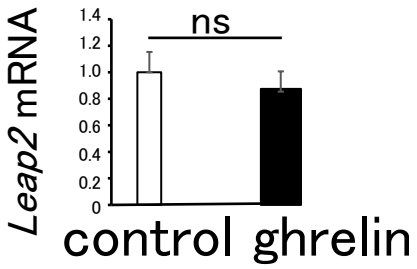
ghrelin



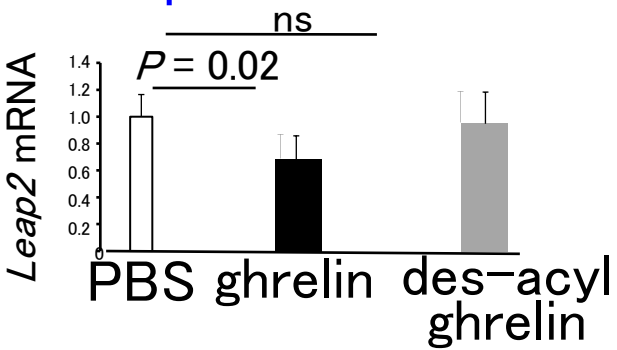
primary hepatocyte (wild type mouse)



primary hepatocyte (GHSR-KO mouse)

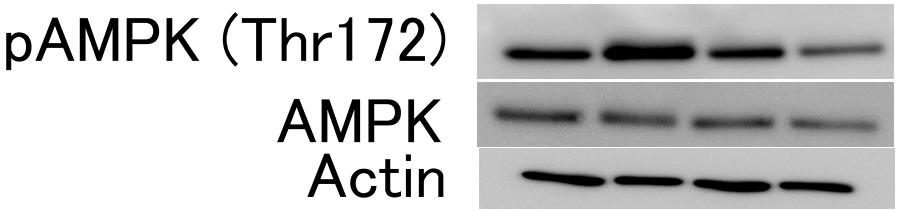


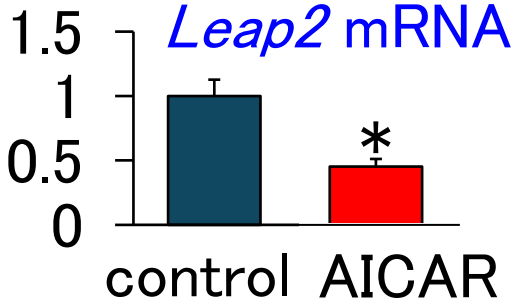
Hepa1-6 cells

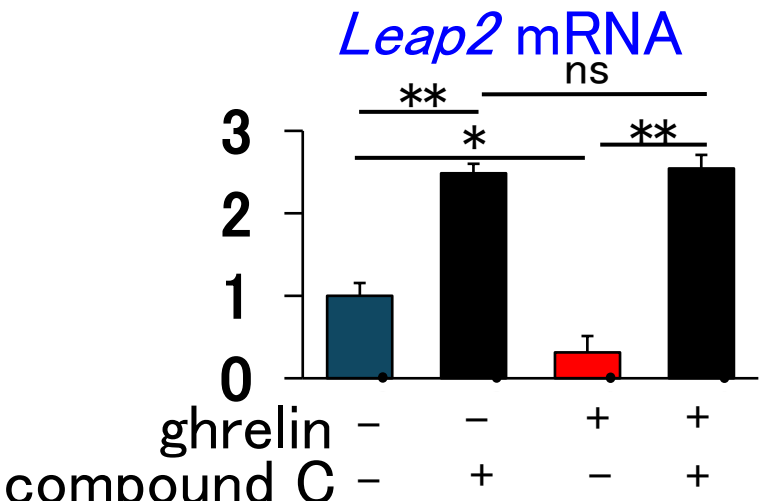


Ghrelin directly suppressed *Leap2* expression in the liver by AMPK- dependent pathway (Hepa 1-6)

ghrelin
↓
GHSR
↓
pAMPK ⬆
⊥
Leap2 expression ⬇





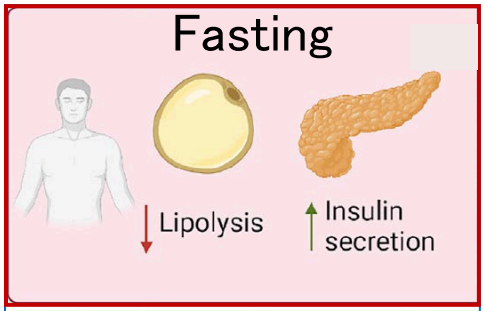


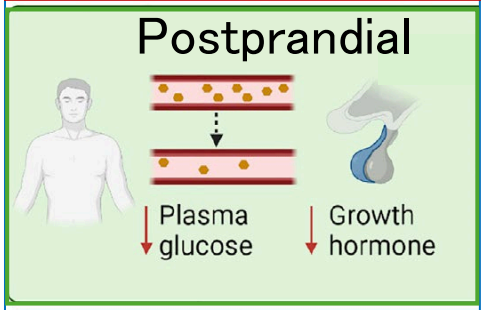
AICAR : AMPK activator
compound C : AMPK inhibitor

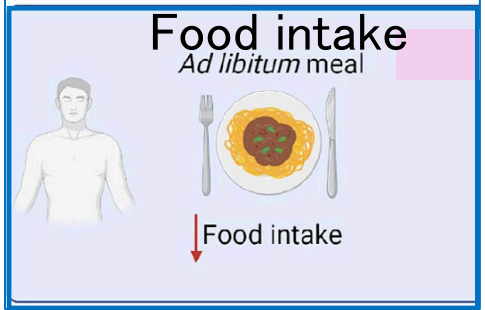
Islam M *et al.*, 2020, J Endocrinol, 244: 13-23

LEAP2 effects in humans (Hagemann et al., 2022, Cell Reports Med 3, 100582)

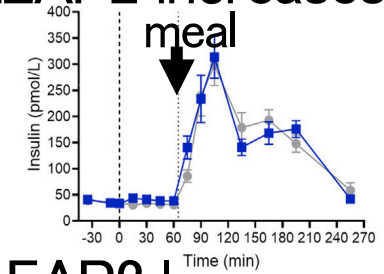
20 healthy young males





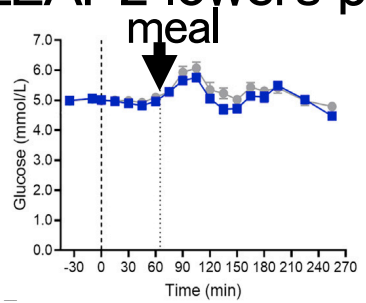


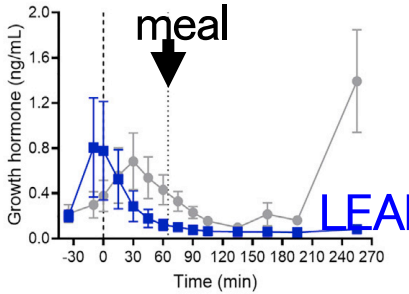
LEAP2 increases insulin secretion during fasting



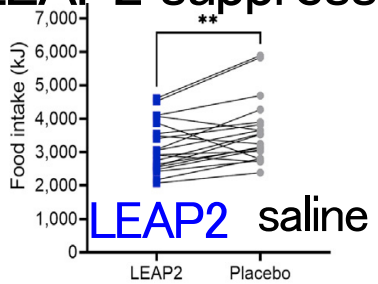
LEAP2

LEAP2 lowers postprandial glucose and GH





LEAP2 suppresses *ad libitum* food intake

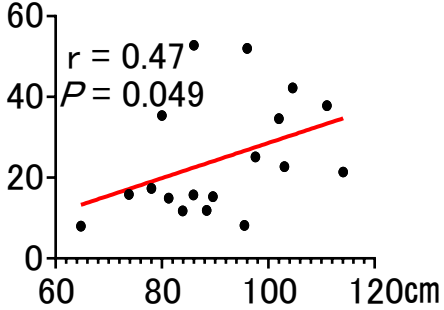


plasma LEAP2
- half-life: 9 min
- peak level: 2.6 -fold

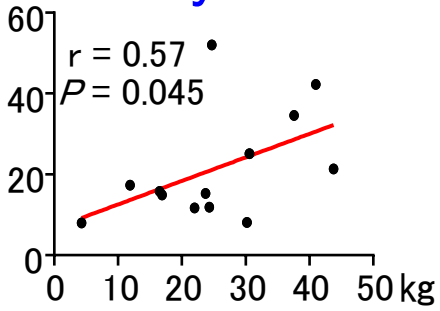
Fasting serum LEAP2 level is associated with insulin resistance and liver steatosis in type 2 diabetes

Age 60.4 ± 2.3, Male 4 Female 14, BMI 27.9 ± 1.2, HbA1c 8.2 ± 0.3 (average±SE)

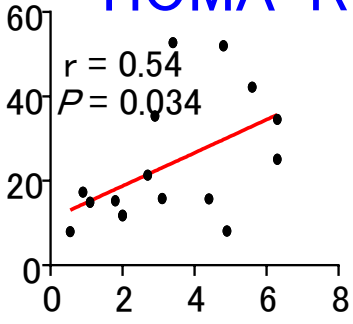
Waist circumference



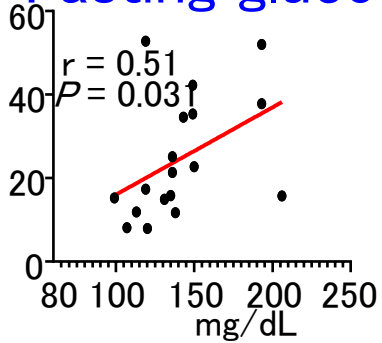
Body fat mass



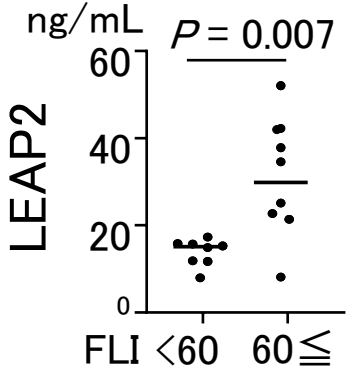
HOMA-R



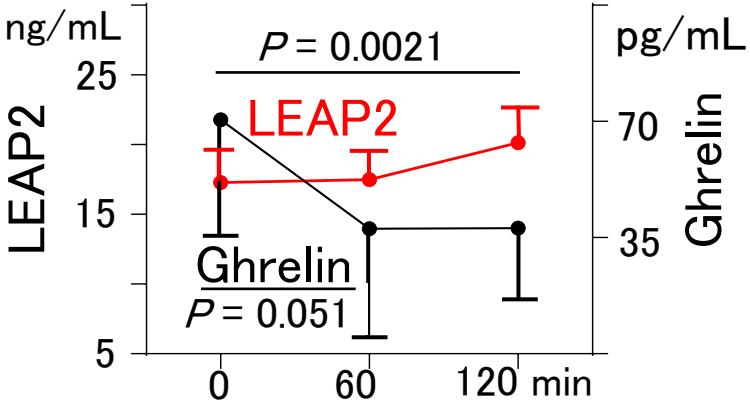
Fasting glucose



Fatty Liver Index



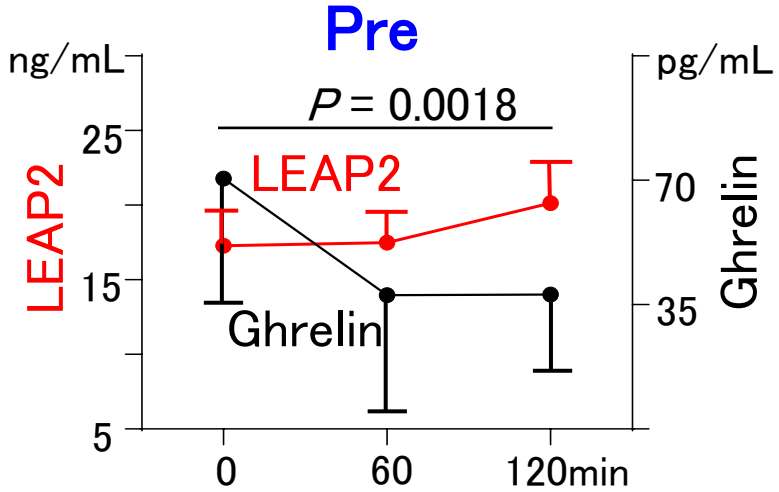
Postprandial change

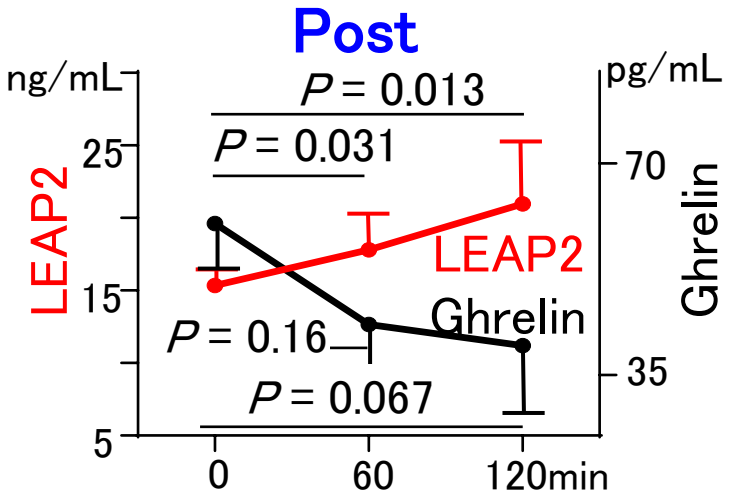


Nabekura H, Nakazato M, *et al., Obesity Facts*. 2023

Delayed postprandial LEAP2 increase in 30 patients with diabetes improved by diabetes treatment (SGLT2 inhibitor and DPP4 inhibitor combination tablets)

	Pre-treatment	Post-treatment
Body weight(kg)	65.2±12.7	63.8±12.5 *
One-day average glucose(mg/dL)	150.5±38.9	113.2±13.8 *





The postprandial increase in LEAP2 was shortened from 120 min to 60 min after 2-week administration of anti-diabetic drugs.

Nurul I *et al.* Sci Rep 2024, 14, 24782.

Effects of weight loss after bariatric surgery on serum LEAP2

	Before surgery	One year after surgery
Age	42.6 ± 1.4	
Sex	19/23 (men/women)	
BMI	44.2 ± 1.5	⟶ 32.0 ± 1.2
Operation method	38/4 (LSG/LSG + DJB)	
Type 2 DM	28	⟶ 6
weight loss effectiveness (Excess weight loss rate, %EWL)		71%

LSG: laparoscopic sleeve gastrectomy DJB: duodenojejunal bypass

Excess weight loss (%EWL) = weight loss/(pre treatment weight −ideal weight)*100

%EWL in this study (71%) is higher than the average %EWL (59%) one year after sleeve gastrectomy

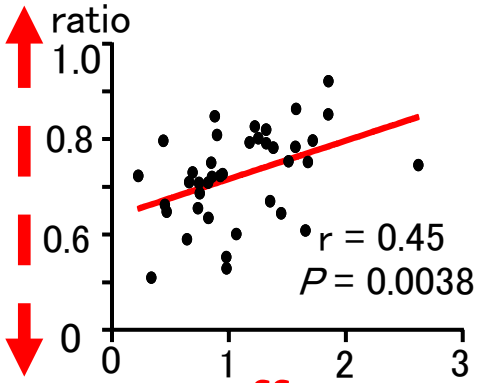
Collaboration with Drs I. Tatsuno and T. Yamaguchi at Toho University Sakura Hospital, Japan

Nabekura H, Nakazato M, *et al., Obesity Facts*. 2023

LEAP2 reduction after bariatric surgery positively correlates to operative effects

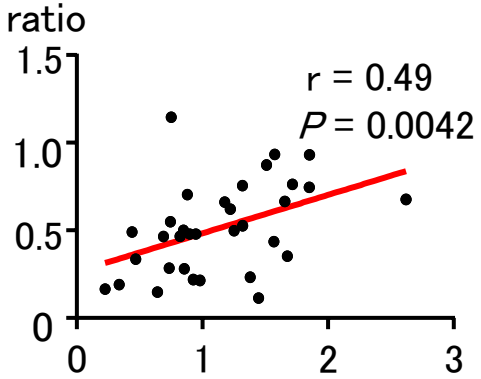
Body weight ratio

less effect

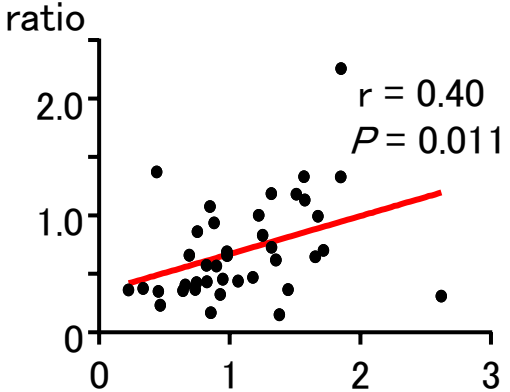


greater effect

Visceral fat ratio



Triglyceride ratio



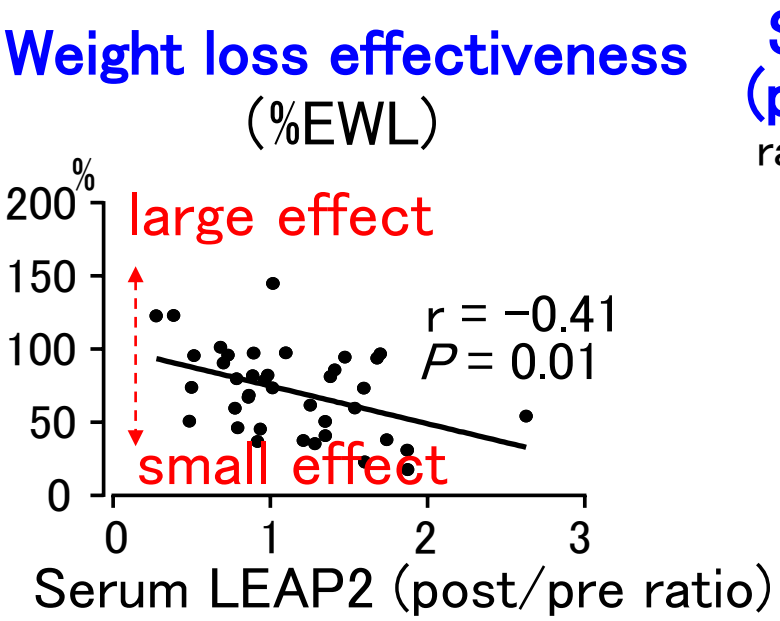
serum LEAP2 (post/pre ratio)

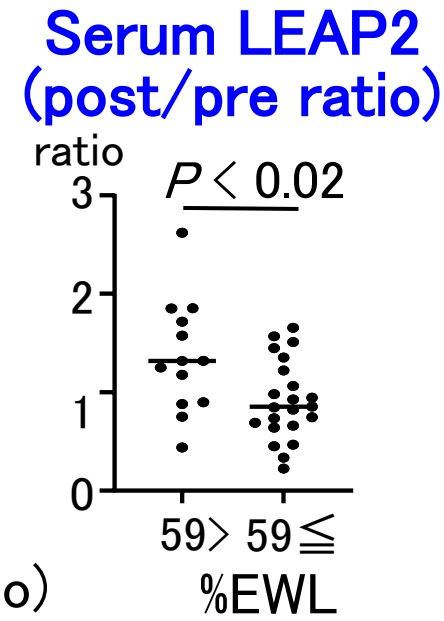
The more body weight, visceral fat, and serum triglycerides were reduced, the more LEAP2 decreased after surgery. Improvements of clinical parameters after bariatric surgery reduced serum LEAP2.

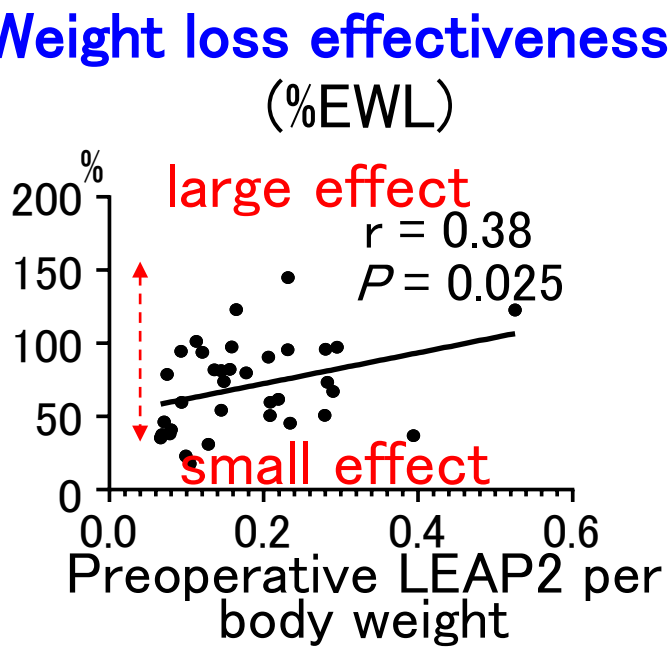
Collaboration with Drs I. Tatsuno and T. Yamaguchi at Toho University Sakura Hospital, Japan

Nabekura H, Nakazato M, *et al., Obes Facts*. 2023

Preoperative high LEAP2 may be a predictor of weight loss after sleeve gastrectomy



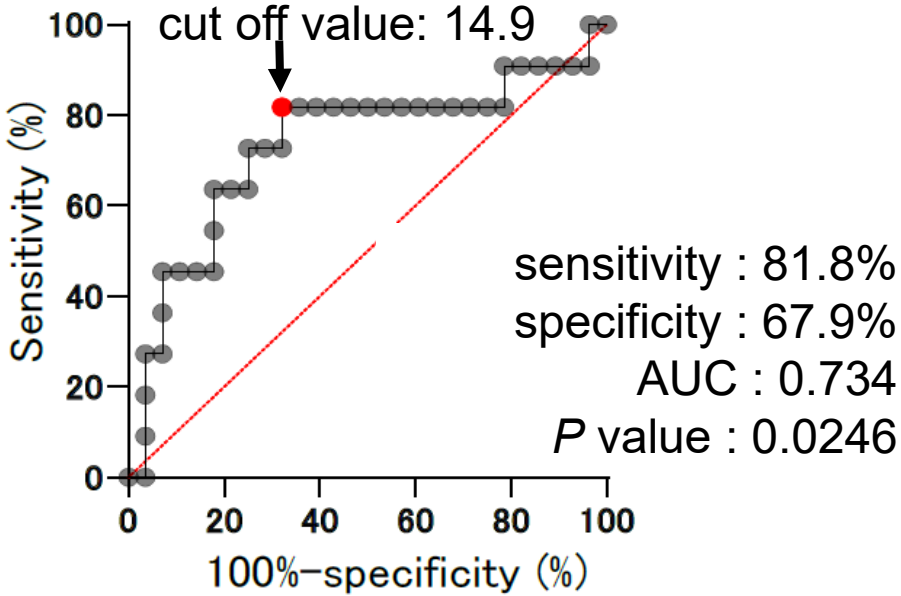




The higher the excess weight loss rate was, the more LEAP2 decreased after the surgery. Preoperative LEAP2 per body weight was positively correlated to the postoperative weight loss effectiveness index.

Nabekura H, Nakazato M, *et al., Obes Facts*. 2023

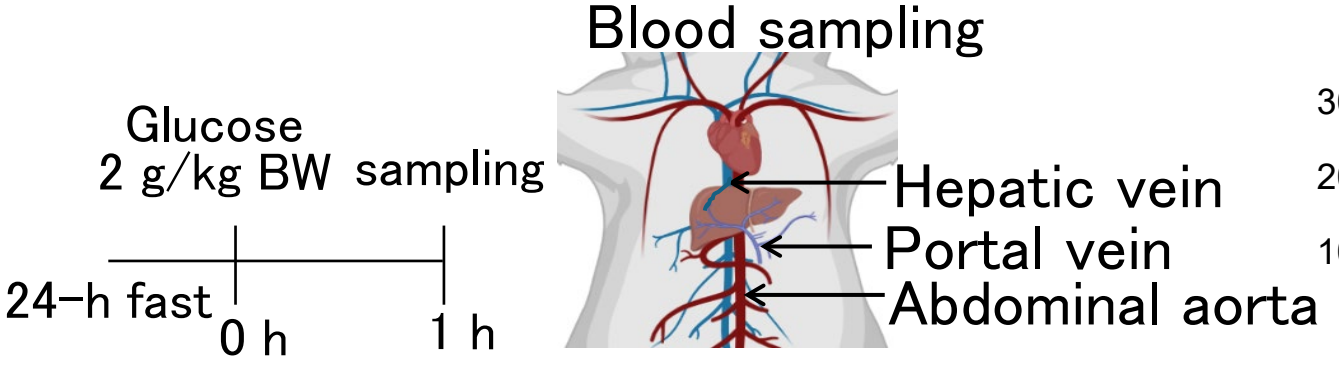
ROC curve of fasting blood LEAP2 / body weight before surgery at optimal cut off level of %EWL 50%

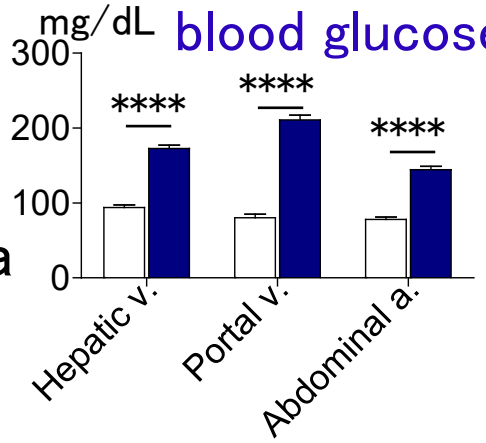


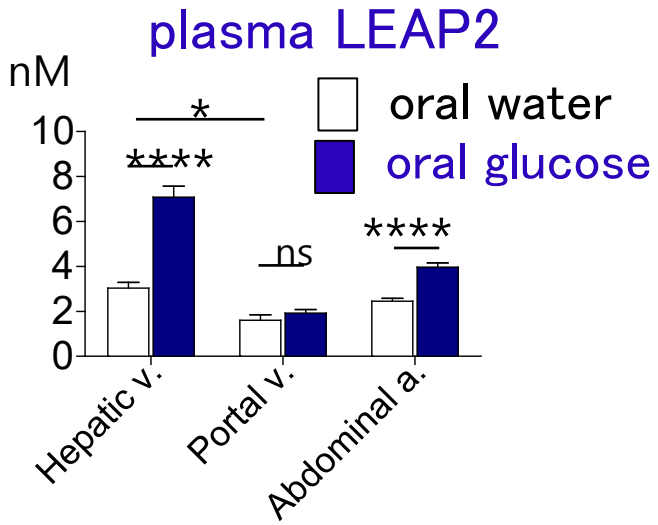
ROC curve analysis of serum LEAP2 per body weight before surgery shows that the value of more than 14.9 predicts weight loss effectiveness by bariatric surgery. LEAP2 determination may contribute to surgical case selection and additional postoperative treatment in the future.

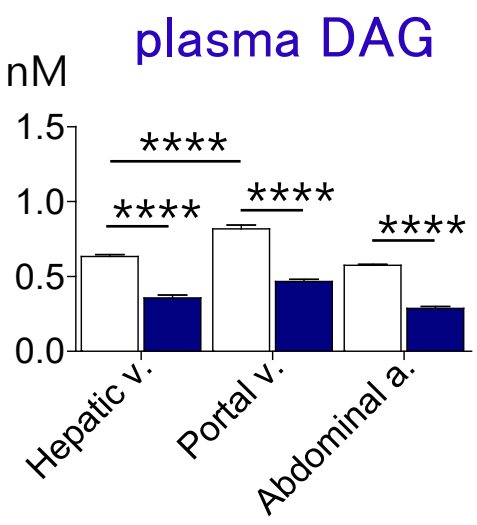
Nabekura H, Nakazato M, *et al., Obesity Facts*. 2023

Liver is the main organ to circulate LEAP2 in the body









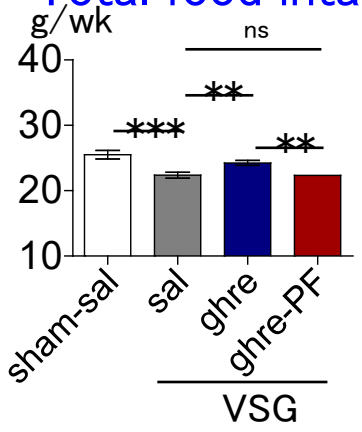
Nurul I *et al.* Sci Rep 2024, 14, 24782.

Ghrelin recovered parameters altered by VSG in chow-fed mice

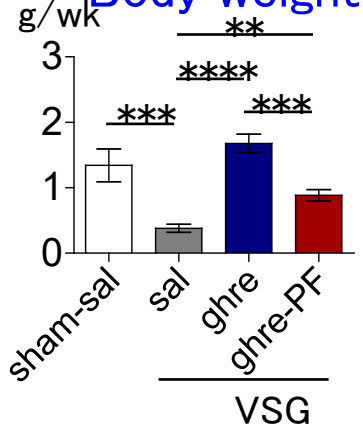
8 W	9 W	10 W
sham	saline	body weight
VSG	saline	food intake
VSG	ghrelin	sampling
VSG	ghrelin-PF to VSG-saline	

ghrelin: 10 nmol/twice/day,7 days

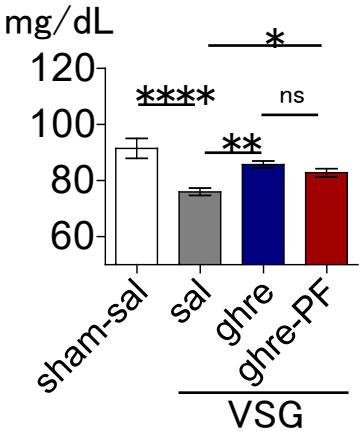
Total food intake



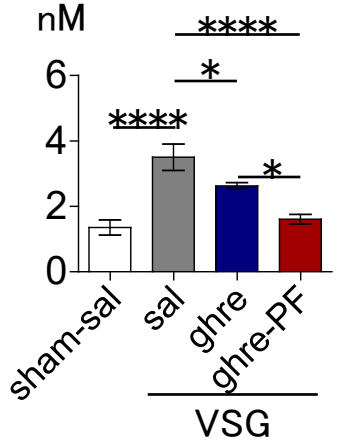
Body weight gain



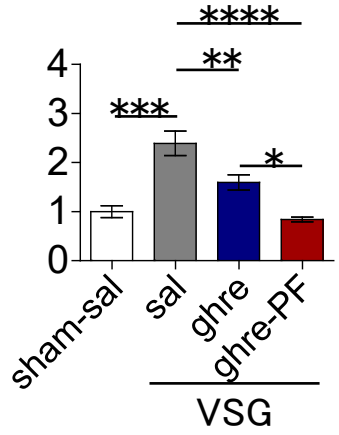
Blood glucose



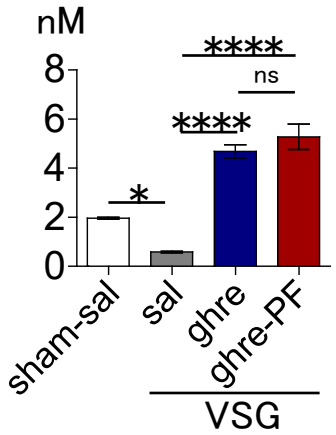
Plasma LEAP2



Liver *Leap2* mRNA

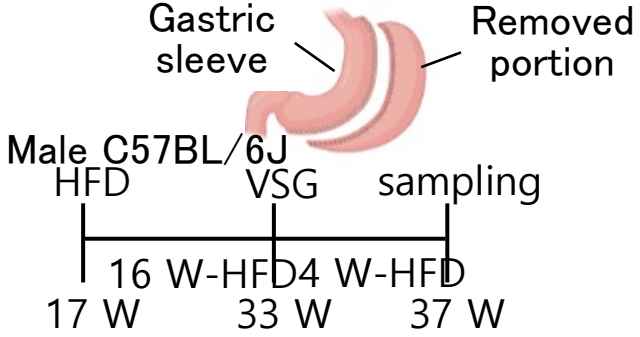


Plasma DAG

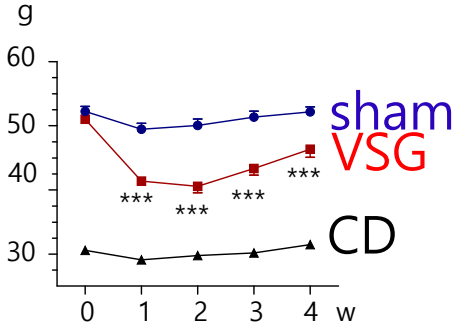


VSG improved metabolic parameters in HFD-fed mice

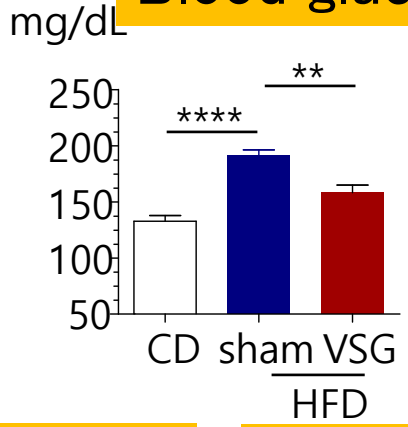
VSG in HFD-fed mice



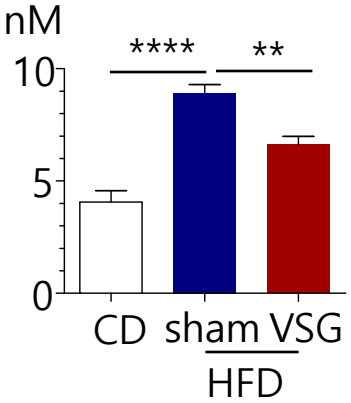
Body weight



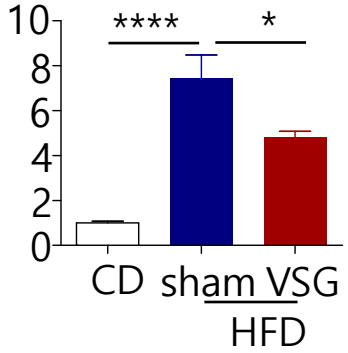
Blood glucose



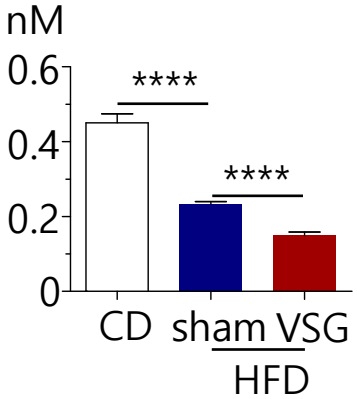
Plasma LEAP2



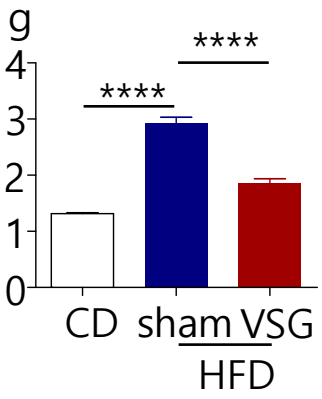
Liver *Leap2* mRNA



Plasma DAG

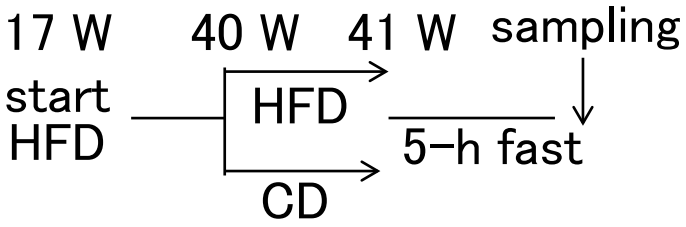


Liver weight

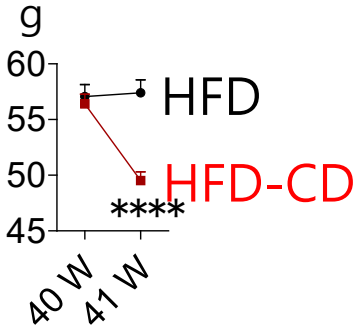


Dietary change from HFD to CD reduced LEAP2

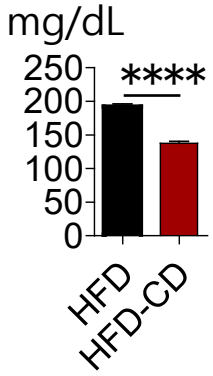
Experimental design



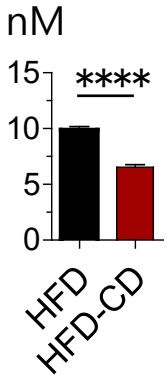
Body weight



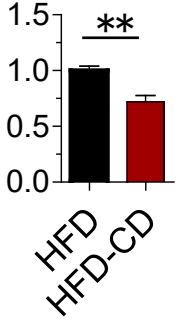
Blood glucose



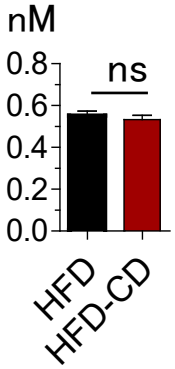
Plasma LEAP2



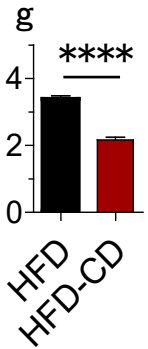
Liver *Leap2* mRNA



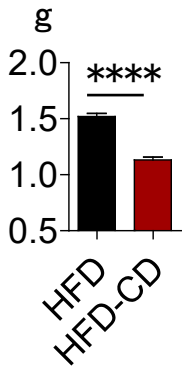
Plasma DAG



Liver weight



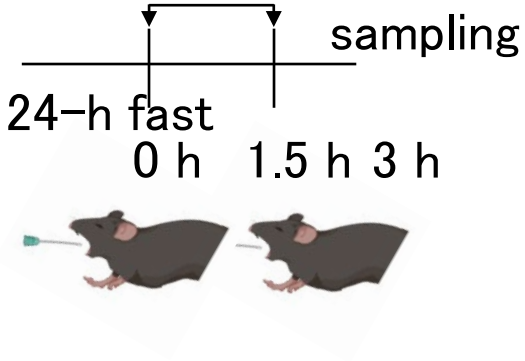
eWAT weight



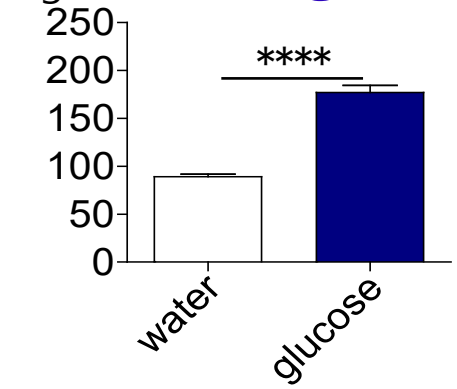
Nurul I *et al.* Sci Rep 2024, 14, 24782.

LEAP2 response to glucose

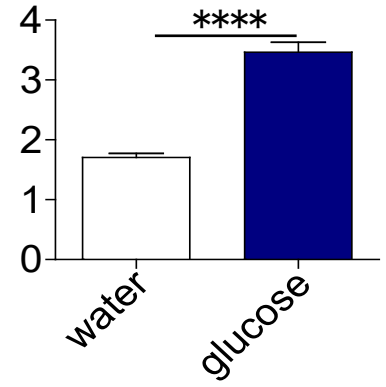
Glucose: 2 g/kg BW



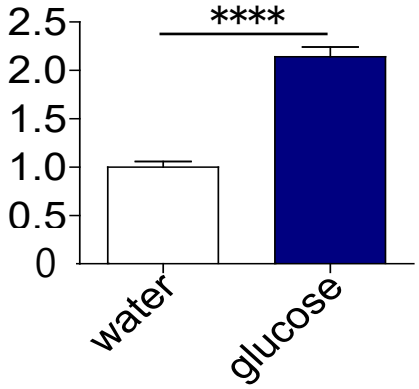
Blood glucose



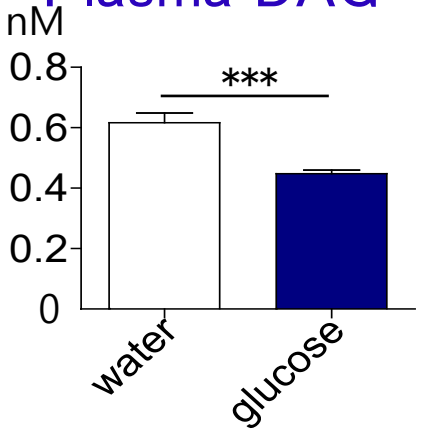
Plasma LEAP2



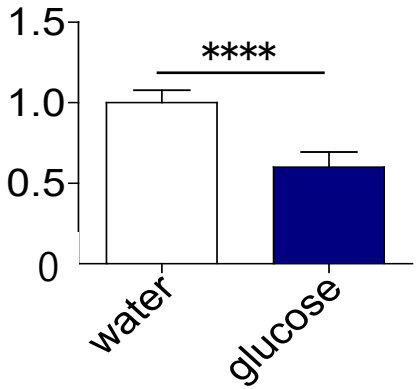
Liver *Leap2* mRNA



Plasma DAG

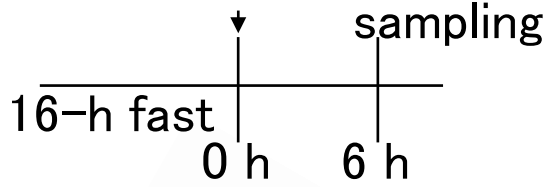


Stomach *ghrelin* mRNA

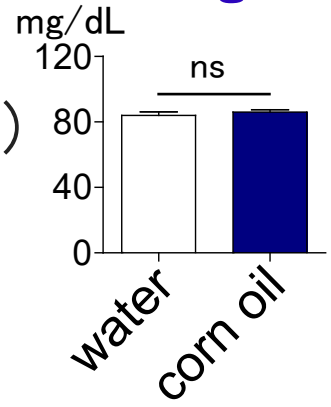


LEAP2 response to fat or fatty acid

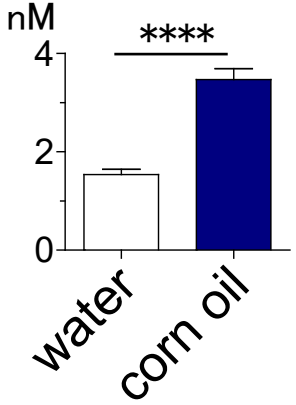
corn oil (100 µl/mouse)
or
palmitate (3 µmol/mouse)



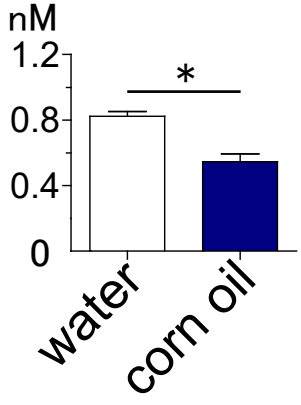
Blood glucose



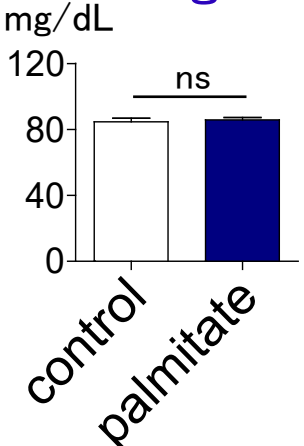
Plasma LEAP2



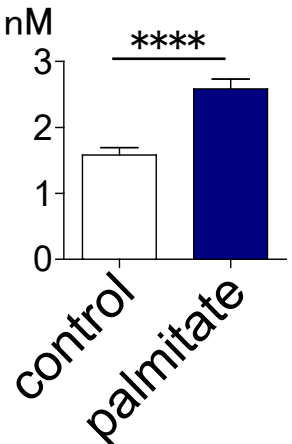
Plasma DAG



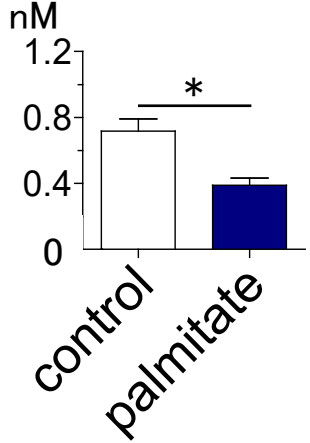
Blood glucose



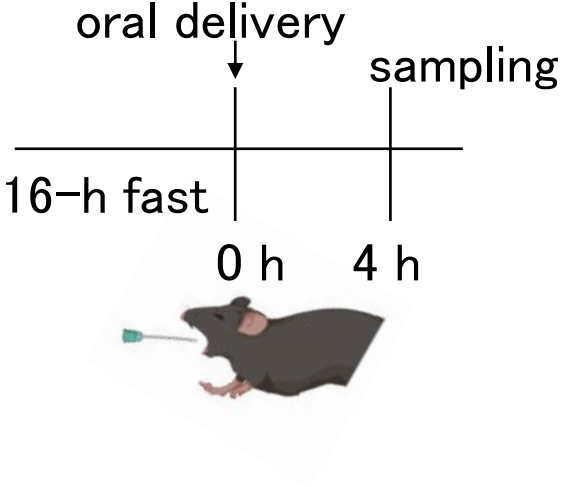
Plasma LEAP2



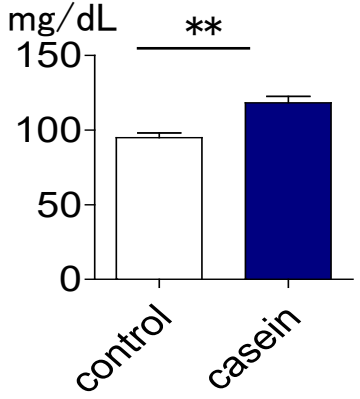
Plasma DAG



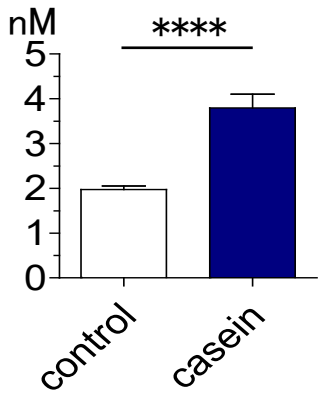
LEAP2 response to protein (casein)



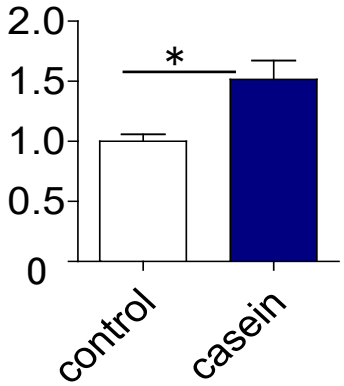
Blood glucose



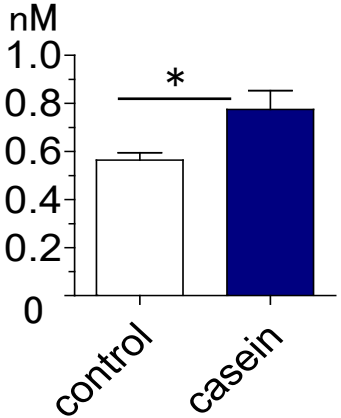
Plasma LEAP2



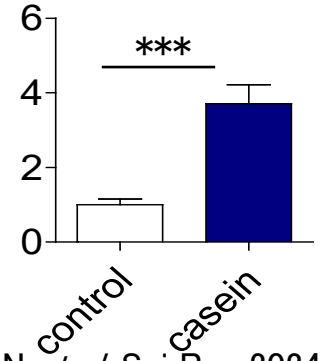
Liver *Leap2* mRNA



Plasma DAG



Stomach ghrelin mRNA



Islam N *et al.* Sci Rep 2024, 14, 24782

Factors regulating LEAP2 and ghrelin productions

LEAP2 ↑

Glucose
Fructose
Fat (corn oil)
Protein (casein)

LEAP2 ↓

Weight loss by VSG and diet switching (HFD to CD)
ghrelin

Ghrelin ↓

VSG
Glucose
Fat (corn oil)

Ghrelin ↑

protein (casein)
weight loss (not VSG)

Nurul I *et al*. Sci Rep 2024, 14, 24782.

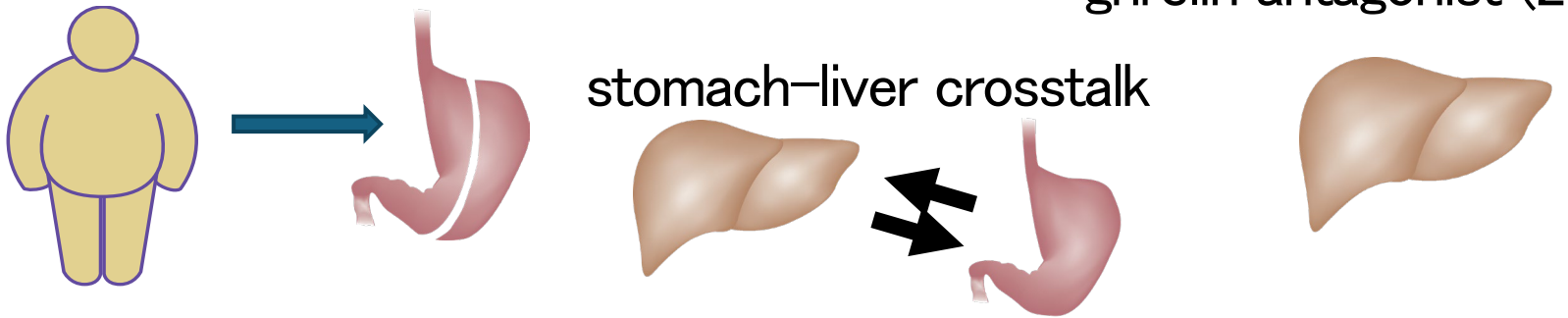
Ghrelin/LEAP-2 interaction serves in stomach-liver crosstalk in the regulation of feeding and GH secretion

peptide hunting of orphan GPCR

discovery of ghrelin (1999)

mediator hunting targeted to bariatric surgery

identification of LEAP-2 as a ghrelin antagonist (2018)



Orexigenic ghrelin suppresses production and secretion of anorectic LEAP-2.
LEAP-2 suppresses ghrelin production.

Ueno H, Nakazato M, *et al, Pharmacol Ther*. 2022