Hormonal and neurotransmitter mechanisms regulating feed intake

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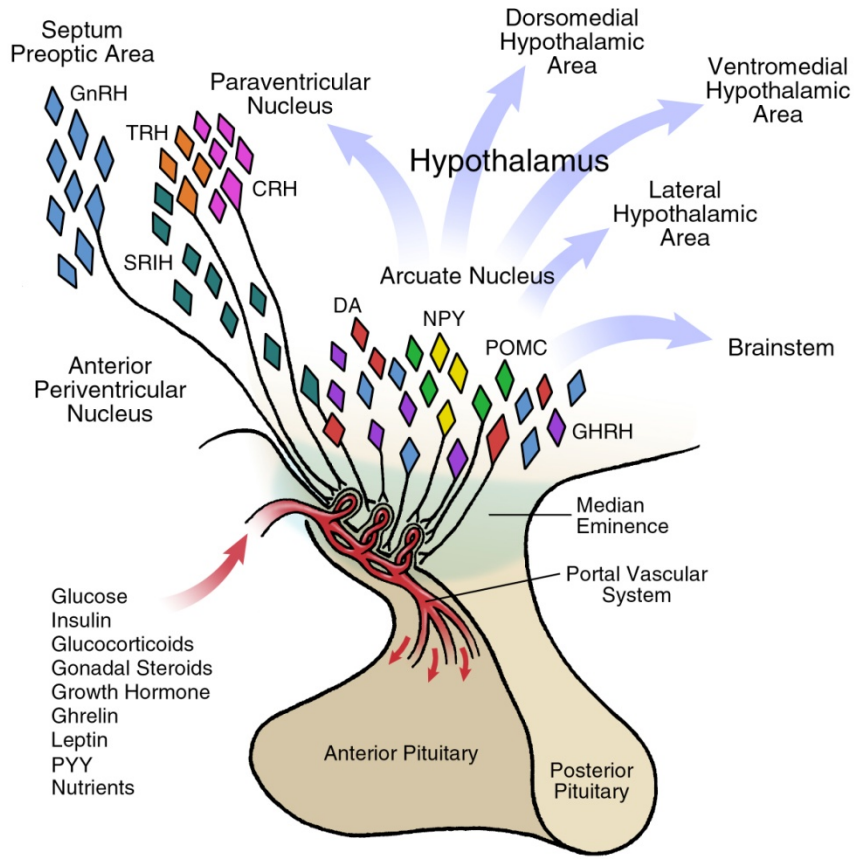
Appetite control in the hypothalamus is regulated by a complex array of molecules



Neurotransmitter and Hormonal Regulators of Appetite

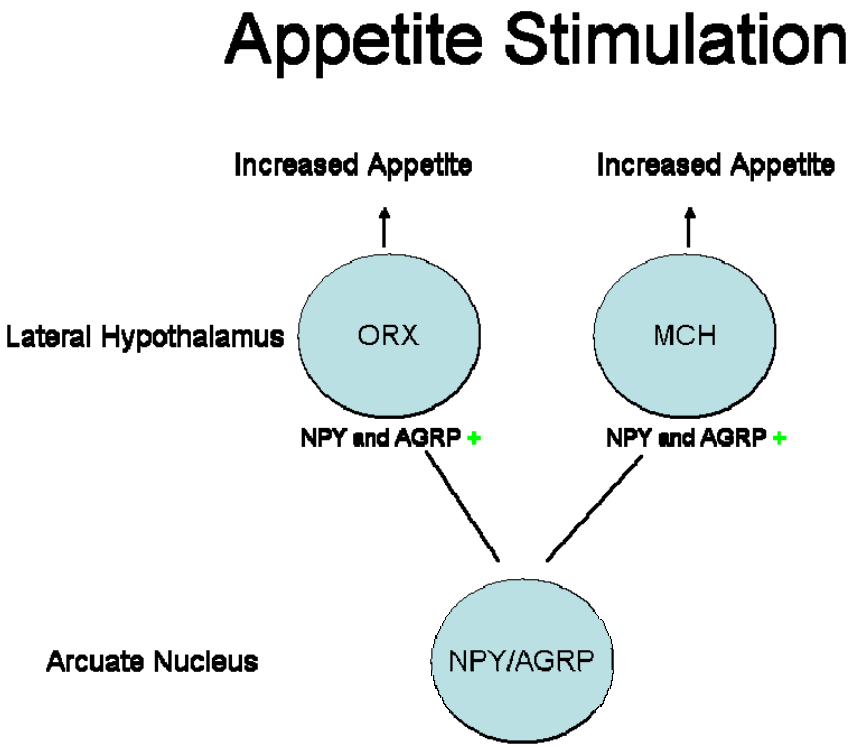
Orexigenic

- Neuropeptide Y
- Agouti related protein
- Melanin concentrating hormone
- Orexin

Anorexigenic

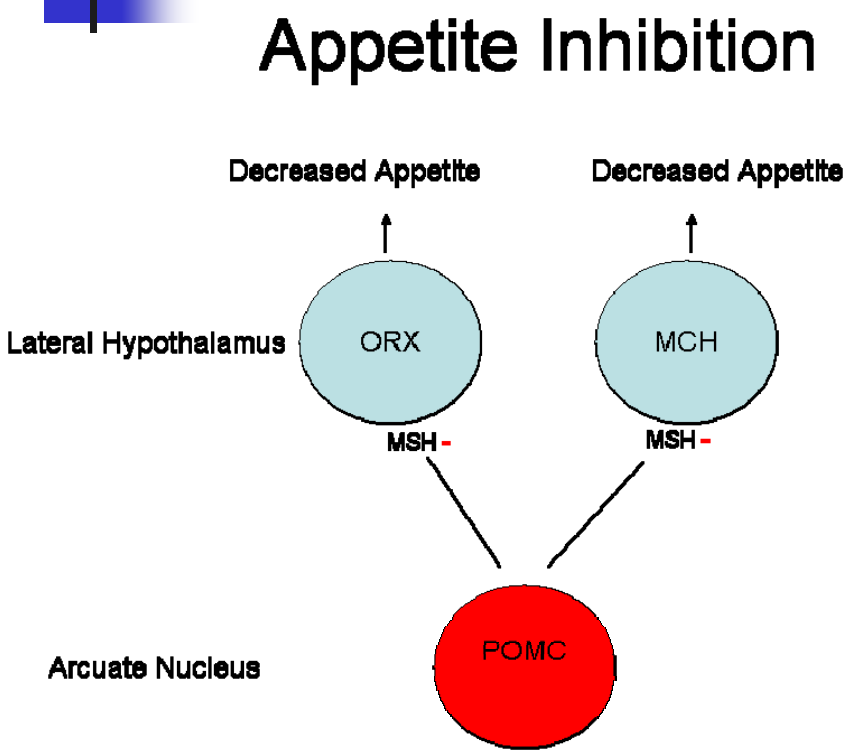
- Leptin
- α-Melanocyte stimulating hormone

Orexigenic Neurotransmitters



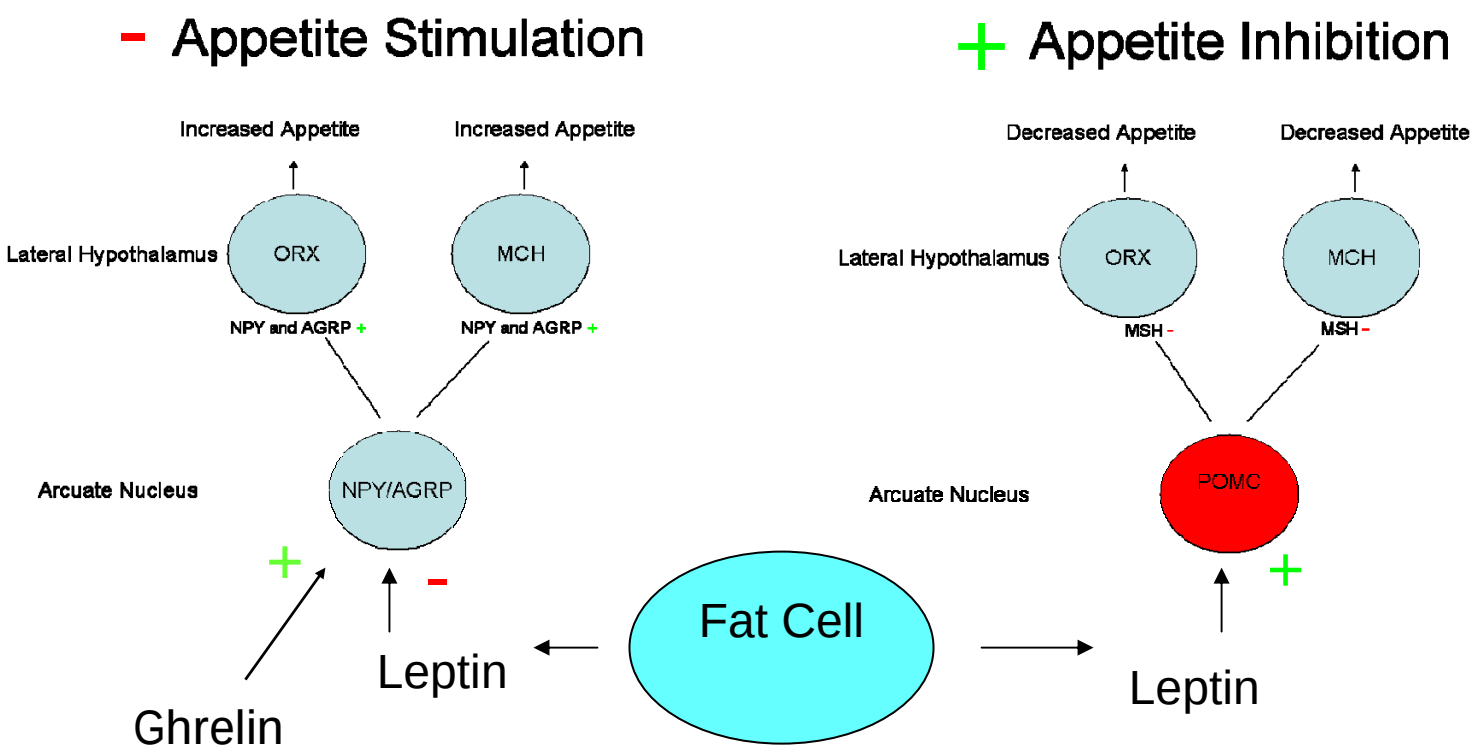
- ORX-orexin
- MCH-melanin concentrating hormone
- NPY-neuropeptide-Y
- AgRP-agouti related protein

Anorexigenic Neurotransmitters



- ORX-orexin
- MCH-melanin concentrating hormone
- MSH- α-melanocyte stimulating hormone
- POMC-proopiomelanocortin

Integration of Central Appetite Control

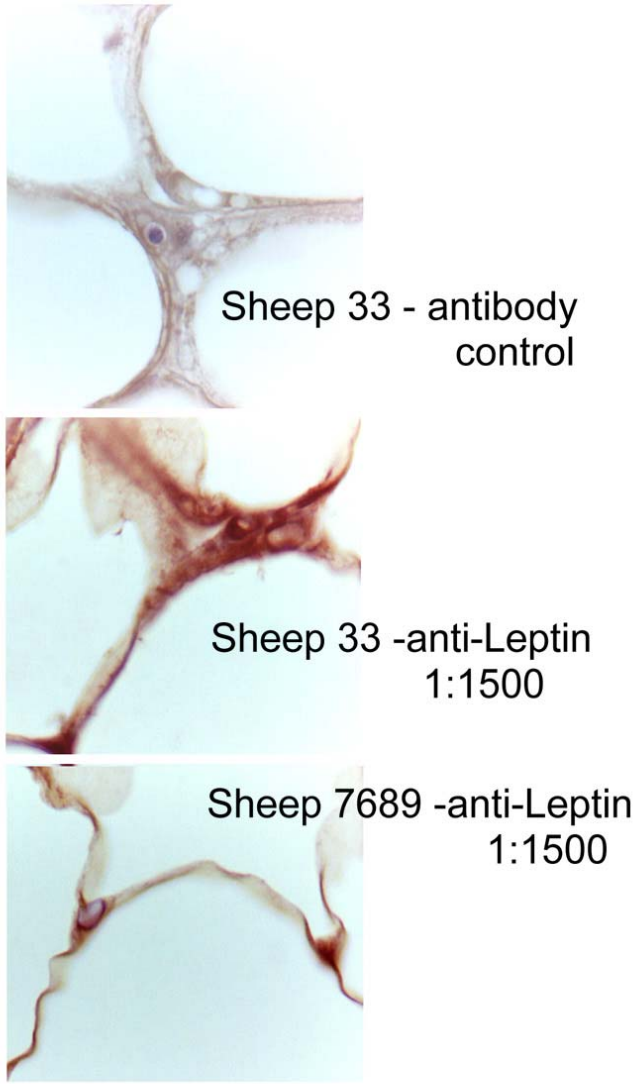


Fat Cell Leptin as a Mediator of Reduced Appetite and endocrine function

Venus of Willendorf. Circa 22,000 B.C.

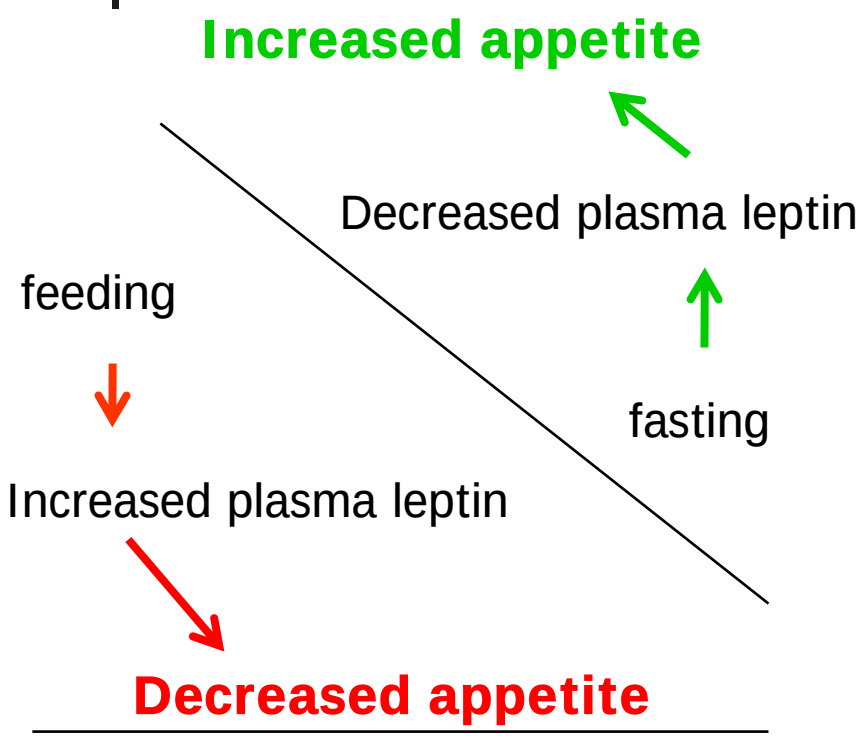
Leptin

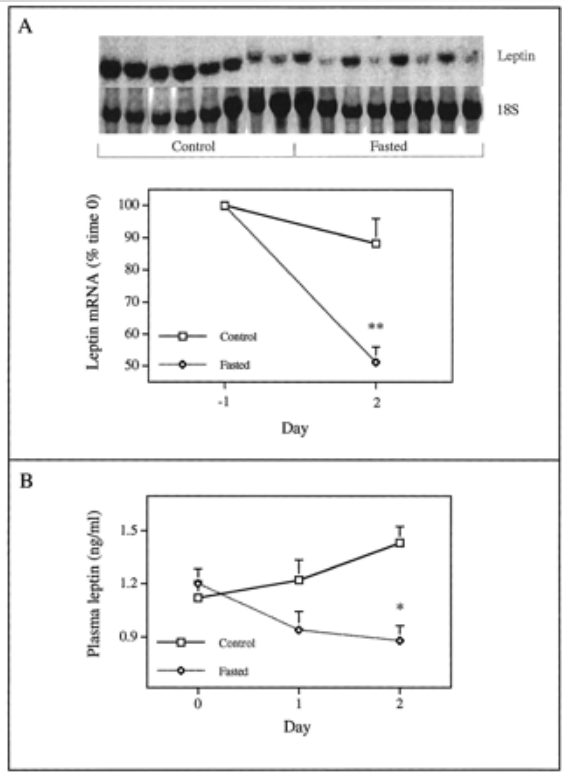
- Leptin is a product of the Ob gene and is synthesized and released from the fat cell of most animals including ruminants.



Daniel et al. J Anim Sci 81:2590, 2003

Fasting decreases plasma leptin concentrations in cattle



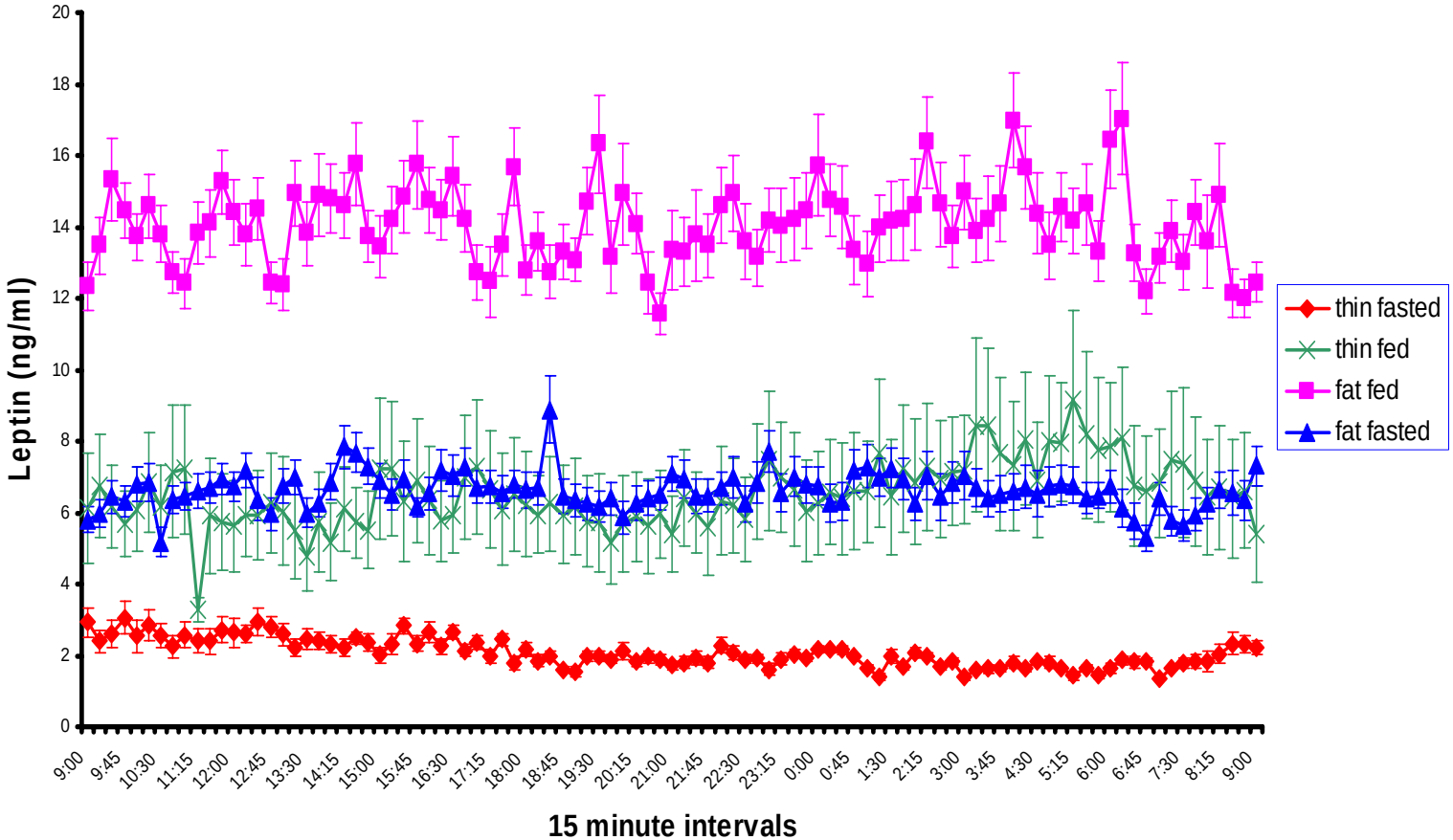


Panel A. fat cell leptin gene expression decreased with fasting and plasma levels also decreased.

Elevated leptin reduces food intake at the hypothalamus.

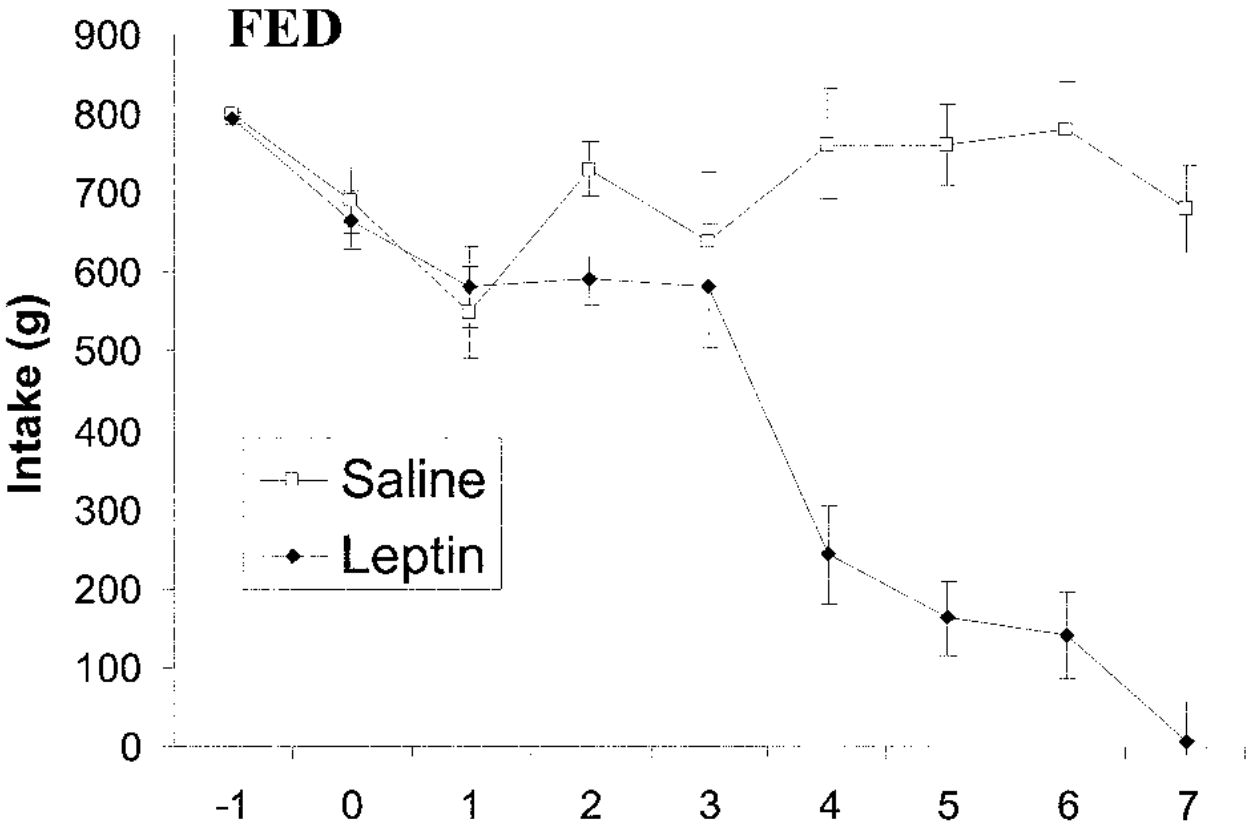
Amstalden et al. Biol Reprod 63;127, 2000
Chilliard et al. Domest Animal Endocrinol 21:271, 2001

Plasma leptin is not photoperiod sensitive but is elevated by obesity and reduced by fasting in sheep



Daniel et al, J Anim. Sci. 80: 1083, 2002

Effect of ICV infusion on feed intake in well fed lambs (P<0.001) but had no effect in fasted lambs

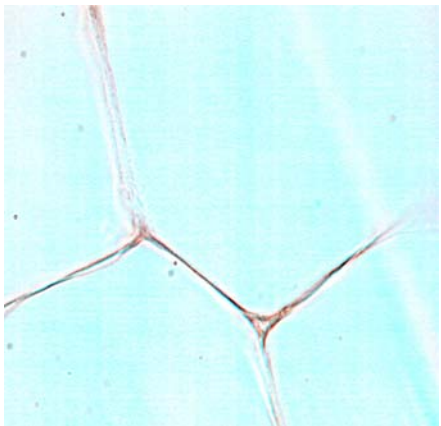


Morrison et al. J Endocrinol 168:317, 2001

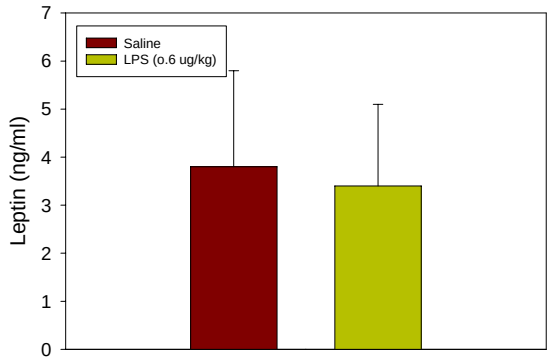
Effect of Endotoxin on Plasma Leptin Concentrations

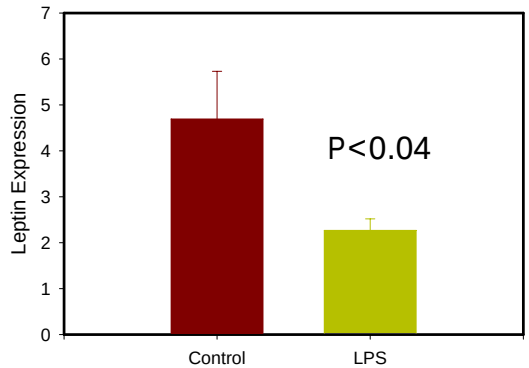
Ovine fat cell CD14 cloned:

GeneBank AY289201



CD14 immunostaining in sheep fat cells. Daniel et al, J Anim Sci. 2003.



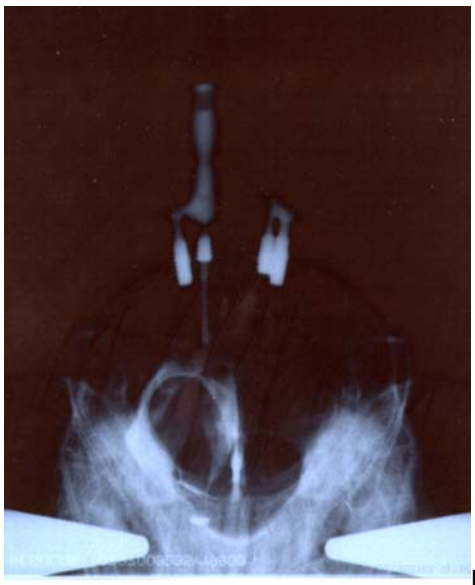


Soliman et al. Jpn J Vet Res. 50:107 2002.
Daniel et al. J Anim Sci, 81:2590, 2003

Conclusions

- In sheep, leptin is regulated by nutrition and elevated leptin inhibits feed intake.
- The targets for leptin may be neurotransmitters or receptors within the hypothalamus.
- Increased leptin may not mediate the inhibition of appetite with disease.

Hypothalamus and Appetite Regulation



Frontal x-ray of sheep brain showing injection into lateral ventricle

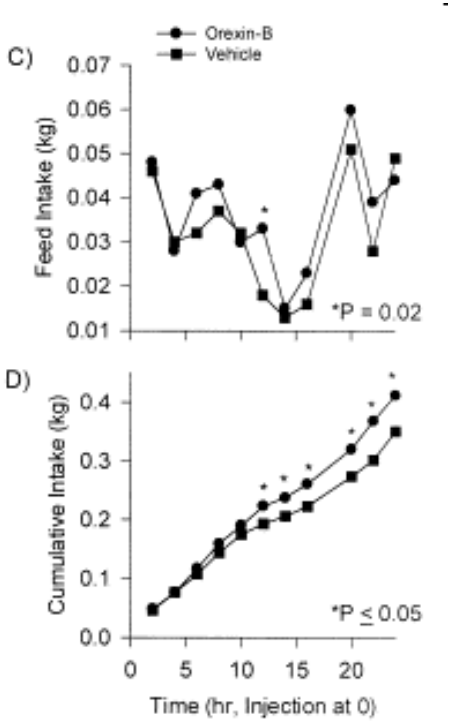
Neuropeptide Y
Agouti Related Protein
Melanin Concentrating Hormone
Orexin

Orexin-B

Orexin courtesy of Dr. Robert Matteri, USDA

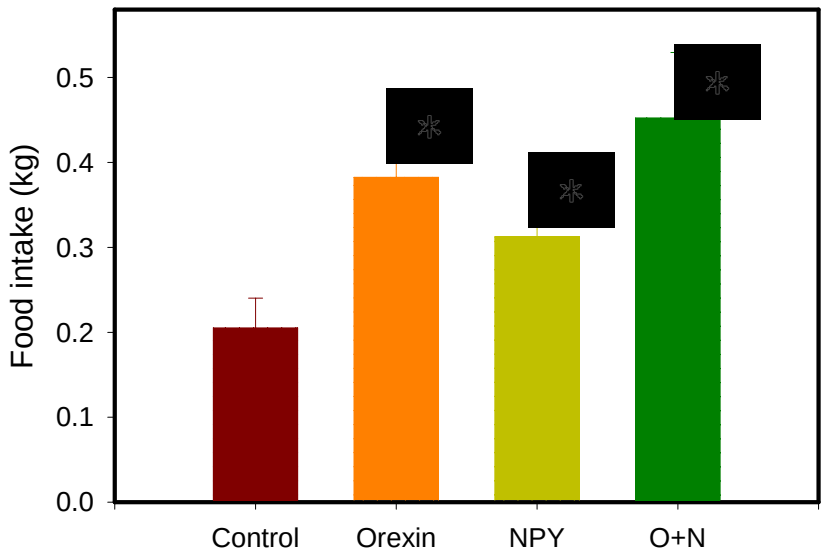
Effects of orexin B on feed intake.

Feed intake in pigs (i.m.)



Dyer et al. Domest Anim Endocrinol 16:145, 1999.

Feed intake in sheep (i.c.v.)

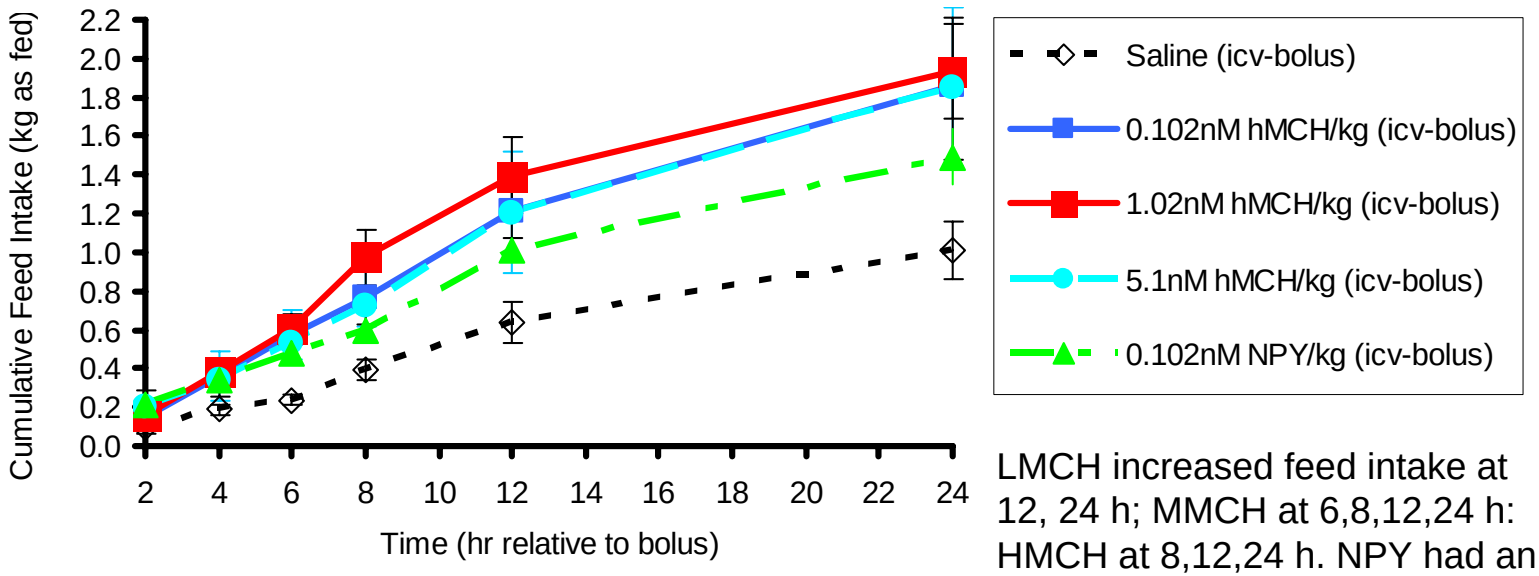


Sartin et al. J Anim Sci, 79:1573, 2001.

Melanin Concentrating Hormone (MCH)

Melanin Concentrating Hormone courtesy of
Dr. Fran Buonomo, Monsanto

Effect of ICV administration of MCH and NPY on Appetite in sheep



LMCH increased feed intake at 12, 24 h; MMCH at 6,8,12,24 h: HMCH at 8,12,24 h. NPY had an Effect at 12 and 24 h.

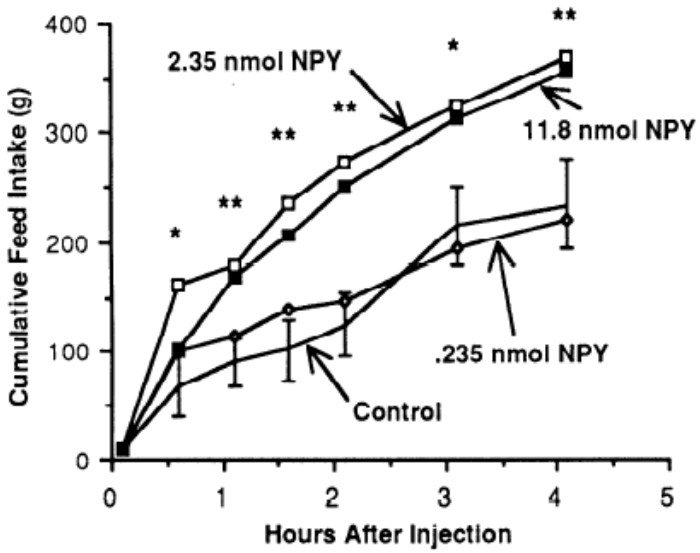
Whitlock et al. Domest Anim Endocrinol 28:224, 2005.

Neuropeptide Y

NPY

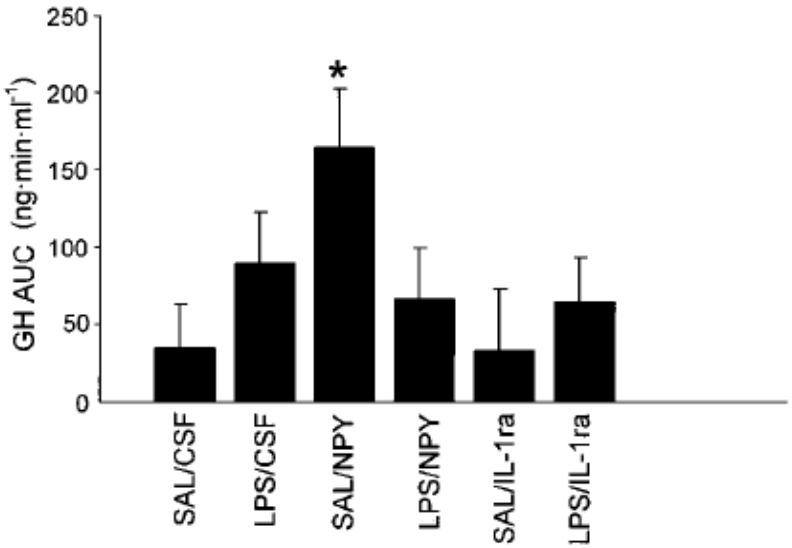
Effect of ICV administration of NPY in sheep

NPY increases feed intake



Miner et al. Amer J Physiol 257:R383, 1989

NPY increases GH release



McMahon et al. J Endocr 161:333, 1999.

Agouti Related Protein (AgRP)

Agouti Related Protein and other MC4R/MC3R drugs courtesy of Dr. Dan Marks, Oregon Health Sciences University

AGRP-background

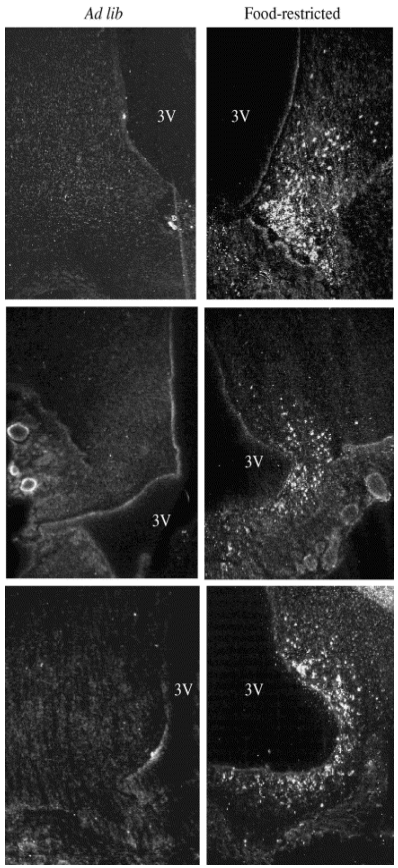
- AGRP is a melanocortin-4 receptor (MC4R) antagonist. AGRP prevents the inhibition of appetite.
- AGRP is colocalized with NPY in the hypothalamic arcuate nucleus.
- AGRP increases feed intake in rats and mice via the MC4R.
- AGRP neurons project to the areas of the hypothalamus that directly regulate feed intake.

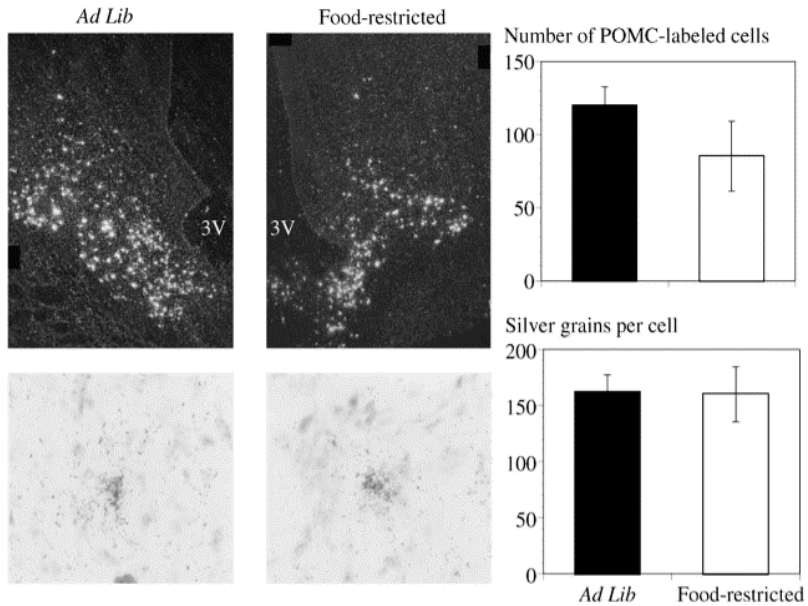
Is AgRP a physiological regulator of appetite in ruminants

- Is AgRP synthesized in the hypothalamus areas regulating feed intake?
- Is AgRP synthesis regulated by a physiological stimulus to feed intake?
- AgRP should increase feed intake when administered into the lateral ventricle of the sheep brain.

Effect of fasting 4 days on POMC and AgRP mRNA in sheep

Henry et al. Neuroendocrinology, 2001





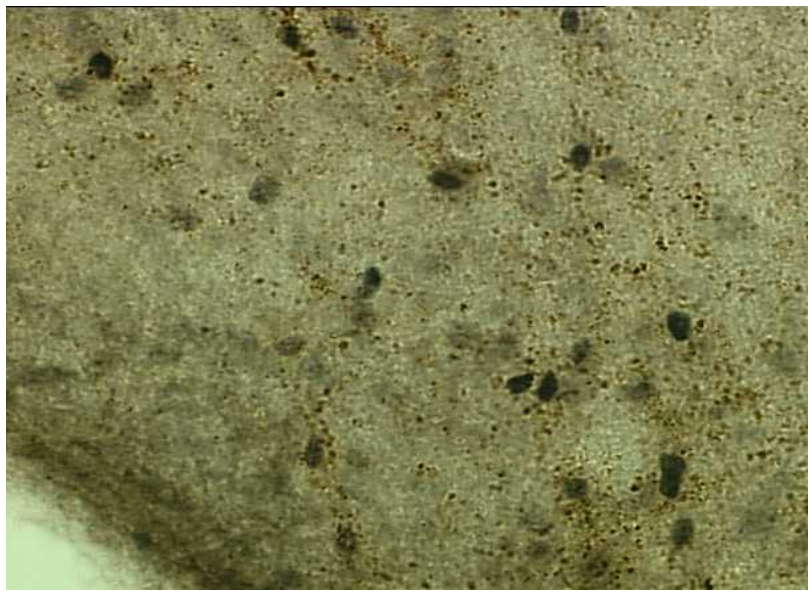
Above: no effect on POMC mRNA.

Left: AGRP mRNA increased.

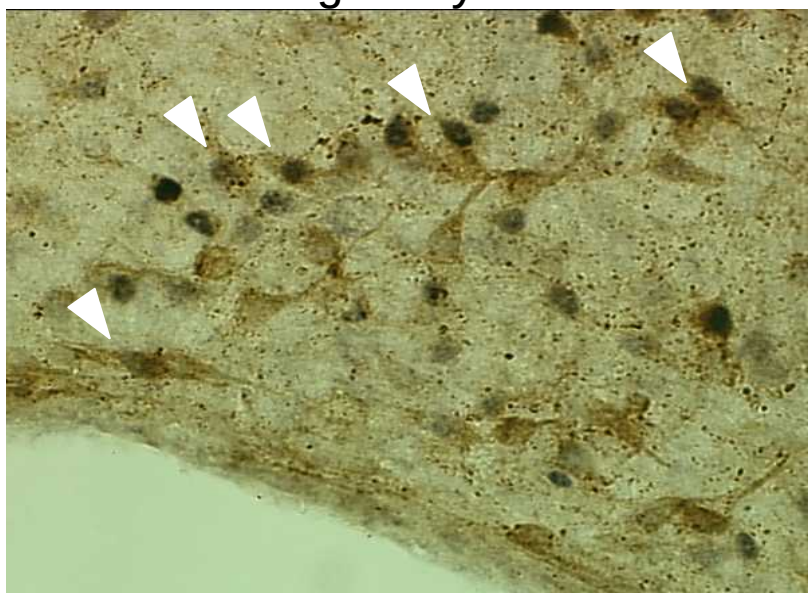
Effect of Fasting on AgRP neuron activation in the hypothalamic arcuate nucleus.

Wagner et al. Neuroendocrinology 80:210,2004.

Control

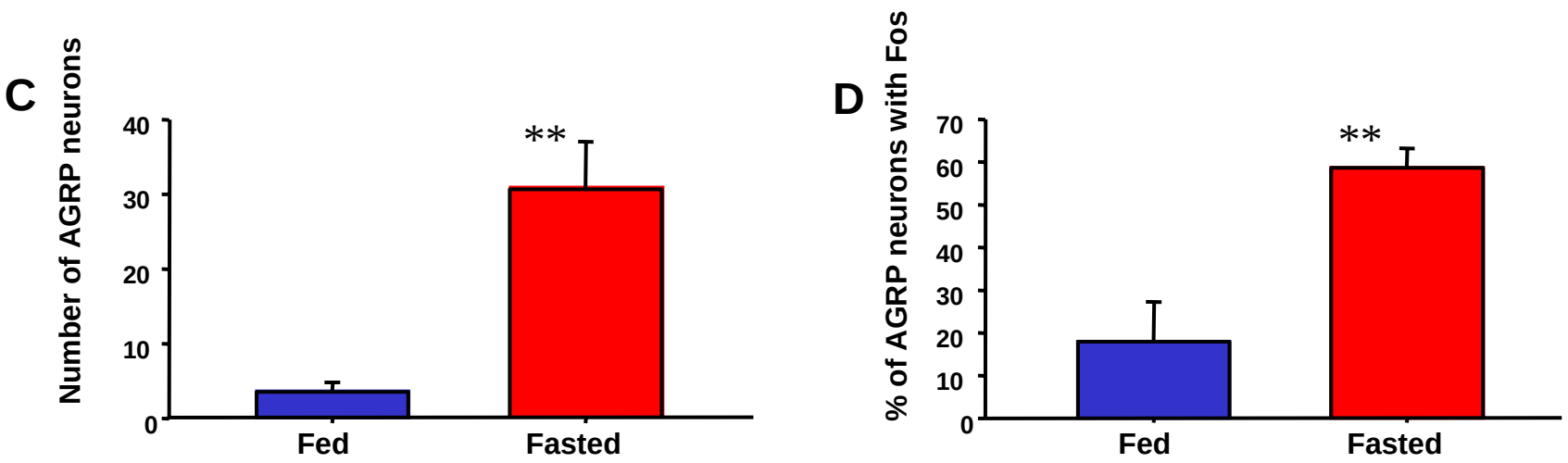


After fasting 3 days

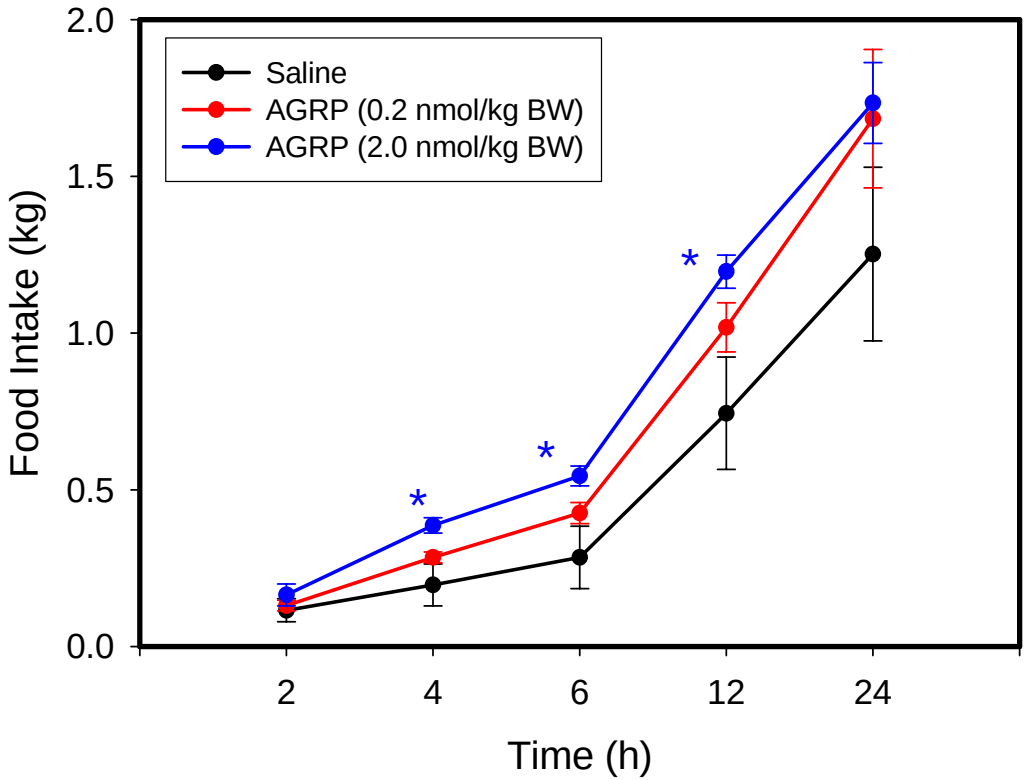


AgRP (cytoplasm; brown stain) colocalization with c-Fos (nucleus; black stain) in the arcuate nucleus of sheep. C-Fos is an index of neuronal activation.

Effect of fasting on AgRP neuron activation in the arcuate nucleus.



Effect of AgRP on Feed Intake in *ad libitum* Fed Sheep



AgRP (2 nmol) differed from controls at 4,6,12 Hours P<0.05.
AgRP at 0.2 nmol did not differ from controls.

Wagner et al. Neuroendocrinology 80:210,2004.

AgRP-conclusions

- AgRP stimulates feed intake in sheep.
- AgRP is synthesized in the hypothalamus.
- AgRP is regulated by a physiological appetite stimulus.

- Therefore, AgRP is an endogenous appetite regulator in sheep.

Can AgRP serve as a target molecule to manipulate feed intake

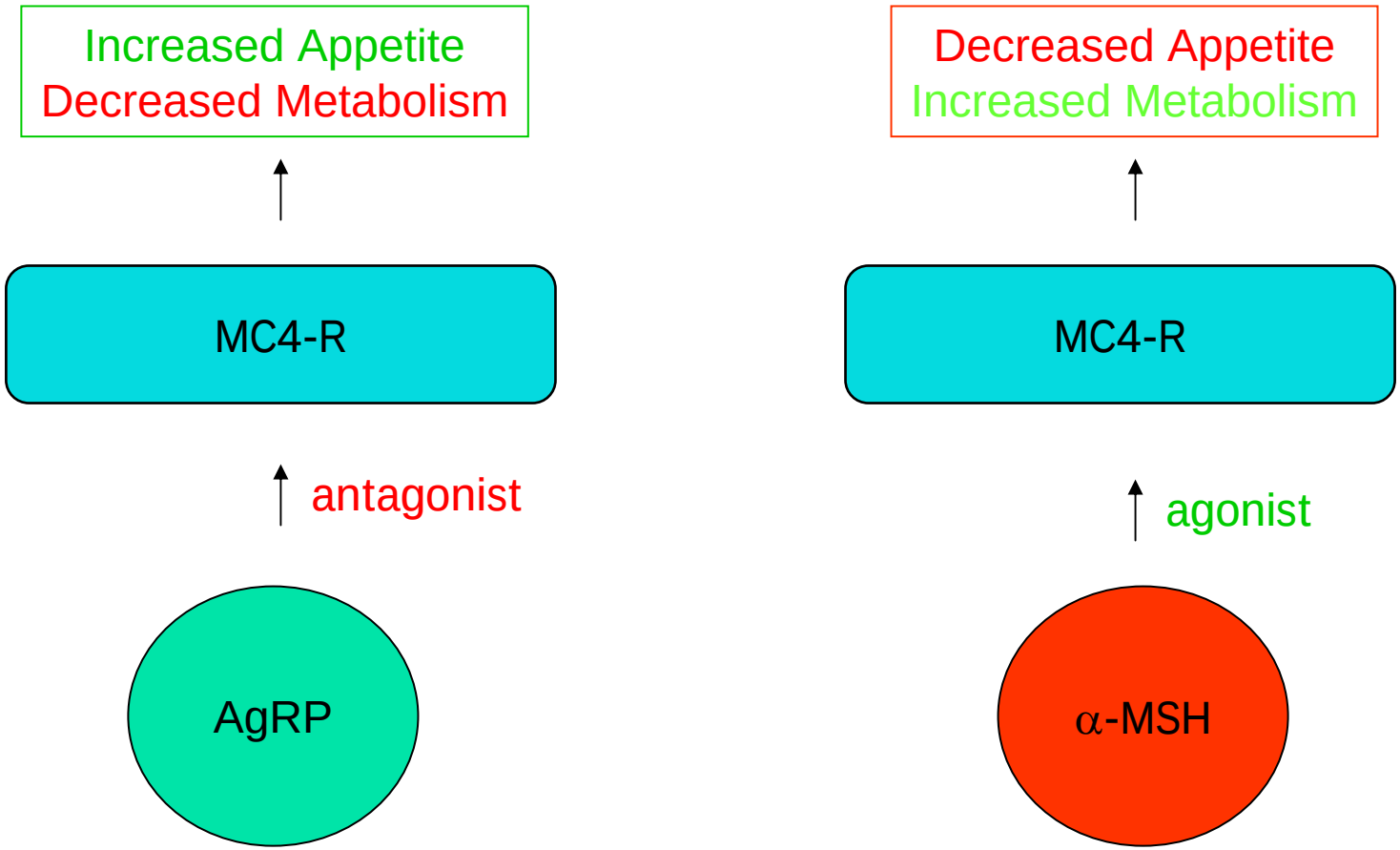
AgRP is an endogenous antagonist to the receptor that inhibits feed intake

MSH signaling through the MC4R and disease

- Cachexia consists of relative anorexia, increased metabolic rate, and pathological wasting of lean body mass.

- Central MSH activation of the melanocortin-4 receptor reproduces all features of cachexia. Thus an antagonist might have a role in modulating appetite during disease.

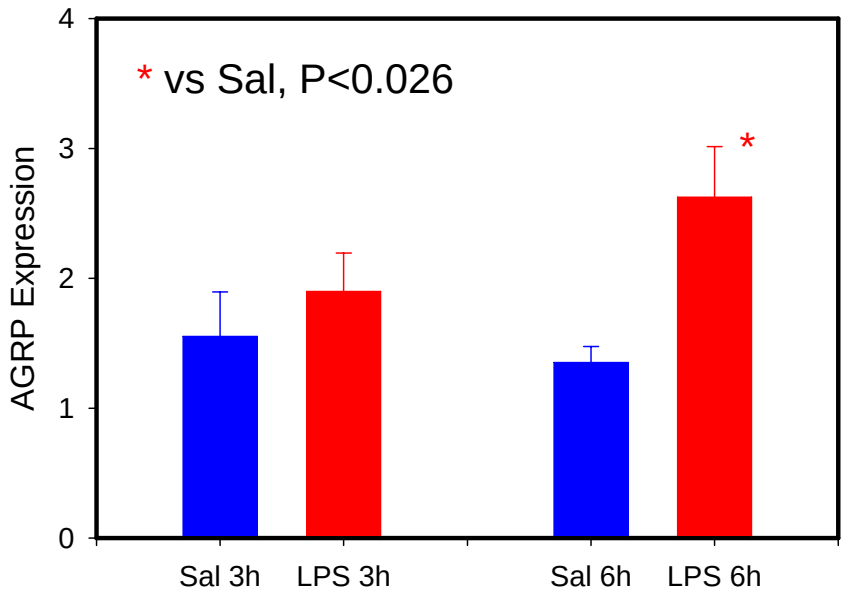
Melanocortin 4 Receptor



MC4-R as a Site for Reduced Feed Intake in Disease Models

- Does endotoxin cause changes in AgRP and MSH expression in the ARC?
- Can AgRP prevent the inhibition of food intake in endotoxemic sheep?

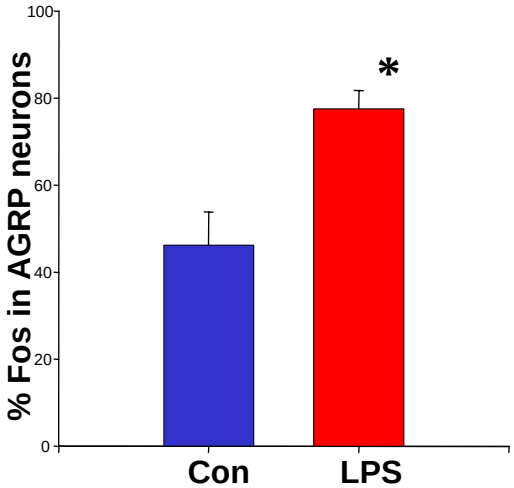
AGRP Gene Expression in Sheep Hypothalami after LPS



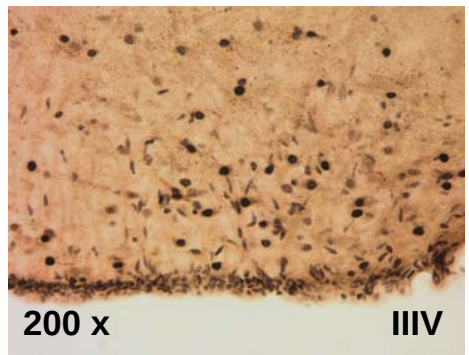
- Hypothalami were collected 6 hours after Saline or LPS injection. mRNA was assayed by real time PCR using ovine specific primers. AGRP expression was increased at 6 h after LPS.

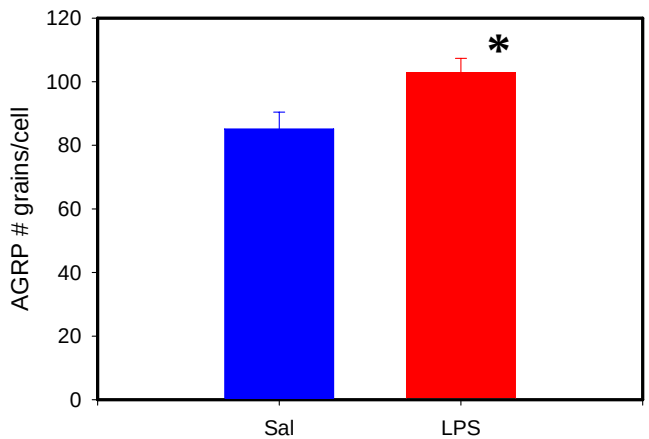
Sartin et al. JAS 86:2557-2567, 2008.

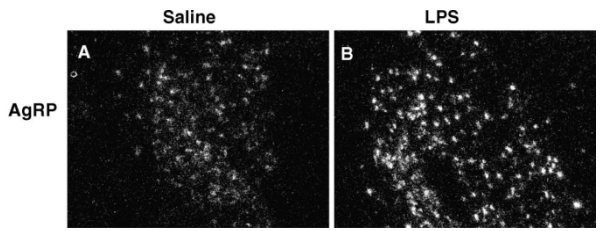
Effect of LPS on AgRP neuron activation (c-FOS) and AgRP gene expression



Dual label AgRP and c-FOS in the ARC, LPS stimulus







In situ hybridization of AgRP mRNA in the ARC

Sartin et al. JAS 86:2557-2567, 2008.

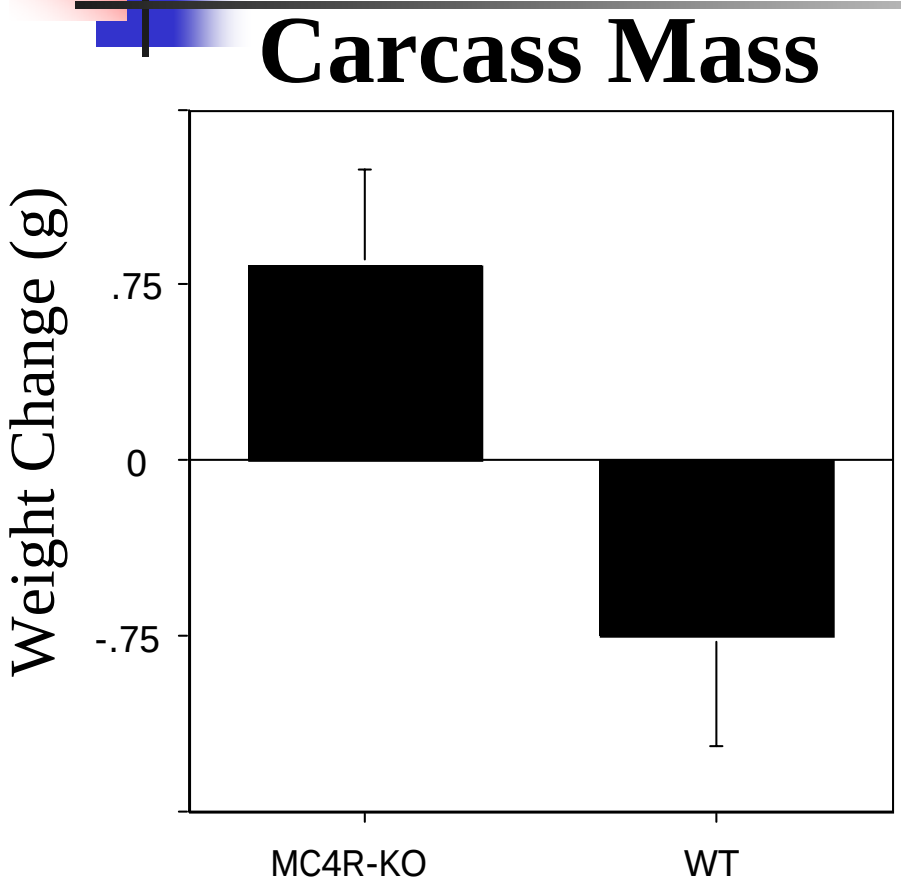
Conclusion

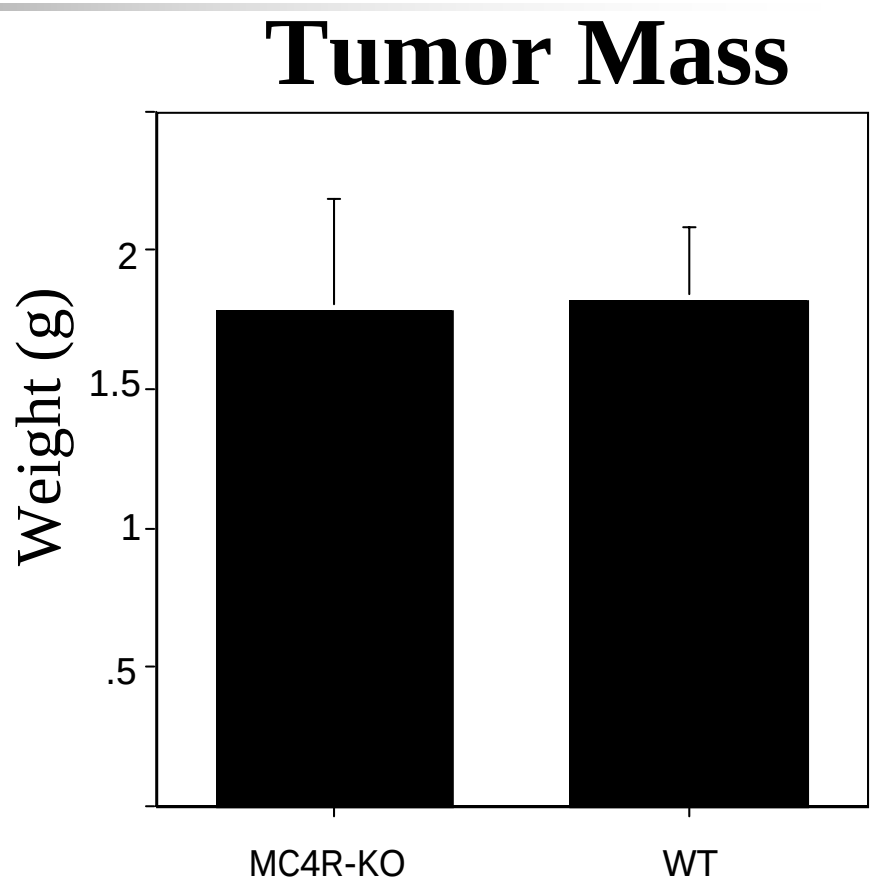
- The increase in activation of AgRP and reduced activation of α-MSH neurons represents an attempt to recover from appetite suppression due to LPS injection.

Agouti Related Protein maintains feed intake in disease

Can appetite regulatory neurotransmitters prevent or overcome reduced feed intake induced by endotoxin?

MC4-R KO Mice Maintain Body Mass with Normal Sarcoma Growth

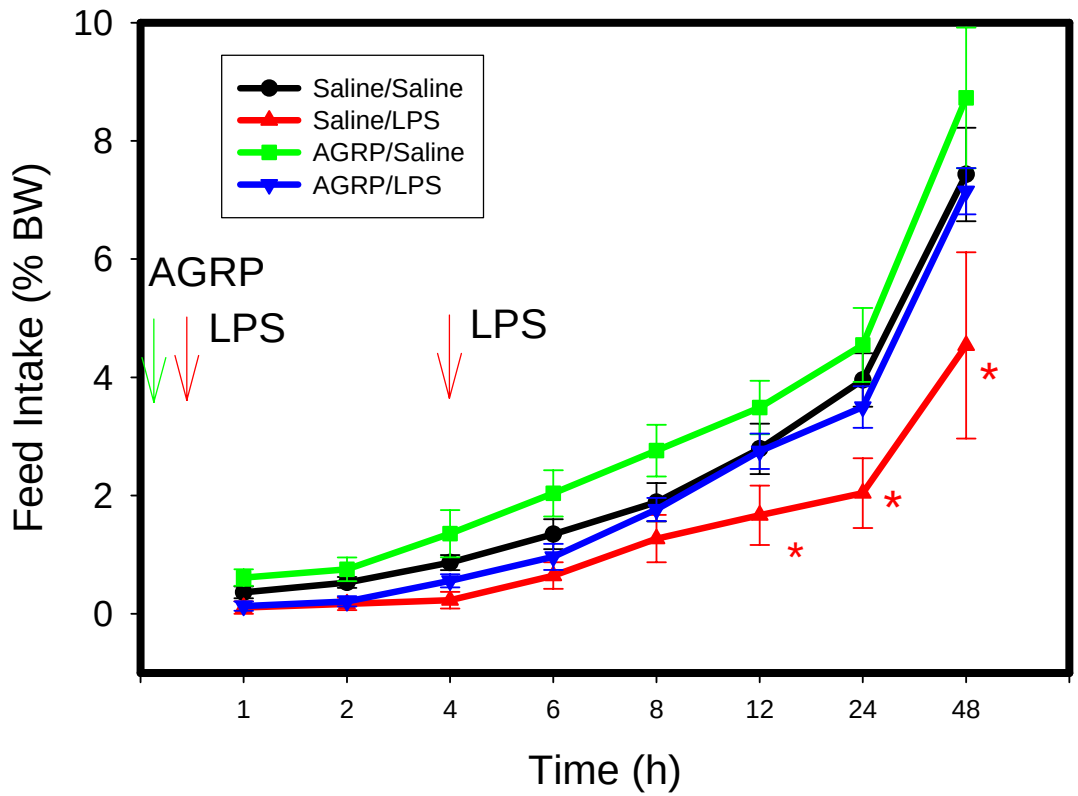




Marks et al., Cancer Res, 2001

Effect of AgRP on endotoxin (LPS)- induced cachexia in sheep

Sartin et al. J Anim Sci 86:2557-2567, 2008.



Effect of treatment: (P=0.0271)
Time: (P<0.001
Treatment by time: (P=0.0081).

LPS reduced feed intake At 12, 24 and 48 h (P<0.05) compared to control and to AGRP+LPS.

AGRP+LPS did not differ from saline feed intake at any time.

AgRP-Conclusions

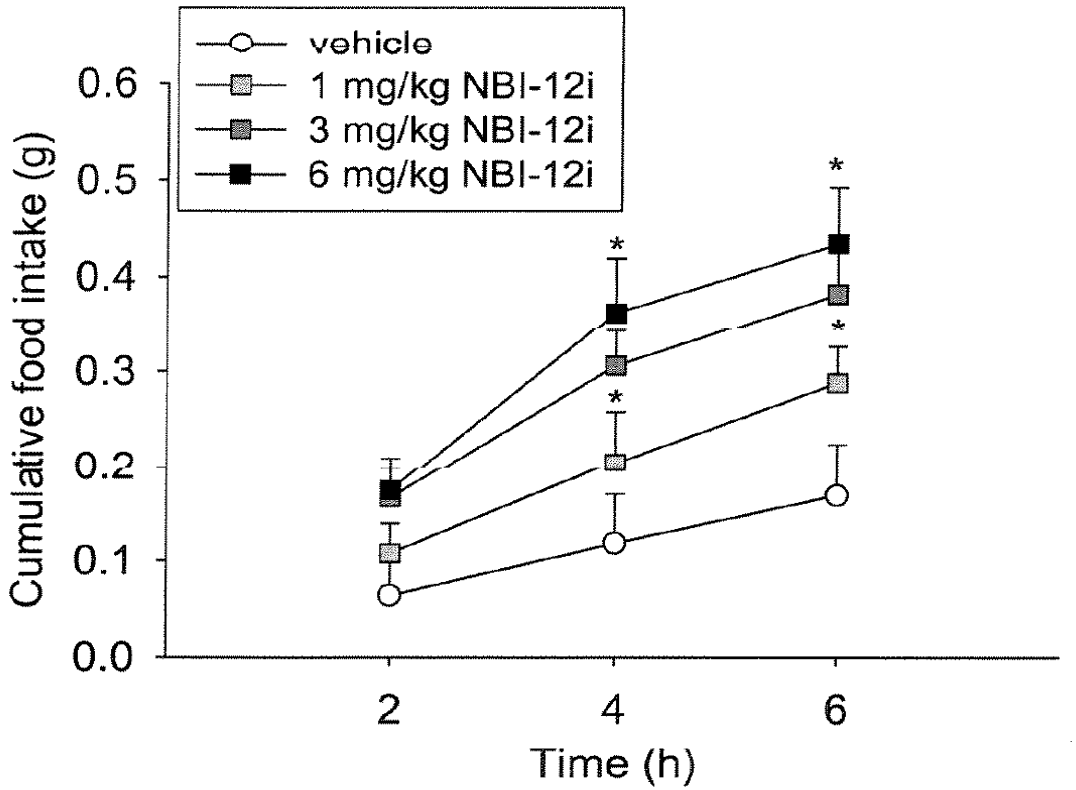
- AgRP is a MC4-R antagonist.
- AgRP prevents LPS reductions in feed intake.

- Therefore LPS actions to reduce feed intake may be mediated at the MC4-R.

Can IV administered pharmaceuticals be developed to improve appetite in disease models

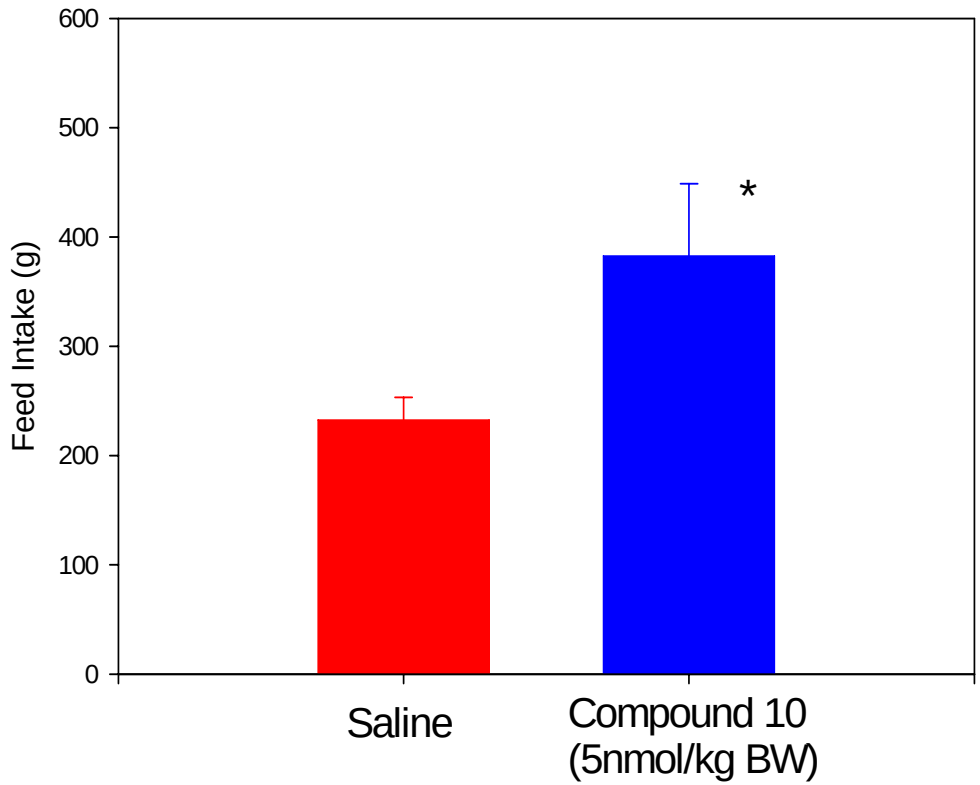
Small molecule MC4R antagonists

Small Molecule Antagonists Increase Food Intake in mice

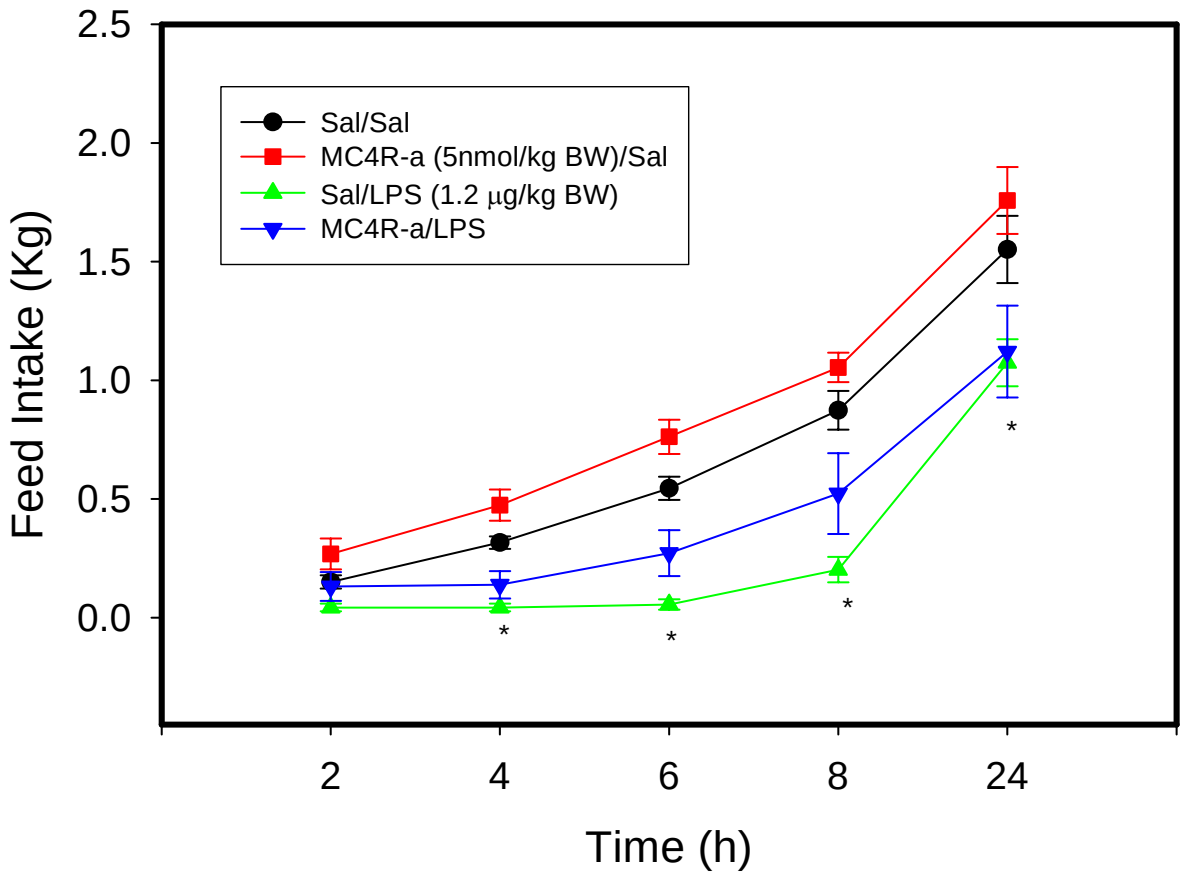


Markison et al, Endocrinology 2005

Effect of an IV MC4R antagonist (6h)



Effect of a synthetic MC4R antagonist on LPS inhibition of feed intake in sheep.



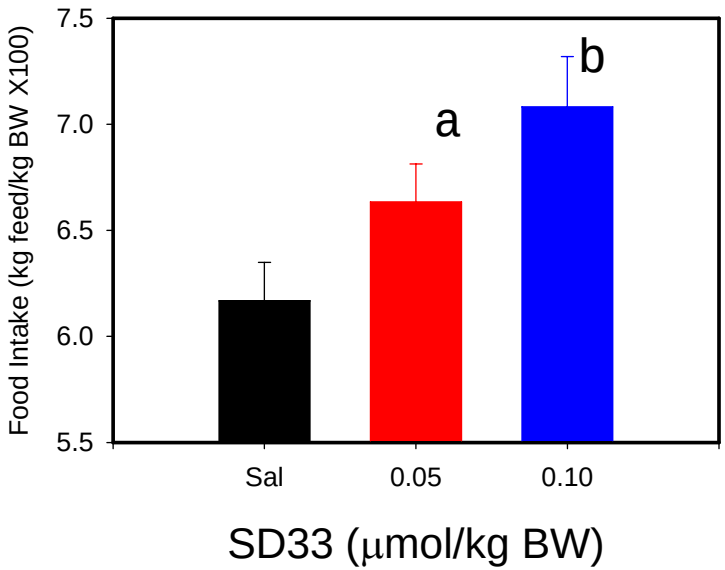
MC4Ra, P<0.001
LPS, P<0.0001
Time, P<0.001
LPS X Time, P<0.0002

Conclusions

- Small molecule MC4R antagonists may have potential for increasing food intake and should be useful in recovering food intake in cachexic disease in domestic animal species.
- These drugs may also be useful in other circumstances requiring increased feed intake, such as early lactation.

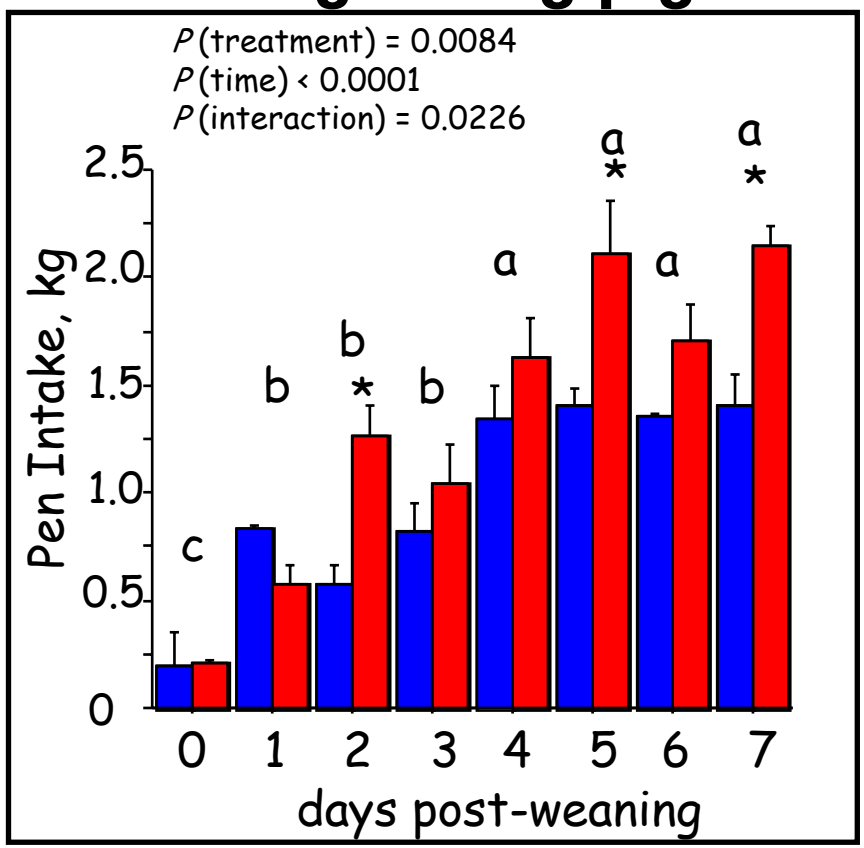
Opioid receptor agonist SD33 increases long term feed intake.

Effect in sheep 48h h after iv Injection.



Obese et al. J Anim Sci 85:111, 2007

Effect in growing pigs



Kojima et al doi 10.2527/jas.2009-2033

Future Studies

- We will examine changes in gene and protein expression of POMC and α-MSH in an LPS model to determine the mechanisms for appetite inhibition.
- We are planning to examine additional MC4R antagonists for their ability to alter feed intake in catabolic disease models.

Acknowledgements

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- Monsanto Company