ENERGY HOMEOSTASIS-CENTRAL REGULATION

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LEARNING OBJECTIVES:

- Describe the source of circulating leptin, and the changes in serum levels anticipated in fasting and obesity. Name the two conditions for which therapeutic leptin is effective.
- Name, then compare/contrast the two critical neuronal cell types in the arcuate nucleus of the hypothalamus that regulate appetite.
- Describe the mechanism of action for Lorcaserin (Belviq®) and list contraindications to its use.

Terms and Abbreviations:

Anorexigenic, appetite-suppressing
Hedonic, pertaining to pleasure or its perception
Hyperphagia, excessive eating
Orexigenic, increasing or stimulating the appetite
Satiety, the state of feeling full after eating
AgRP: Agouti-related protein
CART: cocaine- and amphetamine-regulated transcript
Db/db: the obese mouse model lacking the leptin receptor (LepR)
GOAT Ghrelin o-acyltransferase (enzyme that adds octanoyl fatty acid to ghrelin)
MSH: melanocyte-stimulating hormone
NPY: Neuropeptide Y
Ob/ob: the obese mouse model that lacks leptin
POMC: Pro-opiomelanocortin (source of αMSH)

I. Introduction and history

A. Obesity is a "growing" epidemic. According to data from the U.S. National Health and Nutrition Examination Survey, 2009–2010:
- More than 2 in 3 adults are considered to be overweight or obese.
- More than 1 in 3 adults are considered to be obese.
- More than 1 in 20 adults are considered to have extreme obesity.
- About one-third of children and adolescents ages 6 to 19 are considered to be overweight or obese.
- More than 1 in 6 children and adolescents ages 6 to 19 are considered to be obese.

B. The hypothalamus is fundamental in regulating body weight. It has internal mechanisms for integration of the sensory inputs, involving comparison of the sensory data to a set point, which indicates the ideal levels for body temperature, body weight, serum sodium, etc. The hypothalamus integrates diverse sensory inputs (e.g. smell, taste, gastric stretch, hormones, etc.) into a series of coordinated autonomic, endocrine, and behavioral responses.

1. The importance of the hypothalamus in regulating energy balance was originally confirmed from classic brain lesion experiments of the early 1900's (see Figure 1).

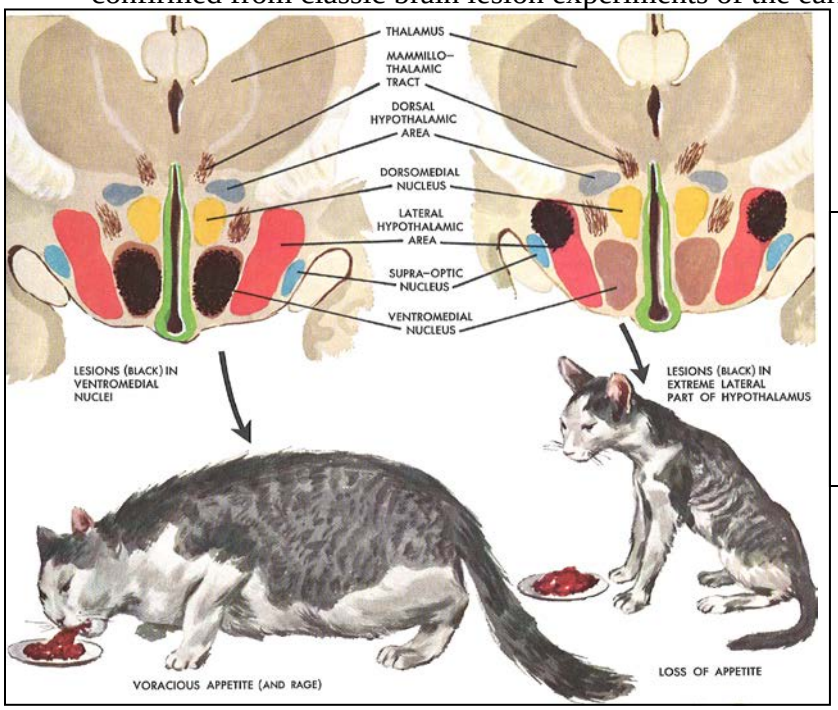


Figure 1. Lesions (indicated in black) of particular hypothalamic regions result in extreme changes in appetite, food intake, and body weight.
From Netter's CIBA collection of Medical Illustrations, Vol. I. Nervous system-Suppl. Plate 8.

2. With advances in mouse genetics, drug design and administration, laser-capture microdissection, and metabolic phenotyping, investigators have now "lesioned" selected cells of the hypothalamus and/or modified metabolic and hormonal pathways in discreet regions of the brain to delineate many of the neuronal circuits required for food intake control (Figure 2).

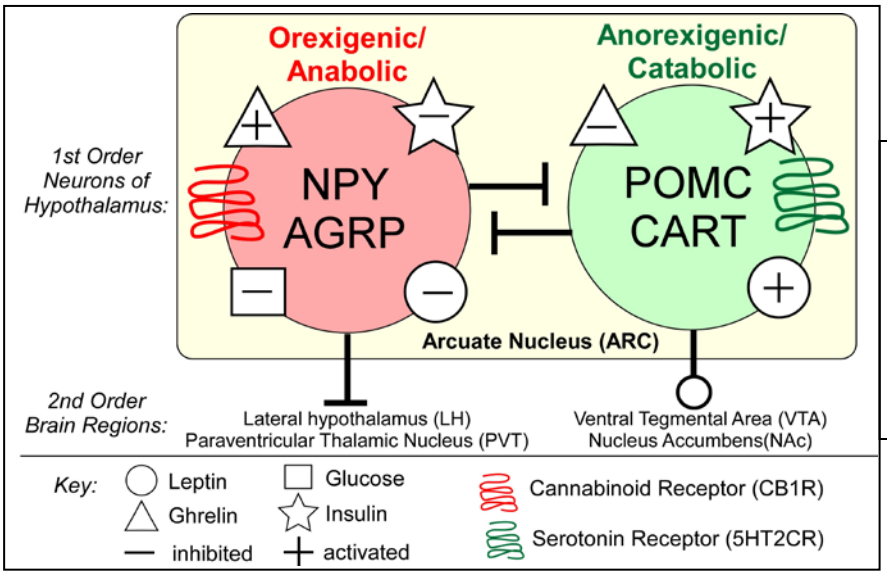


Figure 2. Nutrient and hormonal regulators of the critical ARC neurons (NPY/AGRP and POMC/CART) that control food intake. Adapted from: Ferrario, et al. 2016. Homeostasis meets motivation in the battle to control food intake. *J. Neurosci.* 36:11469-11481.

1. Two cell types located in the arcuate nucleus (ARC) of the hypothalamus are critical. They are defined by the peptide hormones they produce and secrete (Figure 2):
    a. NPY/POMC neurons, when activated, promote feeding (i.e. are orexigenic) and anabolic processes (compatible with the fed-state's "goal" of using/saving calories).
    b. POMC/CART neurons, when activated, reduce appetite (i.e. are anorexigenic) and promote catabolic processes (compatible with changes of the fasted state - utilization of previously stored nutrients).

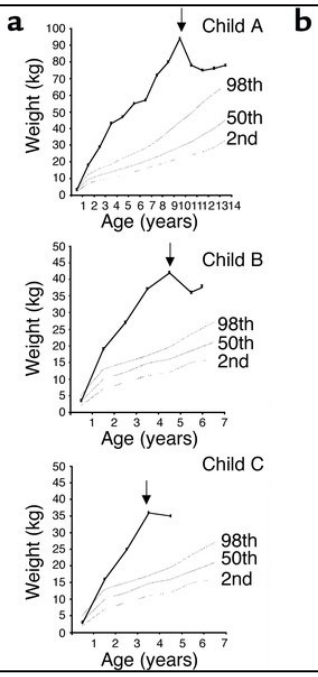
We will continually refer to Figure 2, as we discuss hormones that regulate the action of these neurons, and new FDA-approved drugs that target these neuronal circuitries to decrease appetite and promote weight loss.

II. Hormones that impact food intake via central mechanisms.

A. **Leptin** (from the Greek λεπτοσ (leptos) = thin, as thought to be a satiety hormone)

1. An adipokine exclusively expressed and secreted from adipocytes (see Rana Gupta's adipose syllabus).
2. Discovered by positional cloning of the causative gene responsible for the phenotype (extremely hyperphagic and obese) of the ob/ob mouse model.
3. The leptin hormone is a 167aa protein hormone that circulates both free and bound to serum proteins.
4. The db/db mouse model (like ob/ob, obese and hyperphagic) was used to identify the leptin receptor. This plasma membrane receptor, LepR, uses the JAK/STAT signal transduction mechanism and is highly expressed in hypothalamic neurons.
5. Is leptin a satiety hormone? This is still a controversial topic (e.g. *Cell Metabolism* opinion article July 2017).
   a. Chronically, serum leptin levels reflect adiposity, i.e. obese individuals have very high circulating leptin levels and do not respond to leptin administration (leptin resistant?).
   b. Acutely, serum leptin levels fall during fasting (and sleep deprivation) and some propose that it is these acute changes that somehow impact feeding behavior.
   c. Ultimately no clinical trials of leptin therapy in obese subjects have shown a benefit by decreasing appetite or food intake (bummer!).
6. However, Leptin has received FDA-approval for treatment of two conditions (both of leptin *deficiency*, so this is essentially a hormone-replacement strategy):
   a. In individuals with inactivating mutations of the leptin gene (like the ob/ob mouse), restoring leptin is a "miracle" (Figure 4). Note this is a very rare genetic disorder (see Table 1).

Figure 3. Ob/ob is on left.



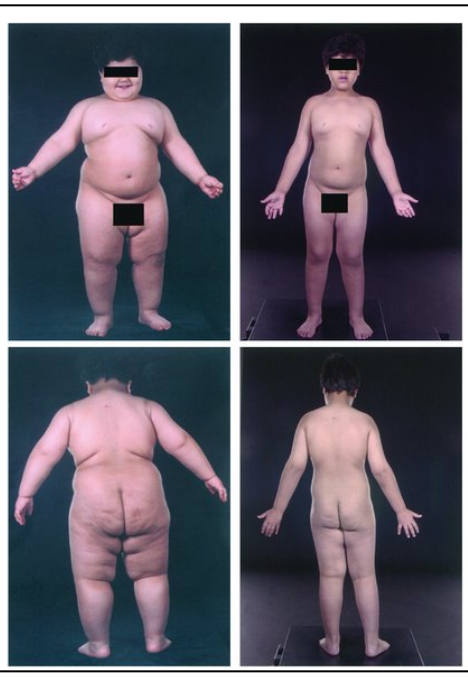


Figure 4. Effects of recombinant-human-Leptin on weight in three children with congenital leptin deficiency.
(a) Weights of child A compared with normal centiles for girls and of child B and child C compared with normal centiles for boys. Arrows indicate the start of rhLeptin therapy.
(b) Clinical photographs of child B before (height = 107 cm) and 24 months after rhLeptin therapy (height = 124 cm). (Farooqi, et al. *J. Clin. Invest.* 110(8):1093-1103, 2002).

b. Here at UT Southwestern, Dr. Abhimanyu Garg, has received FDA approval to use leptin in individuals with lipodystrophy (no adipose = no leptin). Leptin injections improve glucose homeostasis, serum lipid levels, and NAFLD (non-alcoholic fatty liver disease).

B. **The melanocortin system (αMSH/MC4R)**

1. The anorexigenic/catabolic neurons of the arcuate nucleus (the POMC/CART neurons, see Figure 2) express proopiomelanocortin (yes, the same preprohormone you met in the lecture describing the corticotropes of the anterior pituitary). However, in POMC neurons a complement of proprotein convertase enzymes cleave a peptide fragment called αMSH (not ACTH) which is released to bind MC4 receptors in downstream effector neurons to reduce food intake.
2. The importance of this ligand (αMSH) – receptor (MC4R) system on feeding control and obesity is evident by the phenotypes observed when the signaling system is absent (Figure 4).

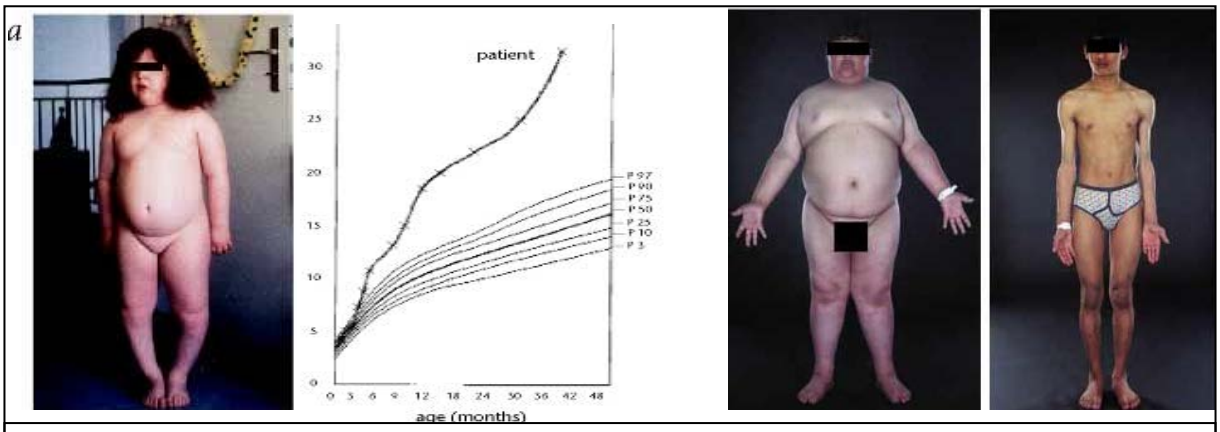


Figure 4. The loss of αMSH (a, POMC mutation) and MC4R (b, with affected brother on left and unaffected sibling on right) result in genetic forms of hyperphagia/obesity. From Krude, et al. 1998. *Nature Gen.* 19:155-157 and Farooqi, et al. 2003 *N. Engl. J. Med.* 348:1085-1095.

3. Characteristics of individuals with relevant POMC mutations and/or MC4R deletion:
    a. Hyperphagia
    b. Hypometabolism
    c. Hyperinsulinemia
    d. Increased linear growth
    e. Often red hair pigmentation (in individuals with POMC mutations that concomitantly affect peptide hormones for MC1R action in melanocytes).
4. A recent report suggests that "hormone replacement therapy" may be effective in individuals with POMC mutations (Kuhnen, et al. 2016 *N. Engl. J. Med.* 375:240-246). A daily injection (for 42 weeks) of an MCR4 agonist in a patient with POMC deficiency resulted in a 33% loss of body weight [from 340 to 230 lb] and a dramatic decline in serum leptin levels [biomarker of adiposity, falling from 45 to 4 ng/ml].
5. Finally, these genetic obesity syndromes also affect our pets, as it was recently discovered that POMC mutations are quite common in labrador retrievers (particularly those bred as assistance dogs) and predispose them to hyperphagia, adiposity and extreme body weight (Raffan, et al. 2016 *Cell Metab.* 23: 893-900).

C. Genetic forms of obesity involving the leptin and/or melanocortin systems are extremely rare (see Table 1). However, they provide critical information on the neural circuits regulating food intake, and may provide clues to new avenues of pharmacotherapy to impact body weight regulation.

Table 1. The prevalence of genetic forms of human obesity.

	MUTATION%	
GENE	GENERAL POPULATION	OBESE POPULATION
MC4R	0.66%	6-8%
POMC (rs28932472)	0.22%	0.88%
PC1 (PCSK1)	0.03%	0.19%
LEP	rare	rare
LEPR	rare	rare

From: Farooqi, et al., (2003). *New England Journal of Medicine*, 348(12), 1085-1095.

D. **Ghrelin.**

1. Ghrelin is a circulating peptide hormone expressed and released by enteroendocrine cells (the A or (X-like) subtypes) of the gut. These enteroendocrine cells are largely located in the stomach and distributed along the upper small intestine.
2. The ghrelin peptide requires a unique post-translational modification (addition of an 8-carbon fatty acid by the enzyme GOAT) to be active and capable of binding its receptor GHSR [growth-hormone secretagogue receptor].
3. Ghrelin is released under low-glucose conditions; blood concentrations of ghrelin increase during fasting; GHSR of the hypothalamus becomes activated (in orexigenic cells) to enhance appetite and promote food intake.
4. Serum ghrelin levels are increased with fasting, sleep deprivation, and in the hyperphagic genetic disorder, Prader-Willi syndrome.
5. Ghrelin is also highly involved in the regulation of glucose metabolism. Independently, and in conjunction with its ability to promote growth hormone release (from anterior pituitary), ghrelin promotes increased serum glucose levels to prevent hypoglycemia.
6. Finally, the ghrelin receptor, GHSR, is located in a variety of other brain regions associated with hedonic (pleasure or its perception) aspects of eating and behavior.

III. Drug therapies that impact food intake via central mechanisms.

An important consideration in targeting the CNS for weight control:

As eating is a complex behavior in humans, the identification of therapeutic strategies to impact only the homeostatic/metabolic/energy central circuitry and not adversely affect the hedonic/pleasure neuronal circuitry has been difficult. For the recently FDA-approved weight loss agents (Table 2) that work by central mechanisms, nearly all include safety warnings to watch for cognitive changes, depression, and suicidality. One of the first "CNS weight loss drugs" (Rimonabant, approved in Europe, but not by FDA in US) was withdrawn from the market because of the significant increase in psychiatric complications and suicides. Finally one of the most common adverse events, albeit rare, following the extreme weight loss observed with bariatric surgery is depression with increased incidence of suicide.

A. That said, from 2012 to 2014, three drugs received FDA-approval (the first such drugs approved in over 14 years): Belviq; Qsymia; and Contrave.

   1. Lorcaserin (Belviq) initially raised concerns because it works much like fenfluramine, a weight-loss drug that was withdrawn from the market in 2007 because it caused heart valve damage. However, Belviq shows improved receptor specificity – activating only the 5HT2**C**R of POMC cells, and not the 5HT2**B**R expressed on cardiac valvular interstistial cells and pulmonary-artery smooth-muscle cells.

   2. Qysmia is a combination drug consisting of phentermine (~amphetamine, a controlled substance with the potential for abuse) and topiramate (an anti-epileptic agent).

   3. Contrave is a combination drug consisting of naltrexone (an opioid receptor antagonist usually used to treat alcoholism) and bupropion (an antidepressant used for smoking cessation).

   4. Oral administration, generally once per day.

   5. All are thought to work primarily by activating the melanocortin signaling pathway (via POMC neurons) to reduce appetite.

Currently FDA-approved anti-obesity drugs that act via CNS					
Name	Mechanism of action	Average weight loss at 1 year vs. placebo	% of Adults achieving >5% loss of body wt at 1 year vs. placebo	Safety warnings	Contraindications
Lorcaserin (Belviq®)	5HT2CR agonist	5.8 kg (12.8 lb)	47.5%	5HT syndrome valvular heart dis cognitive depression hypoglycemia	MAOIs, use with extreme caution with serotonergic drugs (SSRIs, SNRIs) pregnancy
Phentermine/ Topiramate (Qsymia®)	NE transport inhibitor/ GABA agonist	8.1 kg (17.9 lb)	62%	Fetal toxicity Acute myopia Cognitive Metabolic acidosis	Glaucoma, Hyperthyroidism MAOIs pregnancy
Naltrexone/ Bupropion (Contrave®)	Opioid receptor antagonist/ DA reuptake inhibitor	6.1 kg (13.4 lb)	39%	Suicidality BP, HR Seizure risk Glaucoma hepatotoxicity	Seizure disorder Uncontrolled HTN Chronic opioid use MAOIs pregnancy

Ultimately all of these drugs activate POMC neurons in ARC

Taken from Chen, Y. 2016. *Drug Discoveries & Therapeutics* 10(2): 62-73.

IV Endocannabinoids

A. It has long been recognized that tetrahydrocannabinol (THC) the principle psychoactive constituent of marijuana increases hunger (i.e. the "munchies"). In fact, in the states that allow for medical marijuana use (currently 29 states, *not* Texas) one of the main reasons for its prescription is to enhance appetite in those with various cancers and/or undergoing chemotherapies.
B. THC exerts these effects on appetite by binding as an agonist to cannabinoid receptors, in this case the CB1 receptor (a GPCR) of the CNS. More recently, endogenous agonists (the endocannabinoid lipids, AEA and 2AG) have been identified.
C. Pharmaceutical companies have developed antagonists of the CB1R to reduce appetite (by inhibiting the action of the NPY/AGRP orexigenic neurons, see Figure 2). However, as mentioned earlier, the first of this class (Rimonabant, approved only in Europe) was withdrawn because of adverse psychiatric complications, including depression and suicide.

Dr. Repa wishes to acknowledge the contributions of faculty members who have presented this lecture in previous years and, therefore, have contributed to the content and organization of this syllabus: Drs. Jeffrey Zigman, Deborah Clegg (now at Cedars-Sinai), and especially Joel Elmquist [Director of the Division of Hypothalamic Research].

Practice questions:

1. Which of the following hormones is thought to increase appetite:
   A. Alpha-melanocyte-stimulating hormone
   B. Ghrelin
   C. Insulin
   D. Leptin

2. Which one of the following statements is true:
   A. Activation of NPY/AgRP hypothalamic neurons promotes anorexigenic processes.
   B. Blocking the action of POMC/CART hypothalamic neurons promotes satiety.
   C. Leptin therapy has been effective in treating common forms of obesity.
   D. Use of the new long-term "CNS weight loss drugs" (Belviq, Qsymia, and Contrave) is contraindicated in individuals taking monoamine oxidase inhibitors for atypical depression or Parkinson's disease.

Answers
1. B
2. D