Eating and weight regulation
Introduction: weight regulation in an affluent society

- In our society much effort and money is expended on regulation of weight.
- Failure to maintain weight within appropriate limits has resulted in a so-called ‘epidemic of obesity’.
- 30% of the US population is estimated to be obese.
- Since obesity is associated with health problems (type II diabetes, heart disease, etc.) and premature death, this is an important public health issue in the US.
- Of course, much of the world has the opposite problem, that of not having enough to eat. However, given where we live it is still necessary (and valuable for drug companies and people selling bariatric surgery!) to try to address the problems associated with overeating.
• Efforts to treat obesity by manipulating brain systems for weight regulation may be doomed to failure, because such systems were evolved to guard against starvation, not to prevent obesity, which presumably would have been very unusual in cavemen.

• Any drugs which suppress eating by acting on the brain are likely to have major side effects (as has been observed) because they perturb transmitter systems to produce this ‘unnatural’ state; despite early optimism about leptin there is no natural brain mechanism which has been successfully exploited to eliminate weight gain in ‘typical’ obese humans.
Although the role of genes in body fat regulation is now established, it is safe to assume that the rising prevalence of obesity has not been due to a recent change in the genetics of the Western world. It is very likely that a propensity to store fat in times of abundance was a positive trait selected over a long period of human evolution.
Genes and the environment both affect weight

• >30% of variation in body mass index (BMI) is genetically controlled (estimates range from 30% to 80%).
• The remainder is presumably determined by environment.
• This graph shows that obesity correlates with economic status (both of the subject and of her parents). Thus, unless one believes that there is a gene for socioeconomic status, these kinds of results suggest that cultural conditions (food availability and cost, necessity for exercise in daily life, cultural valuation of obesity vs. thinness) are likely to be important in determining the frequency of obesity.
Regulation of feeding behavior in rodents.

- Whether a rat or mouse eats or not is determined by many factors.
- These include the proximity of food, caloric/nutritional value of available food, danger of predation during feeding, seasonal expectations of future food supplies, circadian rhythm, etc.
- The regulation of feeding decisions is one of the central functions of the brain, since eating is essential for survival.
- Regulation of feeding behavior is very complex, involving many neurotransmitters and brain areas.
- We will focus on the hypothalamus in this lecture as its role is best understood.
Hypothalamic lesions and their effects on eating and weight

- Ventromedial (VMH) + arcuate (ARC) hypothalamic lesions produce hyperphagia/obesity
- Lateral hypothalamic (LHA) lesions produce aphagia/weight loss
The concept of the adipostat

- Parabiosis (linking of circulatory systems) experiments showed that when a rat with a VMH lesion is connected to a normal rat, it becomes obese as usual but the normal rat starves to death.

- The VMH-lesioned rat cannot perceive fat excess, but a factor generated by its (fat-filled) adipocytes acts on the normal rat to prevent it from feeding.

- The VMH of the normal rat senses that adequate fat stores exist, and this rat stops eating in order to maintain fat at the appropriate level.
Leptin

- Parabiosis experiments with $ob$ and $db$ mutant mice, which are both very obese, were interpreted as indicating that Ob is a circulating ligand that reports on fat storage, and Db is the receptor for that ligand. That is, $ob$ mice connected to normal mice lose weight, while $db$ mice do not.
- Cloning showed that the $ob$ gene encodes a soluble protein, leptin, which is made primarily by adipocytes.
- Leptin expression is regulated by fat stores. Under conditions where sufficient fat has been stored, leptin is expressed by adipocytes.
- When fat stores decline with underfeeding, leptin expression is switched off.
The leptin receptor

- *Db* mice behave like VMH-lesioned animals in parabiosis experiments; they cannot sense fat storage levels and continue to eat while the connected normal rat starves.
- *db* encodes a transmembrane receptor protein, OB-R, which binds leptin.
- It signals via interactions with a JAK/STAT pathway (JAK kinases interact with ligand-bound receptors, and these kinases directly phosphorylate STAT transcription factors which switch on downstream genes).
- There are two forms of OB-R, which have a long or a short cytoplasmic domain. The long form signals through JAKs.
- The short form could be a transport protein or a protein that removes leptin from circulation through binding.
Effects of leptin administration on mice

- Leptin is primarily a long-term signal for nutritional state of the animal, not a regulator of feeding in the short term.
- Leptin administration to *ob* mice causes rapid weight loss, and leptin given to normal mice also causes some weight loss.
- *Db* mice are leptin-resistant as expected.
- Other obesity models such as Agouti have high circulating leptin levels, and are leptin resistant due to downstream blockage of leptin signaling.
• Leptin enters the brain via the median eminence of the hypothalamus, which is an entry port through the blood-brain barrier.

• Leptin interacts with neurons in ARC and other hypothalamic nuclei that express the long form of OB-R.
Leptin activates and inhibits subsets of ARC neurons

- Hypothalamic neurons that are activated by leptin can be identified because they express the immediate early gene (IEG) Fos rapidly after leptin administration.
- In some OB-R expressing ARC neurons, however, leptin does not induce Fos but does induce another marker, SOCS-3. These neurons are inhibited by leptin.
- One can thus characterize the groups of neurons that are activated or inhibited by leptin by mapping the Fos+ and Fos- SOCS-3+ cell groups after leptin treatment and examining which neuropeptides these cells make.
Neuropeptides downstream of leptin inputs in the hypothalamus

• We will consider 4 neuropeptides in this lecture: NPY and AgRP, which promote feeding and are made by neurons inhibited by leptin (Fos- SOCS-3+), and POMC and CART, which reduce feeding and are made by neurons activated by leptin (Fos+).
Hypothalamic neuropeptides involved in the leptin response: NPY

- Neuropeptide Y (NPY) is an abundant neuropeptide expressed in ARC and in other hypothalamic nuclei.
- NPY is the most potent known stimulator of feeding when injected into cerebral ventricles.
- The *npy* mouse is not hypophagic or especially lean.
- However, the *ob-npy* mouse is thinner than the *ob* single mutant.
- This suggests that NPY is involved in leptin signaling, but the system is redundant.
Hypothalamic neuropeptides involved in the leptin response: AgRP

- Obese Agouti mice have a dominant mutation that causes Ag protein, normally only expressed in the skin, to be expressed by brain neurons.
- Ag is a natural antagonist of melanocortin receptors and thus blocks the action of αMSH.
- A closely related melanocortin antagonist that acts on the same receptors, AgRP, is normally co-expressed in NPY+ ARC neurons.
- AgRP overexpressors are also obese.
Hypothalamic neuropeptides involved in the leptin response: αMSH

- The POMC (proopiomelanocortin) precursor is processed to produce αMSH together with other peptides.
- In the pituitary POMC processing generates ACTH.
- In the hypothalamus, however, ACTH is cleaved to make αMSH.
- MC4R agonists such as αMSH inhibit feeding, and antagonists stimulate feeding.
- MC4R KO mice are obese.
Hypothalamic neuropeptides involved in the leptin response: CART

- The cocaine and amphetamine-regulated transcript (CART) encodes a neuropeptide.
- CART and POMC are coexpressed by ARC neurons that project to the PVH and LHA.
- CART decreases feeding on its own, and also blocks NPY-induced stimulation of feeding (black bars in graphs).
Hypothalamic circuits involving the 4 neuropeptides.
Figure 2. A Leptin-Regulated Melanocortin Circuit Influences Energy Homeostasis and Body Weight
The adipocyte hormone leptin crosses the blood brain barrier (BBB) and acts directly on two populations of neurons within the arcuate nucleus that express NPY and AgRP or POMC and CART. Leptin stimulates production of α-MSH, an agonist for the MC4 receptor (as well as CART), and inhibits production of AgRP, an antagonist for this receptor (as well as NPY). MC4 receptor-expressing neurons receive these leptin-regulated signals, as well as others, such as NPY. Such MC4R neurons are just now being chemically and functionally identified, and include TRH neurons in the paraventricular nucleus (PVH) that regulate the thyroid, MCH neurons in the lateral hypothalamus that regulate feeding, GABAAergic neurons in the PVH that modify other as yet unidentified neurons tied into energy balance, and others. Several outputs of the MC4R-expressing neurons include: endocrine outputs such as thyroid, growth and reproduction, through control of pituitary function; behavioral outputs, including feeding; autonomic output, regulating energy expenditure; insulin secretion; and glucose homeostasis. Sites in the pathway at which spontaneous loss of function mutations have caused obesity in rodents and humans are indicated in yellow, as are sites at which induced mutations have caused obesity in rodents (in blue). Not shown here are potential direct actions of leptin on peripheral tissues.
Leptin regulation of adipostat neurons via GABAergic integrator neurons

- Leptin activates (induces Fos in) ARC neurons that are anorexigenic (their activation tends to block eating), which express CART and αMSH and project to PVH and LHA.
- Leptin inhibits (induces SOCS-3 but not Fos in) ARC neurons that are phagic; these coexpress NPY and AgRP and also project to PVH and LHA.
- These 4 peptides could all have presynaptic effects on inhibitory GABAergic neurons in PVH and LHA that would synapse onto and regulate adipostat neurons. Adipostat neurons would be excitatory and project elsewhere in the brain.
Figure 5. Model for the Integration of α-MSH, NPY, and AGRP Signals at GABAergic Neurons Upstream of the Adipostat

(Top left) Arcuate POMC neurons, arcuate NPY/AGRP neurons, and NPY neurons from other sites such as the brainstem project to GABAergic interneurons in the mpPVH. These neurons provide inhibitory input to the adipostat neurons. Other inputs (urocin, CRH, galanin, GLP-1, neurtensin) and brainstem NPY neurons may also send projections to the PVH GABA interneurons or directly to the PVH adipostat neurons.

(Inset) Melanocortin receptors and NPY receptors in the GABA interneurons may regulate GABA release directly via their opposing action on adenyl cyclase. Additional signaling pathways for influencing GABA release include Ca^{2+} channels. Melanocortin and NPY receptors may
Regulation of adipostat neurons by GABAergic neurons that integrate neuropeptide inputs.

- The GABAergic neurons might integrate many anorexic and phagic signals in deciding whether to fire and inhibit adipostat neurons. Many other peptides could be involved downstream or in cross-regulation in the hypothalamus. There are at least 10 others implicated by the literature.

- However, if we consider only the 4 neuropeptides we discussed here (NPY, AgRP, αMSH, CART), there are two opposing states of the GABAergic PVH neuron, and therefore of the adipostat, that these produce.

- NPY + AgRP turn on the NPYR, and block the MC4R, leading to a decrease in AC activity and cAMP, closing Ca^{2+} channels, making it less likely that the inhibitory neuron will fire, thus increasing the activity of the adipostat and stimulating feeding.

- αMSH (+ CART?) turns on MC4R, elevating cAMP, opening channels, increasing inhibition, decreasing adipostat activity and decreasing feeding.
Model for adipostat regulation in wt and *ob*- mice.

- This model can explain why the *npy*- mouse is not lean but the *npy- ob*- mouse is thinner than the *ob*- mouse.
Regulation of hypothalamic neurons by insulin and ghrelin

• Insulin is released from the pancreas after eating and decreases blood sugar.
• It also acts together with leptin to inhibit the NPY/AgRP neurons, which are phagic.
• Another hormone, ghrelin, is made by the stomach when there’s a negative energy balance; that is, it is produced by the empty stomach but not by the full one.
• Circulating ghrelin also acts on neurons in the arcuate nucleus.
Effects of ghrelin and insulin on ARC neurons.

- Circulating ghrelin activates NPY/AgRP cells, stimulating feeding.
- It thus acts in opposition to leptin and insulin.
• Ghrelin stimulates firing of yellow NPY/AgRP cells, while leptin inhibits these cells but stimulates firing of green POMC/CART cells.
Figure 1. Components of the Energy Balance System

The energy balance system involves long-term afferent signals from fat (leptin) and pancreatic β cells (insulin) and short-term, meal-related afferent signals from the gut, including inhibitors of feeding (PYY, GLP-1, and CCK), and the stimulator of feeding (ghrelin). These inputs are integrated within the brain. Efferent outputs regulate appetite, energy expenditure, hormonal milieu, energy partitioning, and the status of reproduction and growth.
Hormone-induced synaptic plasticity in the hypothalamic circuit

- In addition to direct activation/inhibition of signaling within arcuate neurons, leptin and ghrelin can also change synaptic connections.
- *Ob*- mice have more excitatory synapses on NPY/AgRP neurons than do normal mice, and more inhibitory synapses on POMC neurons, consistent with the obese phenotype.
- Administration of leptin to *ob*- mice causes a rapid increase in inhibitory relative to excitatory synapses on NPY/AgRP neurons (phagic) and an increase in excitatory relative to inhibitory synapses on POMC neurons.
- These changes precede the effects of leptin in decreasing feeding.
- Ghrelin administration (to wild-type mice) produces an increase in inhibitory synapses on POMC (anorexigenic) neurons, consistent with ghrelin’s role in promoting feeding.
Leptin and ghrelin regulation of synaptic inputs in normal animals

- Perhaps fluctuations in leptin levels that occur in normal animals also change synaptic weights on a daily basis.
- In full animals, leptin is high relative to ghrelin, so the balance might be shifted toward activation of POMC neurons and inhibition of NPY neurons.
- Conversely, when leptin is low and ghrelin is high, the excitatory vs. inhibitory balance would be shifted in the opposite direction.
Fig. 2. Simplified representation of potential action of gut peptides on the hypothalamus. Access circulating agents into the arcuate nucleus of the hypothalamus is facilitated by a relaxed blood-brain barrier. Primary neurons in the arcuate nucleus contain multiple peptide neuromodulators. Appetite-inhibiting neurons (red) contain pro-opiomelanocortin (POMC) peptides such as α-melanocyte-stimulating hormone (αMSH), which acts on melanocortin receptors (MC3 and MC4) and cocaine- and amphetamine-stimulated transcript peptide (CART), whose receptor is unknown. Appetite-stimulating neurons in the arcuate nucleus (green) contain neuropeptide Y (NPY), which acts on Y receptors (Y1 and Y5), and agouti-related peptide (AgRP), which is an antagonist of MC3/4 receptor activity. Integration of peripheral signals within the brain involves interplay between the hypothalamus and hindbrain structures including the NTS, which receives vagal afferent inputs. Inputs from the cortex, amygdala, and brainstem nuclei are integrated as well, with resultant effects on meal size and frequency, gut handling of ingested food, and energy expenditure. →, direct stimulatory; ←, direct inhibitory; ↔, indirect pathways.
The leptin system and obesity in humans

• Rare mutants exist in inbred humans that are equivalents of ob (leptin-) and db (receptor-). These patients are morbidly obese from an early age.
• Treatment of ob humans with leptin causes rapid weight loss, as in mice.
• Pedigree and weight graph of *leptin-* humans
Other components of the leptin/POMC circuit are more frequently mutated in obese humans (but these mutations are still quite rare)
Leptin resistance

- Most obese humans have high leptin levels, and weight regulation is leptin-resistant, as in \textit{db} mice. However, these people do not have leptin receptor mutations, or mutations in other components of the leptin system.
- Leptin has little effect on weight in patients in clinical trials.
- This suggests that some pathway downstream of leptin is desensitized.
• Less food in wild;
• Switch primarily thrown this way
• Animal very sensitive to leptin

• More food in civilization;
• Switch already thrown this way; leptin is high
• More leptin has no effect
• However, other (downstream) factors prevent hypophagia and weight loss
Consequences of attenuation of leptin action in obese individuals

- (a) shows the idea that leptin signaling has less effect as the amount of leptin increases with increasing fat.
- (b) shows that attenuation of leptin action can allow obese individuals to still be stimulated to eat by ghrelin produced by an empty stomach, even when excess leptin should inhibit phagic NPY/AgRP neurons.
Hedonics

- An area that is highly relevant to human weight regulation, since weight is determined by both environment and genes, is the influence of hedonic inputs (nongenetic drives that affect food consumption).
- This is due to interactions between cortical and midbrain reward circuits linked to voluntary behavior and the (involuntary) circuits of the hypothalamus and other areas.
- Hedonic inputs (voluntary drive to consume food) may outweigh adiposity and satiety signals.
Regulation of energy output

- Body weight remains stable by matching energy output to energy intake.
- Hibernating rodents and human infants use brown adipose tissue (BAT) to generate heat.
- In BAT the levels of uncoupling protein (UCP-1) are high, and cause the proton gradient in mitochondria to dissipate as heat rather than generating ATP. This makes metabolism more inefficient, generating heat to keep the animal warm in the absence of activity.
• Ablation of BAT in mice with a transgenic toxin causes weight gain, showing that increasing the efficiency of metabolism by reducing uncoupling has an effect on weight.

• Adult humans don’t have BAT, but they do have 2 UCPs that are expressed in muscle and elsewhere.

• Polymorphisms in UCP2 are associated with excess weight in Pima Indians.

• Drug companies are searching for specific activators of UCPs that would decrease metabolic efficiency in skeletal muscle, thereby increasing energy expenditure without increasing physical activity. However any such activators would also cause heat generation in muscle, and this could have deleterious consequences.
Variation in human weight gain after overfeeding.

- Do differences in metabolic efficiency account for variations in human propensity to gain weight?
- There is wide variation in weight gain (1.4 kg-7.2 kg; 0.36-4.23 kg fat) after 1000 calorie/day overfeeding for 8 weeks.
- However, there was little variation in basal metabolic rate (BMR) among these people (BMR would include changes in uncoupling).
- Exercise was monitored and kept constant.
Watching your weight. (Top) When energy intake exceeds energy expenditure, weight is gained, with most of the extra energy stored as body fat and smaller amounts as lean tissue (protein and glycogen). When energy expenditure exceeds intake, weight is lost, with most of the loss of energy as body fat and smaller amounts of lean tissue. Physical activity can be divided into the energy used for conscious activities (mostly volitional) and for NEAT or nonexercise activity thermogenesis. The bottom panel summarizes the results in (2). In response to overfeeding, an average 39% of the excess calories were stored in the body as fat and 4% as lean tissue (protein and glycogen). The remaining excess calories were dissipated by thermogenic mechanisms [8% in RMR; 14% in TEF (proportional to the increased intake); and 33% in physical activity, both volitional and nonvolitional (defined by the authors as NEAT)]. Most of the increase in physical activity was accounted for by an increase in NEAT because volitional activity was kept constant. The variability in the gain of fat was inversely related to the subjects' ability to increase NEAT. RMR, resting metabolic rate; TEF, the thermic effect of food (energy required to absorb and store the ingested calories).
Energy expenditure by NEAT

- Since they could find no differences between those who gained weight and those who did not, the investigators decided that remainder of the energy difference must be used up in NEAT, or non-exercise activity thermogenesis. They hypothesized at that time that the major contribution to NEAT was fidgeting.
- Later studies suggested that this was incorrect, but have not provided a better explanation for NEAT.
- Perhaps NEAT is a myth, invented to explain their failure to find metabolic differences among the subjects.
A leptin signaling system in *Drosophila*?

- Mutations in the fly receptor, JAK, and ligand genes all cause excess fat deposition, as does turning down JAK/STAT signaling in specific fly brain neurons whose activity regulates fat content.

(Bader Al-Anzi and K.Z.)
Recent reviews on feeding


*Required reading*
Papers for student-led discussions on feeding behavior


