Hormone circuits Lecture notes

Uri Alon (Spring 2021)

Lecture 1

Hormone Theme song (Animals animals)

There's hormones in the thyroid for our metabolic rate
And gonadotropins that help women ovulate
There's hormones in our kidneys and hormones in our brains
Hormones help us sleep at night
And help us stand the pain

There's hormones in the pancreas to control metabolites
And leptin form the fat to control our appetites
There's feedback loops and circuits
That are beautiful to watch
And in this course we'll study them a lot
But not too much

Hormones, hormones, Hormones here and there
Hormones x4 everywhere

Frame Setting for the course

Hi I'm Uri Alon, a professor at molecular cell biology. I did a PhD in physics, and fell in love with biology. The first decade of research defined the basic circuits inside cells, using bacteria as simple model organisms. Ten years ago I fell in love again, with human medicine and physiology, and how physics-style thinking can help us make sense of our bodies in health and illness.

I'm excited to start this course with you, on hormone circuits.

We will see the amazing world of hormones and their impact on the basic questions of life, sex, food, agression, fear, love, reproduction, growth.

It's good to think about the goal of this course. **At the end, the goal is that you have learned how to think in a new way about biological circuits, and can use simple but powerful mathematical models to describe them.**

The models are powerful because they turn a bunch of facts and details into useful understanding, and hopefully new ways to think about treating diseases. And hormones play a role in some of the most deadly and common diseases: diabetes, thyroid problems, depression, bipolar disorder, autoimmune diseases. We will understand these diseases in the course.

I'd like to describe the logistics of the course. There will be 12 lectures of about 90 minutes. Every lecture or two will have an exercise that should take a few hours to solve.

There is no final exam, instead a final project that you can do in groups of 2-3. It is pass/fail.

The final grade will be determined by the exercises.

The teaching assistants are Avi Mayo and Alon Bar. You can reach them at the course email (urialonsb@gmail.com). Please write Avi or Alon with any questions.

The lectures are recorded and will be available to watch. I intend to later post the lectures on youtube.

Some Rules:
Lets begin with our first feedback loop (Fig. 1). We can be in a relaxed state of mind, which is good for listening,learning and memory. In the relaxed state our body behaves in specific ways. For example we take slow deep breaths. The wonderful thing is that as human beings we can decide to take a deep breath, and this increases the chances we will enter the relaxed state.
Because the relaxed state is so good for learning, we will practice taking nice deep sighs of relief in this course from time to time. Let's practice now: you don't have to, but if you do, I promise you will enjoy it- lets all together take a nice deep sigh of relief.

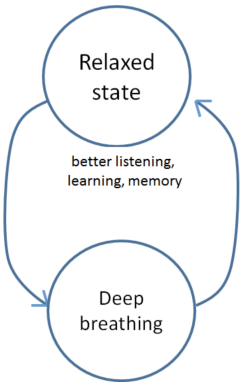

Fig. 1

About your roles:
You are from biology, physics, math, chemistry and other subjects.
This is a heterogeneous class. Some things are basic for some of you and hard for others. For some of you the following equation is easy, some need brushing up

$$dx/dt = -\alpha x$$

Whose solution is

$$x(t) = x(0)e^{-\alpha t}$$

This equation will play a role in this course describing degradation of molecules and cells.
For others, the difference between 'transcription' and' translation' is obvious, for others mysterious. Transcription is decoding DNA into RNA, translation is decoding RNA into proteins.

We will use our heterogeneity to our advantage! I will do breakout rooms where you can learn from each other.

Notice we have a photographer filming us, Uzi - hello :)

In this class I want to start light and demonstrate some basic tools and concepts. And also learn a fascinating bit of physiology. So let's take a nice deep sigh of relief- here we go!

Weight song (Streets of london)

Have you ever wondered how our weight stays nearly constant
Give or take 5 kilos
Over decades it's the same
Of course there are exceptions, there are times we fluctuate
But overall it seems that there's a setpoint for our weight

So if you want to know the answer
And you have a curious mind
Let me take you by the hand and walk you through the leptin circuit
I'll show you you something that may help you understand

Leptin and weight control
It seems we have a weight setpoint. I have been about 85 kilos from age 25 to now, age 52. Where is this weight setpoint written down in our body? We need to exactly balance our energy

intake and energy expenditure, which is remarkable given that a person with enough access to food eats about a million calories per year.
Weight control is an important basic question of biological control, and an example of more general principles we will expand later in the course. It is also a major health concern. Overweight is growing in the world, including children. Overweight can be defined as BMI>25, where BMI is weight in kilos divided by height in meters squared. Obesity for adults is BMI>30.

- In 2016, more than 1.9 billion adults aged 18 years and older were overweight. Of these over 650 million adults were obese.
- In 2016, 39% of adults aged 18 years and over (39% of men and 40% of women) were overweight.
- Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2016.
- The worldwide prevalence of obesity nearly tripled between 1975 and 2016.

What causes obesity and overweight?
The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been:

- an increased intake of energy-dense foods that are high in fat and sugars; and
- an increase in physical inactivity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing, and education.

What are common health consequences of overweight and obesity?
Raised BMI is a major risk factor for noncommunicable diseases such as:

- cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2012;
- diabetes;
- musculoskeletal disorders (especially osteoarthritis – a highly disabling degenerative disease of the joints);
- some cancers (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon).

No discussion of weight is complete without talking about dieting. When we diet we lose weight, but when we stop the diet, we overeat and return to the setpoint. We will understand why in this lecture.
Human beings have a weird effect that if you diet for 6 months or more, you start to regain weight despite the diet and go about halfway back to where you were before. This is not understood.

So we need to consider the weight set point, how it arises and how it breaks down.

Weight is controlled by feedback loops whose general idea is: the more you eat, the more processes are activated that reduce food seeking behavior (Fig. 2).

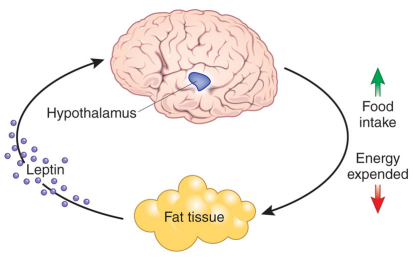

Fig. 2

One of the basic feedback loops was discovered when obese mice mutants were studied. These mice eat alot and have 250% more fat mass than normal mice. It turned out they were missing a hormone called **leptin**, or were missing the receptor for leptin.
Leptin is a hormone - a hormone is a molecule made by one organ and secreted to the blood to affect other organs. Leptin is predominantly produced by white adipose (the scientific name for fat) tissue and secreted into the blood circulation. Leptin regulates metabolism and appetite by inhibiting food intake, lowering body weight and increasing metabolic rate. Circulating leptin levels grow about quadratically with body fat percentage and body mass index (BMI) (Fig. 3). Leptin also responds to acute changes in energy balance: fasting decreases and feeding increases leptin levels. Obese people have high circulating leptin levels suggesting decreased sensitivity to leptin, which is referred to as leptin resistance.

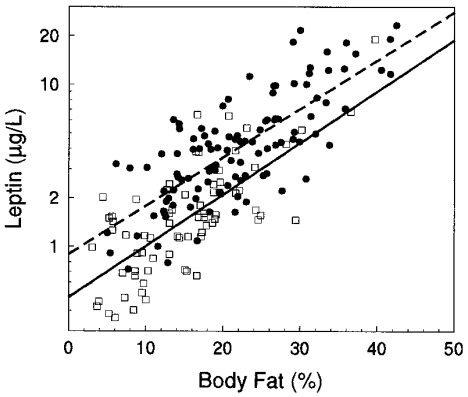

Fig. 3

So this feedback loop specifically controls the amount of fat (Fig. 4). Fat cells used to be considered as simple containers for fatty acids, a storage unit that can deploy those fatty acids for use as fuel for the body in times of need. The discovery of leptin added to fat the role of an endocrine organ- a smart organ capable of communicating with the brain.

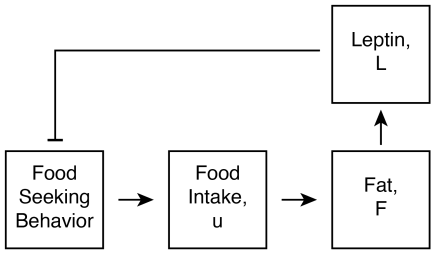

Fig. 4

The way that leptin acts in the brain is, like most questions about the brain, still shrouded in mystery. Leptin interacts with neurons in the hypothalamus, a brain region we will see is like the body's thermostat that integrates many signals to keep our physiology balanced.
In this lecture we will elegantly sidestep the brain by discussing this feedback loop in a graphic analysis based on two curves that can be readily measured experimentally.
We will also avoid the complications of human psychology and culture linked to weight, and talk in this lecture about rodents - mice and rats.

The leptin feedback loop was developed over evolution because it is important for fat amounts to be kept under control. Too low fat levels make the organism vulnerable to starvation. Too low fat levels also prevent reproduction. On the other hand, too high fat levels make the organism prone to predation. There is a sweet spot in the middle, and the feedback loops job is to keep that sweet spot.

To understand the feedback loop, we break it down into two arms. The first arm is the way that food affects fat percentage. Suppose we keep an animal on a certain amount of food intake, u grams/day. After a few weeks we measure its fat percentage. The more food, the more fat. So we can plot this curve, the diet curve, on an important plot called a **phase portrait** (Fig. 5)**.** The axes are food intake u versus fat percentage F.
This line, which we call the diet line, intersects the x axis not at zero: if we restrict food at a too low level, below the level

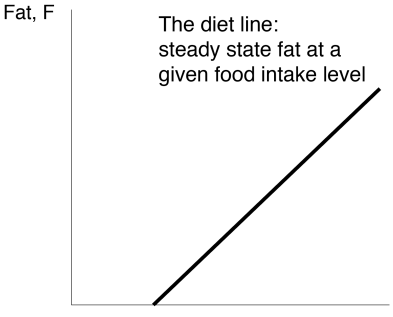

Fig. 5

needed to support basic metabolic costs, we go to zero fat: the animals starve to death.
The diet line thus describes the food-->fat arm of the feedback loop.
The second arm describes how fat inhibits food seeking behavior, namely fat--|food, where the blunt headed arrow --| is our symbol for inhibition. We'll call this the **appetite line** (Fig. 6)**.** Experimentally, we start with an animal that has a fat level F, perhaps reached after a specific diet. We then let it eat as much and as often as it wants over a 24h period, technically called eating *ad-libitum*. The more fat, the more leptin, and thus the smaller the appetite, and the less the animal eats. Plotting this on the phase plane we have a downward curve shown in red here[1].

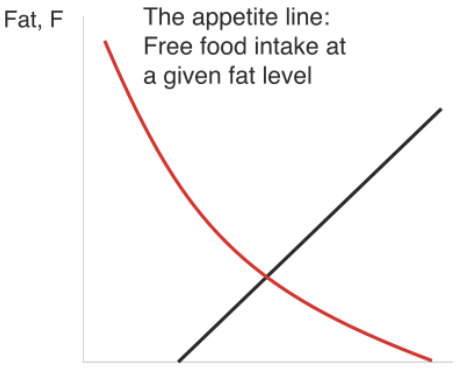

Fig. 6

Now the interesting point to watch is where the two lines intersect. This is the **weight setpoint**. At this point, the food intake and the appetite match exactly. It defines a steady-state fat level F_{st}, and a steady-state food intake level, u_{st}.
So our first concept here is that negative feedback can be broken into two arms. Each arm can be measured by keeping one variable constant and measuring the other at steady-state. In the language of dynamic systems these lines are called nullclines, and their intersection is the fixed point, where the system does not change, a set point.

The dynamics of dieting include an overshoot of eating
Now let's discuss dynamics and dieting. Suppose we diet for a while, eating less than our normal intake u_{st}. The amount we eat is u_{diet}. We move away from our set point. After a few weeks, we get to F_{diet} which is lower than our normal fat setpoint. We know what F_{diet} is from the diet line. That is how the diet line is defined in the first place. Great. What happens when we stop dieting?

To understand this we need the concept of **separation of timescales.** The two processes, weight gain and appetite, have very different timescales. Fat changes over weeks, much more slowly than appetite which happens over the course of a day. Leptin changes over a timescale of an hour. So after we stop dieting, and allow ourselves to freely eat, our intake in the next 24h will be determined by the appetite line. Because fat is low after the diet, appetite is higher than our setpoint. We eat more than we used to before the diet- we overshoot. After a while we gain fat. The next day we are a little fatter. Our appetite changes accordingly, crawling along the appetite line. We crawl along the appetite line until we return to the setpoint (Fig. 7). We can say the stepoint is defended by the feedback loop against changes. It is globally stable.

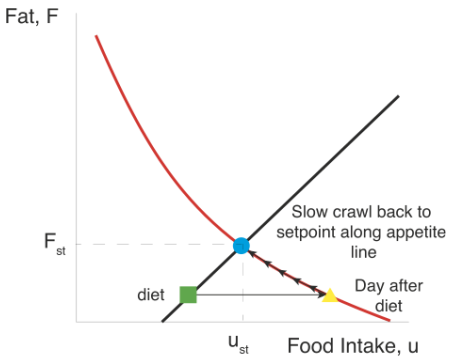

Fig. 7

[1] The appetite line might seem counter intuitive- shouldn't a fat animal have more appetite than a thin one? This may be true, but these two animals have different appetite lines as well see below. For a given animal, the more fat the lower the appetite.

To see this in a different way, we can plot our food intake as a function of time (Fig. 8).
After the diet there is an overshoot in eating, and then food intake crawls back to baseline. Fat drops slowly during the diet, then climbs back slowly to the setpoint after the diet is over. This is exactly what is seen in experiments.
The phase portrait can also show us what happens when instead of a diet, we overfeed the animal for some time, as in tube feeding experiments. After overfeeding is stopped, and the animal can eat freely, there is an undershoot in food intake. And this is precisely what is seen in experiments on rodents (Fig. 9).
We will use separation of timescales extensively in this course. It is nature's gift to theorists.

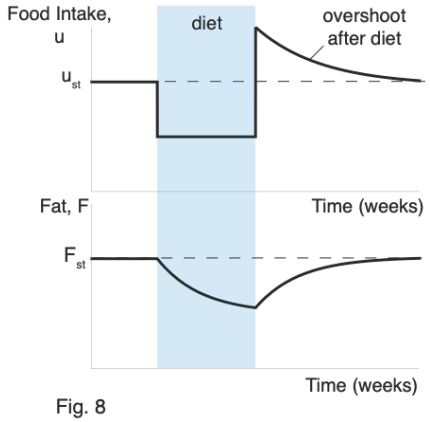

Fig. 8

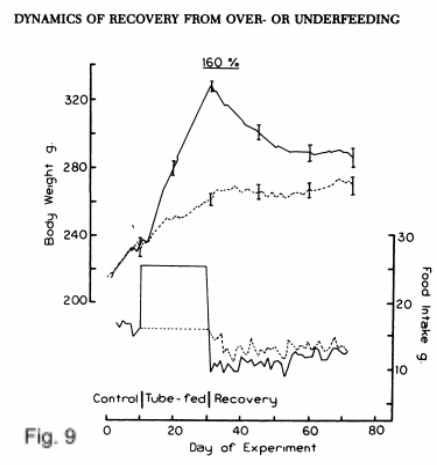

Fig. 9

Exercise shifts the setpoint

Let's use our phase portrait to analyze some interventions. We start with exercise (Fig. 10). Exercise increases the metabolic rate due to activity (eg running 10Km uses about 700 calories), and builds lean body mass, mainly muscle. The extra muscle burns more energy even when we rest. This affects the diet line: if we keep an animal on a certain daily food intake, and let it run on the wheel, it will have less fat than an animal without a wheel. Thus exercise shifts the diet line downwards. In fact it shifts it to the right as we will see later.

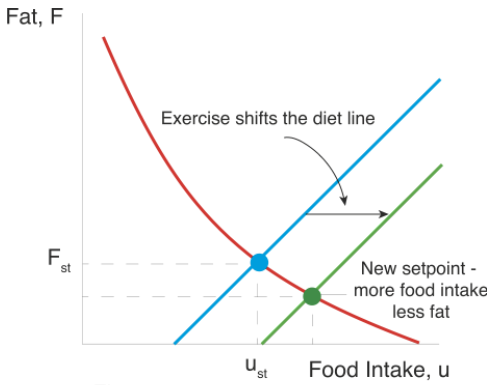

Fig. 10

The set point- the crossing point of the appetite and diet lines - also shifts. It shifts to less fat but more food intake. Eat more and lose fat. And that is precisely what happens when rats are given a running wheel: they lose 30% fat and eat 20% more!

In fact, any intervention that moves the diet line will have a paradoxical effect in which eating and fat move in opposite directions. For example in hyperthyroidism, when we have too much thyroid hormone (which will start in a future lecture), our metabolic rate is too high- heart beats fast, we feel hot. A common symptom is ‘I eat much more but I'm losing weight’. This is because the dietline shifts in a way similar to exercise. The opposite condition, hypothyroidism in which there is too little thyroid hormone (a very common disease causes this in about 2% of the human population), metabolism is slowed. There is often constipation and sensitivity to cold. The diet line shifts up (opposite of exercise). There is a paradoxical effect where we gain weight despite eating less.

Obesity is due to a shifted appetite line caused by leptin resistance

What happens when we shift the other line, the appetite line? This can happen when leptin works less effectively, a phenomenon called leptin resistance (Fig. 11). Leptin is a hormone, a molecule that flows in the blood. It is sensed by its target cells, mainly neurons in the brain, by nanometer sized sensors caller **receptors**. Receptors are proteins that stick out the cell across its membrane. They can bind a specific molecule like a lock and key, and activate processes inside the cell. Leptin is sensed by the leptin receptor on specific neurons. Each neuron has tens of thousands or leptin receptors. The effect of leptin depends on its concentration, the higher the concentration, the more receptors it binds on the cell surface. The more lepin-bound receptors, the more they affect the brain to reduce food seeking behavior. Plotting the output, food seeking behavior, as a function of leptin concentration gives us a decreasing curve. Its halfway point is at a leptin concentration denoted K_L, the binding coefficient of the leptin receptor.

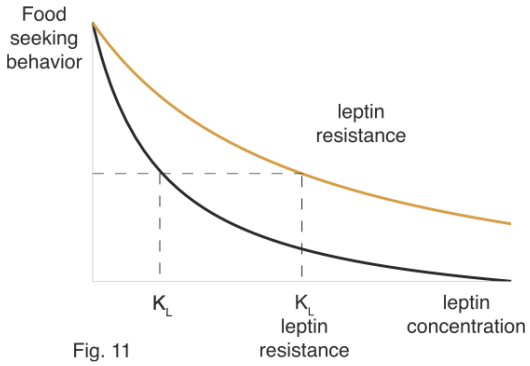

Fig. 11

For reasons not fully clear, people can develop leptin resistance. Their K_l rises, and it takes more leptin to have a given effect. Each unit of leptin, the buzz-kill for food, is less effective. Since leptin is secreted by fat, this means that at a given fat level, appetite is higher. The appetite line shifts to the right (Fig.12).

Notice the effect on the weight setpoint. Both food intake and fat increase. This is what is seen in leptin-resistant rodents. In fact, when the leptin receptor is mutated and made dysfunctional, which is the ultimate resistance, the animal is 250% of its normal weight.

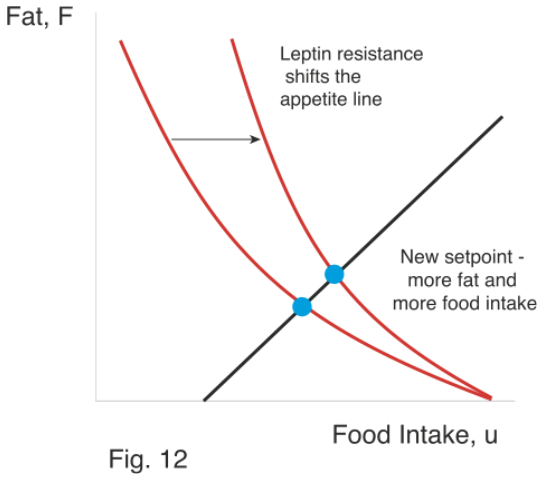

Fig. 12

In general, any perturbation that affects the appetite line will have a coherent effect on eating and fat: both rise or both fall. This is in contrast to shifting the diet line which has opposite effects on fat and eating. The difference is due to the upward and downward slope of the lines. Thus, seeing the effects on eating and fat can help diagnose an intervention to see which nullcline it affects.

Leptin resistance was a source of disappointment for researchers and clinicians who originally hoped that leptin would be a good treatment for obesity. When leptin is injected to obese mouse mutants who cant make leptin, the mice lose weight dramatically. Similarly, in very rare human patients with a mutation in the leptin gene (congenital leptin deficiency, CLD fund in about 100 people on earth) leptin injection is a life-saving treatment: individuals with CLD eat huge amounts of food and have morbid obesity and immune problems, which leptin injections resolve. However, for the vast majority of obese people, leptin has almost no effect due to leptin resistance.

Weight song part 2

So how can I tell you that I lost weight
And I finally fit those jeans?
Yes I know I'll keep those brown sacks
Pretty soon I'll gain those pounds back
It's a cycle that never really ends.

Mathematical analysis of the diet line

So far we used a graphic approach, with the phase portrait and the nullclines. In this course we will back up our graphical approaches with equations. The reason is that equations can help you ask new questions, and make more precise predictions. The equations we will use are the simplest ones that capture the essence of the system; many details with more minor effects are ignored for the sake of understandability.

So let's write an equation for the control of fat mass by food intake- an equation for the diet line. Fat mass F is increased by food when fatty acids are stored in fat cells. The cells get bigger. Fat mass is reduced when fatty acids are secreted from the fat cells, in order to supply the body with fuel. Thus fat mass is a balance of storage and of use for the body's energy needs. The rate of change of fat, dF/dt obeys

dF/dt=(fat gain from food) - (fat removal for the body's energy needs).

The rate of fat gain from a food intake of u grams/day is $\alpha_F u$. The parameter α_F is the rate of fat production from a gram of food, and depends on the type of food - α_F is higher for food rich in fat, for example, than for low-fat food. The rate of fat removal has two parts: there is the energy cost of the body, for the functions of the liver, brain, kidneys and other organs, denoted γ_E. Incidentally, the organs use energy in the following order: liver (30%), brain (20%), muscle (20%), kidneys (10%), heart (10%) others (20%). This energy cost includes both **basal metabolic rate** (BMR) when the body is at rest, about 2000 Kcal/day, and the energy needed to move and exercise. The exercise cost is smaller than the BMR, for example a 10Km run costs about 700 Kcal. BMR is high in children and drops with age, becoming roughly constant from age 20-50, dropping again at ages above 50 (Fig. 13). The second part of the energy cost is the metabolic cost of fat itself, which is proportional to the amount of fat $\gamma_F F$. Putting this all together we obtain:

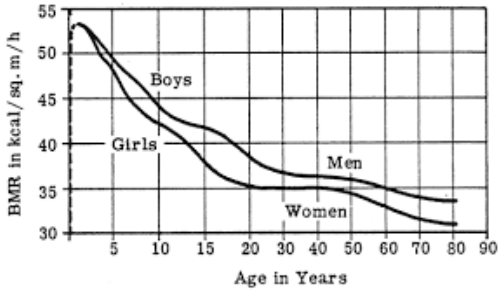

Fig. 13

$$dF/dt = \alpha_F u - \gamma_F F - \gamma_E \qquad (1)$$

To calculate the diet line, recall that we fix food intake (by imposing a diet or by tube-feeding experiments) and we wait until steady-state, which means until fat stops changing. Steady-state thus means zero rate of change, namely that dF/dt=0. Solving (1) at steady state, $dF/dt = 0 = \alpha_F u - \gamma_F F - \gamma_E$, provides an equation for fat as a function of intake u, the diet line:

$$F = \alpha_F/\gamma_F \; u - \gamma_E/\gamma_F \qquad \textbf{\textit{The diet line}}$$

This is a straight line that has slope α_F/γ_F. It intersects the x axis at a point where food intake balances the energy cost γ_E, namely $u = \gamma_E/\alpha_F$. Thus, if we exercise, we increase the energy cost γ_E, the intersect point gets larger (moving the line to the right) but the slope doesn't change. The shifts just as shown in Fig. 14.

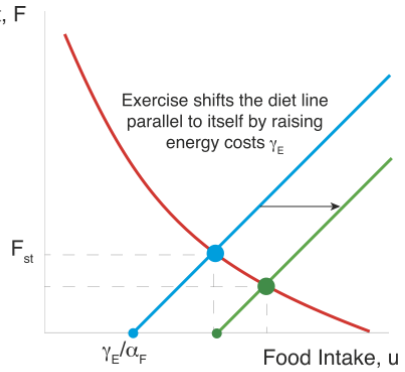

Fig. 14

Interestingly, switching to high-fat food (increasing α_F) increases the slope of the diet line and pushes the intersect point to the left, making the diet line steeper. The weight setpoint rises to higher fat. Makes sense- fatter food, you get fatter. But to understand the set point completely means we need to think also about the appetite line. How does it shift? Does high-fat food affect it too? What about exercise? To understand this, we need to have an equation also for the appetite line. For these and other weighty questions, tune in next week.

Weight song part 3

So what can we do if we can't diet,
And we don't have a weight loss pill?
Exercise and eat good food,
and maybe walk the buddhist path:
accept your setpoint,
and learn to love yourself.

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Leptin resistance
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BMR is not directly regulated by leptin
https://academic.oup.com/ajcn/article/82/5/941/4607670

Here leptin pump, and food intake seems to be a temporal derivative of leptin:
https://www.pnas.org/content/94/16/8878