

The Science of Fat (2004)
Lecture Four—Exploring Obesity: From the Depths of the Brain to the Far Pacific
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1. Start of Lecture Four (00:16)

[ANNOUNCER:] *From the Howard Hughes Medical Institute... the 2004 Holiday Lectures on Science. This year's lectures, "The Science of Fat," will be given by Dr. Ronald Evans, Howard Hughes Medical Institute investigator at the Salk Institute for Biological Studies, and Dr. Jeffrey Friedman, Howard Hughes Medical Institute investigator at the Rockefeller University. The fourth lecture is titled "Exploring Obesity... "From the Depths of the Brain to the Far Pacific." And now, to introduce our program, the grants program director of the Howard Hughes Medical Institute, Dr. Dennis Liu.*

2. Introduction by HHMI Program Director Dr. Dennis Liu (01:10)

[DR. LIU:] Welcome back to the final presentation in this year's Holiday Lectures on Science on the science of fat. In his previous lecture, Jeff Friedman told you about a powerful hormone called leptin that was first discovered in mice and then later found to regulate body weight in humans. In this talk, Jeff is going to take us into the depths of the brain to understand how this hormone affects specific neural circuits. And it turns out that understanding these neural circuits has in turn led to the discovery of more genes and molecules that affect this tricky situation of weight balance. Then, from the depths of the brain, Jeff is going to take us out to the far Pacific, where he's been studying a population of islanders that seem to be plagued with obesity. And he hopes to... by understanding the problem these people have, to understand better some of the evolutionary implications of obesity and also to discover new genes which in turn will hopefully give us insights to new therapies for obesity. After Jeff's lecture, HHMI president Tom Cech will return to close our talks, and as usual, we'll have a video to introduce Jeff now.

3. Introductory interview with Dr. Jeffrey Friedman (02:30)

[DR. FRIEDMAN:] Doing science nowadays is technical, and there is certain base knowledge you have to have, but I think what you need more than anything is a passion for it and a willingness to learn what you need to learn or to move the question you want to answer forward. I think, obviously, you need a certain level of insight or ability to assimilate facts, but I think it takes a lot of traits, actually. The first thing is, you know, to sort of not foreclose any possibilities. I think that academic success in the conventional sense doesn't necessarily correlate with success in science. I mean, I was a good student, but I wasn't an exceptional student or going through... going through my education. I think that I would leave open the possibility that one could discover things about oneself if exposed to the right environment and if the right opportunities are presented. You know, I think it's important, whether you go into science or not, to make sure you're open to life's possibilities. It takes certainly a passion for it and an ability to focus in on a question or a set of questions that are personally exciting and worthy of all the passion you could bring to it. I think it takes a willingness to do what it takes to address that question, you know, a certain openness intellectually to go in whatever direction the work takes you. I think it also, in modern times at least, to a large extent, takes a certain level of interpersonal skills as well. I think science, at least biology nowadays, is very often, at least at the level of a laboratory, a team effort. And then, of course, I think you have to be willing just to work hard and, you know, devote yourself to that, and to have a—I think really good science is easily described. The less verbiage it takes to describe what you did, the better the science in some ways. So, I think, you know, really good science is comprehensible if, you know, someone takes the time to explain it. You know, obviously there are technical aspects to it, and there's jargon, and there are different levels of understanding. But I think, at

its core, good science addresses sets of questions that everybody can relate to, both with respect to the question itself and the answer.

4. Outline of Lecture Four (04:54)

Well, welcome back, everybody. In yesterday's lecture, I tried to develop a conceptual framework, a biological framework with which we could begin to think about the problem of obesity, or more correctly, the biological system that controls weight, and today what I'd like to do is drill down in more detail to tell you about some of the surprising directions that those initial studies led us. In the first segment, I'll be telling you about the neural pathways that we're studying in an effort to understand better how leptin acts and why it doesn't act so well in the obese. And in the section lecture, I'll tell you about genetic studies that I and colleagues have been conducting for some time to try to identify new genes and put in an evolutionary framework the biological system I've been telling you about.

5. Obesity is usually caused by leptin resistance, not leptin deficiency (05:50)

I think the single image that I find most compelling in thinking about the biology of obesity is this one-- the comparison of this same child before and after treatment with the hormone leptin, and it's highly instructive, I think, to just keep in mind the fact that obesity can be caused by genetic alterations and can, as you just heard, be resisted by other genetic alterations. Now, in this particular case, the genetic alteration affected the ability of this child to produce this hormone, leptin, and the importance of that hormone to the regulation of weight, it becomes immediately evident when you look at what the child looks like before and after replacement of the encoded protein. But it's also the case that only a couple of dozen children or adults worldwide have been found to be... were leptin deficient. And so while leptin plays an important physiologic role, deficiency for it is not the principal cause of human obesity. Rather, in most cases, obesity is associated with some insensitivity to leptin that impairs its ability to activate those neural circuits that regulate food intake and burn energy, in turn, also sending signals to these peripheral tissues which include, as you might expect, modulation of PPARs, described so wonderfully by Ron a moment ago. So what we'd really like to begin to understand now is how does leptin normally activate signaling pathways in the brain, and what's different about its ability to do so in the obese state? And the supposition here is from this will come a higher level of understanding, not just of what causes obesity and how we might treat it, but also how we regulate a complex behavior, keeping in mind, of course, that we are not metaphysical beings. We are composed of molecules.

6. How does leptin act on a neuron? (07:38)

Well, in thinking about how leptin works, there are 2 questions-- one, how does it modulate the neurons on which it acts? And then 2, what is the nature of the neural circuit into which that neuron is wired? First question-- how does leptin act? Leptin is a class of hormones that was described for you earlier that act via what's known as a cell surface receptor. Here's leptin, the receptor. Leptin binds, changes the conformation of the receptor, and thus activates a series of downstream cellular elements, the names of which are in some of these boxes, and I'll just list them for you. There's a transcription factor that turns genes on and off known as STAT-3. There's a kinase. This is an enzyme that puts phosphates onto different proteins and regulate other proteins in so doing. And then SOCS-3 is another molecule that shuts the system off, so that the signal is not propagated indefinitely. And it turns out now in recent years, these and other signals have been shown to modulate leptin sensitivity. So, for example, mice that are missing this SOCS-3 become more leptin sensitive, and that property is also exhibited by other genes. And so one level of understanding will require that we know in great detail exactly how leptin changes the activity of the nerve cells in which it acts. And there's already reason to believe that doing so will lead to a better understanding and perhaps treatments for leptin resistance and obesity.

7. Animation: Neuroanatomy of the mouse hypothalamus (09:00)

The other question is, where does the leptin act? What are the nature of the neural circuits on which it acts? And the answer to that-- and leptin acts at a number of sites. Could you roll the tape, please? But the most prominent one is a region of the brain that I've referred to on a number of occasions known as the hypothalamus. So we're going to now go inside the brain of this animal and section it here, and this is the hypothalamus, at the base of the brain, just above what's known as the optic chiasm, which is where the optic nerves cross. This is the hypothalamus. Interestingly, the hypothalamus has hardly changed at all throughout all of vertebrate evolution.

8. Functions of the hypothalamus (09:40)

So, there's a tremendous similarity of this structure in all vertebrate animals, all of whom share the same basic drives. Let's start by asking, what are some of the basic drives that the hypothalamus controls? Anybody want to hazard a guess about that?

[STUDENT:] I would say appetite.

[DR. FRIEDMAN:] OK, so it definitely includes feeding centers, and they're localized in particular nuclei here-- the ventromedial nucleus and the arcuate nucleus. The nuclei in the hypothalamus are collections of nerve cell bodies.

[STUDENT:] Does it regulate thirst?

[DR. FRIEDMAN:] Thirst is regulated there. Any others?

[STUDENT:] Possibly breathing?

[DR. FRIEDMAN:] Actually, breathing is more brain stem, but that's a reasonable guess, and I probably led you to assume it would be based on everything I said. A couple more.

[STUDENT:] It's the body's thermostat.

[DR. FRIEDMAN:] That's right. Body temperature is controlled in other nuclei in the hypothalamus.

[STUDENT:] Regulates sleep.

[DR. FRIEDMAN:] Sleep is regulated through the hypothalamus. That's correct. And...

[STUDENT:] Doesn't it regulate the calcium... amounts of calcium in the bones?

[DR. FRIEDMAN:] It can indirectly regulate bone, and I'll just give you the last answer. It actually also plays a role in regulating aspects of reproduction and sexual behavior. So the hypothalamus is the center, to a very large extent, of all of our basic drives. Now, we study feeding, and so we'd like to know more about the circuits that regulate feeding in response to leptin and other molecules.

9. The leptin circuit in the hypothalamus (11:12)

So, in the hypothalamus, you could imagine that leptin might act on nerve cells and might activate the activity of some and inhibit the activity of others. And we're actually learning a lot about what the specific neuronal classes that respond to leptin are. In one case, there's a molecule called NPY. NPY is

expressed in neurons together with this receptor for leptin, and leptin inhibits the activity of these neurons. Now, normally the neurons signal electrical signals to downstream neurons that express other receptors. And ultimately, these neurons, when they fire, stimulate feeding. There's another group of neurons that express the leptin receptor and a molecule called MSH. This is the same molecule that causes pigmentation in your skin, but in the brain, it changes neural activity, again propagating an electrical signal and when these neuronal pathways fire, feeding is inhibited. So leptin acts, not surprisingly, by inhibiting pathways that stimulate feeding and activating pathways that inhibit feeding. Well, some exciting results have become evident in recent years, because it turns out that mutations in this pathway cause human obesity. And this includes not only the mutations in leptin that I told you about, but mutations in its receptor, in this MSH molecule, and the receptor for MSH, the MC4 receptor. And so single-gene mutations in any of these 4 components have been shown to cause human obesity, and in aggregate, account for 5% of morbid obesity. So 5% of the morbidly obese people in our population have a genetic alteration in a single gene, the majority of which in this pathway are actually in this MC4 receptor. That's pretty high. That's actually higher than the number of simple genetic contributors to other complex traits. Keep in mind that these are single genetic alterations. As we'll come to in the second segment, in the rest of the population, there's a combination of genes collaborating, as it were, with environment to lead to obesity. Now, I want to emphasize to you that more is known about these neural circuits, but one of the exciting prospects here is that by modulating the activity of these molecules and the neural circuits that they in turn modulate, we might be able to develop a new class of anti-obesity agents. And so a number of drug companies are engaged in trying and developing molecules that activate this receptor or inhibit these receptors. Other components of this system have been identified, including what are known as endogenous cannabinoids. And some of you may have heard that new drugs that target these endogenous cannabinoids which normally stimulate appetite, are also in development, and they're also part of this neural circuit the leptin signals through. So there's a great deal of excitement and promise, I think, in developing new classes of agents that modulate the activity of these neural circuits.

10. How to label two neuronal types differently (14:13)

Now, you might imagine that the activity of this circuit would be different in leanness versus obesity, and so that's at least a question we've set out to address-- what is different about the circuit in lean versus obese animals, at least at this stage of the endeavor. Now, in order to study this, we're going to have to look in greater detail at these NPY and alpha-MSH and other neurons, and it's here that we run into a problem. If you look at a section of the hypothalamus, as shown here, you can't tell one neuron from the next. And knowing that each of these neuronal classes have opposite effects, it's pretty important to know which neuron you're studying when you study it. And so if we were to simply look at the hypothalamus as per the previous slide and couldn't tell these neurons apart, we're going to run into difficulties interpreting our results. So we set out to solve this problem by labeling the neurons. Can anyone think of a strategy by which you might be able to mark or distinguish one neuron from the next?

[STUDENT:] Maybe you could use a dye that is ingested and then it can run through the blood and you can see it.

[DR. FRIEDMAN:] So, the use of dyes is actually the strategy you'd want to use, but then the challenge is, of course, to get the dye into precisely the neuron you're interested in, not the neuron you're not interested in.

[STUDENT:] If the molecular compositions of the 2 molecules are different, they could use radioactive tagging in one of them or in both with different tags.

[DR. FRIEDMAN:] Actually, that's a good idea. If you knew the different cell-surface receptors that distinguish the neurons, you could probably target them that way. One other...any other ideas? Well, I'll tell you what—

[STUDENT:] Maybe you could use a dye in those people who have a leptin deficiency, and you could use a dye to identify the nerve cell and the ones that are working and then use process of elimination to find the other different kinds.

[DR. FRIEDMAN:] That's basically right. I think the strategy I'm going to tell you about now makes use of a particular type of dye that in this case is not a dye the way you think of it, but rather a protein that spontaneously fluoresces, and these actually come from jellyfish which fluoresce green. And you can get these jellyfish proteins in different colors, with different spectral characteristics, and so the idea was to make animals in which that dye, that protein dye, was introduced in a distinguishable way into each of the 2 neuronal classes, and the way you do that is as follows. Genes are composed of 2 segments. Part of the gene codes for the protein, but part of the gene includes DNA sequences that turn that gene on and off in the right place and at the right time. These are so-called regulatory sequences. So the idea here is, take the regulatory sequence that specifies expression of this gene and its cells and this gene and its cells, put fluorescent proteins under the control of that regulatory sequence, and inject that into embryos of mice such that that DNA is now going to be carried through the germ line through development into the organism. So we did this, and here's what you get. Now this is that hypothalamus looked at under a microscope that detects fluorescence, and the MSH neurons are labeled in red, and the NPY neurons are labeled in green. Now we have something we can study.

11. How does leptin affect the activity of hypothalamic neurons (17:37)

And so what we wanted to do is use this tool, this ability now to study each of the neurons independently to ask... how does leptin actually work on these different neurons? And the first experiment was to ask, what's different about these neurons and their activity with and without leptin? Now let's think again-- what would happen if leptin were missing? Well, you'd no longer be inhibiting these neurons. They'd be activated, and feeding would increase. And you would no longer be activating these neurons, and that would decrease the inhibitory effect on feeding and in both cases, feeding would increase. So how is leptin going to change the activity of these neurons? Well, there are 2 general possibilities. One would be that it could directly affect the electrical firing of those neurons, and that was actually what we thought would happen, but there's another possibility that I'm going to tell you about. It turns out to be an important component, which is that leptin could change the wiring of those neurons, remembering that these neurons get inputs from other neurons, and to a very large extent, it's the composition of those inputs that regulate the activity of the cells.

12. Animation: Leptin rewiring neuronal connectivity (18:51)

So I'm going to show you a video to tell you what Shirley actually found when she did this experiment by counting synapses to tell us about what the inputs to these neurons are. So the NPY neurons normally get some excitatory and some inhibitory inputs. The POMC neurons the same. They get a little more excitatory inputs and fewer inhibitory. Now you look in the ob mouse, and what do you see? More excitatory inputs that would tend to activate the neurons that stimulate feeding, and in the POMC neurons that express MSH and that repress feeding, you have fewer excitatory inputs. So you compare ob to wild type, and what you begin to see is the wiring diagram is different in ways that would favor activation of NPY and disfavor activation of the neurons that express POMC, the precursor of MSH. So now the question is, what happens when you give leptin? Well, you rewire the brain, it turns out, very quickly, removing the excitatory inputs to the neurons that increase feeding and adding excitatory inputs to the neurons that suppress feeding. These, I should mention again, are the MSH neurons. So, what do

we have? We have an ob mouse, like those animals there. We give them leptin, and in a very short time frame, you rewire the synaptic inputs to those neurons. Now, this was a rather novel and surprising result because it wasn't thought that there would be so much what's known as plasticity underlying the function of this circuit.

13. Leptin rapidly rewires feeding circuits (20:22)

And this has important implications that I'll come to, the first being is that the effect that we observe is very rapid. This rewiring that's evident by counting synaptic connections, occurs within 6 hours at a time frame before there's any change in behavior. We believe this would indicate that it underlies the behavioral change that we're studying. And I also want to point out that the effects of leptin on these neurons are reciprocal. So it's changing the inputs in opposite directions, and this further justifies the need to label and study the neurons individually because if we'd not gone to the trouble of labeling them independently, the effect would have averaged out, and we wouldn't have seen anything. So a really exciting question now is, what's the mechanism of this? How does a molecule change synaptic configuration with that level of rapidity? And that's something we want to understand more about. Now, another issue that emerges from this is the following. I mean, think about a plumbing system and flow through it. You could change flow through the plumbing system either by inserting a valve to change the flow, or you could add new pipe and change the flow. And we have just made the simple assumption that leptin was going to act by changing the flow, by changing switches as it were. It's a less parsimonious explanation to suggest the laying down of new pipe, but it turns out that's a lot of how leptin works on these neurons. It acts by adding new pipe.

14. We need to understand the overall wiring diagram (21:48)

Well, what does that mean? Now we need to understand the broader wiring diagram. It's not going to be enough just to study how leptin acts on the cell. We're going to have to understand the wiring diagram that it's integrated into and in particular understand is the wiring diagram different in the lean and the obese? Are the different drives to eat driven to some extent by more or less excitatory versus inhibitory inputs to these key neurons that we're interested in studying? So, how are we going to get at this? Well, we need to understand what the wiring diagram is, which further means we need to know where do these neurons get their inputs from, and where do they send their outputs to? Now, this leads us into very complex terrain. It leads us into the area of complex motivational behaviors. Now, complex motivational behavior has a very specific term, and it's distinguishable from a different type of response or behavior, which is a reflex. Can anyone tell me what the definition of a reflex is?

[STUDENT:] Isn't it a... action that is not fully run through the integral... or the integratory... or the center of the brain that distinguishes one action from another?

[DR. FRIEDMAN:] Yeah, that's right. It's a biological response that's sort of unconscious and automatic, meaning a defined stimulus invariably gives a particular result, like when your doctor hits your knee with the hammer, your knee's going to pop up, whether or not you want it to or not. That's not the case for feeding.

15. Feeding is a complex motivational behavior (23:24)

There are a number of factors that influence the likelihood that you'll eat, but no single factor will guarantee that you'll eat. And so leptin's clearly an important signal, but so are visual factors. When you look at something, you make a decision about whether it's palatable or not. Smell has a very powerful effect on the likelihood that at any moment you'll eat. Same, of course, with emotion, as well as volition, such as the conscious desire to resist the food and not eat it, such as you all exercised by not eating your

Twinkies yesterday for the duration, with one exception. So let's think about this in a more concrete way. Imagine that you hadn't eaten for an hour or 2, and the only food that was available was rancid. It's highly unlikely that you would eat that food. But now let's take it a day out. You haven't eaten for a day, and the food is rancid. You might actually eat it, but I would postulate that by 3 days, you would be willing to eat food that you would never eat at one hour. So, somehow you're balancing, as you embark on that behavior, your hunger driven by leptin and other metabolic signals against smell. And you can build similar scenarios for fear and any of these and other factors that influence the likelihood that you'll eat. Now, this has led to a theory that there must be some integratory center in the brain that integrates all these signals and makes what turns out to be a binary decision. Either at any moment in time, we either eat or we don't eat. Not only do we not know how this information is processed, we don't even know where it's processed, and I would propose to you that someday some of you will solve this problem because this is one of the frontiers of scientific research-- how are complex decisions made? And the first question would be, where is it made?

16. Method for tracing connected neurons using the Cre recombinase (25:13)

Well, the idea that Jeff DeFalco and the lab had was that maybe if we could follow neuronal pathways downstream for all of these relevant inputs, the point at which the neural pathways converged would be a candidate as being this integratory center. Now, that's a hypothesis, but if we knew what the center was, we could test its candidacy as being such an integratory center. And so Jeff set out to do this. But that experiment requires that you be able to trace neural pathways across the neural network down multiple sets of connected neurons, and this was no easy task. Francis Crick is known for many things-- the discovery of the structure of DNA as well as the... eating cheeseburgers, as you saw yesterday, and he also has been interested in neural pathways and so laid out the importance of trying to find a means of staining all neurons connected to one another. In order to do this, our laboratory made use of herpes viruses. These are DNA viruses that are only propagated across connected neurons, but there's a problem with this, and that is, if we just injected this virus into the hypothalamus, all the neurons would be infected. So the challenge was to develop a virus strain that can be only activated in specific neurons, and this was done by using a particular enzyme known as CRE-recombinase as a key. What was constructed was a viral strain that is inert, that is inactive because of a sequence that's been inserted that can be removed by this CRE-recombinase. So the scheme goes something like this. We can insert CRE into the leptin receptor neurons in the same way as we inserted these fluorescent proteins. If we now use that virus to infect these animals, it's only activated in these neurons, so it will light up that neuron. The virus is called Bartha 2001, and all the connected neurons light up.

17. Where are the neuronal inputs to the leptin circuit? (27:11)

That experiment was done, and it worked. And so what we're mapping here are... where are the neural inputs to these hypothalamic neurons that sense leptin signals? So where are the chains of connect-- where is the information coming from? Well, you follow over time and ask, "Where does the virus appear?" And the first place it appears, after the experiment is done, is within the hypothalamus itself. There are neurons that are regulating one another there. You wait a little longer, where does the virus go? To the amygdala, the region of the brain that controls emotion. Wait a little longer, pyriform cortex, the region of the brain that controls smell. And finally it propagates to higher cortical centers that are the mouse equivalent of centers that control what might be volition. So, what does this mean? All these factors or all these regions appear to be talking to one another. So the leptin centers are receiving signals from other relevant centers. And another key question is gonna be do these other centers receive signals from the leptin neurons? And so what's beginning to emerge is that there's a distributed system here that processes this information, that's going to create a lot of challenges that I think are going to be soluble using the sorts of methods I told you about. Overall, we have a really wonderful opportunity, I think, here to study a complex behavior because we have a defined input in the form of leptin, a discrete

number of responding cells leading to a measurable behavioral output. And I think the challenge for us and many others thinking about this field is to find out what happens in between, the idea being that this will, in time, explain more about how complex behaviors are regulated and have an impact on new therapies. In addition, I think this is going to be a way to think about how brain centers that represent the conscious wish to lose weight interact with basic drives and leave perhaps some people to be better able to resist the hunger signals than others.

18. Q&A: How did you initially isolate NPY and MSH neurons? (29:11) So I'll stop here. I think this is a chance to take a few questions from any of you. I don't think we have much time for too many questions.

[STUDENT:] I'm still a bit confused about how you isolated these 2 neurons--POMC and NPY. And one of the stated reasons--or methods was finding people who were deficient in leptin or are obese and therefore they have less leptin, but these pathways are also the root cause of these issues. So it seems like you're using conclusion to figure out a cause, and I just don't know how you're able to separate the two from the beginning. You have a hypothesis about the existence of 2 neurons, 2 different types of neurons, but how are you able to distinguish them? And how are you able to separate something and use phosphorescence to mark them?

[DR. FRIEDMAN:] So the original observation was that if you give leptin to an animal, you change the level of expression of these genes, and then by other assays, you could show that neurons that express those genes were changing in other ways. But the key experiment was to have a hypothesis that these are neurons that are going to respond and then label them so we could test it directly, and we tested that in other ways that I didn't tell you about. So you're right. There was only an inference that these would be responding neurons at the start, but that allowed us to generate a hypothesis that you could test only when you label them in the way that we did. OK, I think I'll take one more question.

19. Q&A: Connections between skin pigmentation and eating? (30:32)

[STUDENT:] I was wondering, have you found any connection between skin pigmentation and inhibiting feeding?

[DR. FRIEDMAN:] There are connections between those in particular strains of animals that affect MSH expression in all places, and in those animals--they are hypopigmented and obese-- no equivalent mutations, however, have been found in humans like that... although the individuals who were deficient for MSH, who are obese, are also hypopigmented, but I don't think in the general population there's any connection of that sort. OK, I think there will be time for more questions at the end.

20. How does variation in genes lead to obesity? (31:10)

So now we've completed vignette one. Let's go to vignette 2 and now talk about this broader pathway between fat and the brain, back to the periphery. And I want to emphasize to you this connects up in a sense what you heard in the first lecture and leptin, because the brain centers control metabolism in these cells, undoubtedly in part through PPARs, and this in turn can change the level of signals back to the brain. And the question we want to ask now is, how does variation in these genes lead to differences in weight? And what I want to argue for you is that the genes that compose this pathway come in different variants, and that different variants lead to differences in the phenotype of people who carry them. And underlying this is a hypothesis, and that is that susceptibility or resistance to obesity can each confer an evolutionary advantage depending on the environment. What do I mean by this? Well,

21. Hunter-gatherer and Fertile Crescent genes (31:59)

to explain this, I need to go back in time and remind you that we are derived from hunter-gatherers. In pre-industrial or pre-agricultural times, we foraged for food. We hunted, grabbed berries and nuts. And a theory emerged that in this environment, where food was often in short supply, owing to famines and so on, that genes that predisposed to the development of obesity latently confer a survival advantage, because that would allow the individual to be more likely to survive the next famine. And the idea has been put forth that these individuals now when exposed to free access to calories become obese, such as is the case for the Pima Indians. This sort of theory could explain why there are obese people today. These genes are carried from antiquity to modernity. But this does not explain why anybody is thin. Why might that be? Well, we're not all hunter-gatherers. Proximally speaking, in terms of our history, many people are derived from the Fertile Crescent, where plants and animals were first domesticated in large numbers 10,000 years ago. Now, let's imagine that when plants and animals were domesticated, food can now be stored. People are buffered against starvation. And they're also going to become obese in the way that the hunter-gatherers might when given free access to calories. So they'd get obese. But now they'll develop the consequences of Syndrome X. This will have deleterious consequences, and genes that now resist obesity when calories are freely available will develop-- will become prevalent. And so the idea here is that perhaps this hypothesis-- the obese individuals today, all of whom were exposed to free access to calories, carry hunter-gatherer genes at the same regions of the chromosome that the lean people carry these Western genes.

22. Pacific island of Kosrae: A site to study genetic variation (33:43)

Well, this is a hypothesis. We're not going to be able to address this until we know what the genes are. And to try to get to what the genes are, we turn to populations similar to what you heard about today and yesterday. And in this case, not the Pima Indians, who exhibit this, but rather Pacific Islanders on the Pacific island of Kosrae. And this is a collaborative study among my group, the Kosrae Department of Health, other collaborators at Rockefeller as well as Hughes investigators and others elsewhere. So where is Kosrae? Kosrae is in the middle of the Pacific. It's east of the Philippines and Papua New Guinea, about halfway between Sydney down here and Hawaii up there. Geography is important-- it was the last island in the far Pacific that had a really good harbor. That'll become relevant here, because Kosrae, which was settled first by islanders 2,000 years ago, was contacted by Westerners in 1824, who liked the idea of parking their boats there in preparation for the long trip to Hawaii. So over the course of the 1800s, Kosrae became a popular whaling spot. Whalers brought with them communicable diseases that decimated the native population, which then fell from 3,000 to under 300. Or under 350. So this is what's known in genetics as a big bottleneck. The population then grows, perhaps with some Caucasians marrying in, the extent of which doesn't appear to be very dramatic, and the population is furthermore said to be relatively thin. Until 1945, when the U.S., in exchange for free access to their naval waters, begins to give them foreign aid in the form of a Western diet, largely Spam, hamburgers, and turkey tails. Anybody notice a tail on their turkey at thanksgiving? I doubt it. They all get shipped to Micronesia. These are all fat. And this in turn led to the development of Syndrome X on the island, on the part of many of the people and many of the conditions you've heard about. And it was at that point in 1994 that we began to collaborate with the population on a study that tried to identify the genes that lead to differences in weight. And so to do that, to identify these genes, we worked with the island population to screen the entire adult complement of individuals, 3,212 individuals, and catalogued them with respect to whether they developed some or all of these components of Syndrome X so we could analyze the data the way geneticists do.

23. Tracing inheritance of single nucleotide polymorphisms (SNPs) (36:10)

And the question we would like to answer in my laboratory is for the BMI distribution on the island, what genes account for the fact that in this relatively uniform environment, some people qualify as being

morbidly obese while a significant number remain lean. What are those genetic differences? How do we do this? Well, the standard way for a single gene is to track the disease through families. So the gene is on a chromosome, and the elemental principle of genetics is that if you can follow genes from generation to generation or traits, traits that appear together from generation to generation are often linked together on the chromosome. Now, in recent years, the markers or the traits that we study are not always visible traits. They're DNA differences in the form of what are known as single nucleotide polymorphisms. We're controlling--our DNA is composed of a 4-letter alphabet, but the alphabet is variable. In some of us, we might have a "C" at a particular position that influences the way a gene is decoded, and in others, a "G." And that actually has phenotypic consequences that account for the variations, to a large extent, among us. Now, the human genome has 3 billion such bases. About 1 in 1,000 bases are different on average, so actually, we're very much more alike than we are different at the DNA level. But overall, between any two individuals, there are about a million differences in aggregate. And so what the challenge to geneticists is is to ask which of those million differences account for the phenotypic differences, for example, the differences in weight that I told you about. And so what one would do in such cases is track these DNA differences empirically from generation to generation, and ask, "Does a particular DNA difference always get co-inherited with the disease you're interested in studying? And if it is co-inherited, you can infer that they're near one another on the chromosome and possibly causal. It's exactly this general conceptual approach that we use to clone the ob gene in those animals. Those animals were sitting around on the shelf at the Jackson Laboratory in Maine, known to be genetically obese, but the cause of their obesity was not known, and the technology we applied was a more primitive version of what's done today, which is to follow bases, these base spelling differences at work from generation to generation.

24. Using DNA chips to analyze many SNPs at once (38:26)

Fortunately, in modern times, one can catalog these so-called SNPs or spelling variations in great numbers using DNA chips, as they've come to be known. And through these technologies, it's actually possible to analyze hundreds of thousands of these SNPs in parallel and associate individual SNPs with individual diseases. The basic approach goes something like this: let's imagine that gene "A" came in two variants. And the different colors refer to different spelling differences or DNA sequence differences. So gene "A" could be yellow or blue, and let's compare now a group of obese or lean individuals. In this case what you'll note is that the blue and the yellow colors are equally distributed among the lean and the obese. Does anyone think that the way I drew it that gene "A" is likely to have anything to do with obesity? No. Compare that now to gene "B." it comes in two variations: white or orange. And now most of the obese people have the orange variant, and most of the lean people have the white variant. Anyone think that this may have something to do with obesity? Yes? That's--you know enough to start your genetic research now, I think. And this is what you do. And one of the advantages of an approach like this is that it's robust enough to detect genetic contributors in cases where multiple genes contribute. So it doesn't have to be a single gene disorder anymore. Any gene that has this characteristic can be inferred if the experiment is done properly with appropriate statistical analyses. One can identify genes that contribute to differences in weight. So we're setting out to do this on Kosrae asking the question, "Are there differences that distinguish this group from this group?" So we go to our pedigree. This is the pedigree on the island. Everyone on the island has been placed into this single large pedigree. And we can begin to ask, are there DNA differences that are associated with different diseases? And here's a list. And so in each case, we can narrow the search for diseases or genes that affect BMI, leptin level, cholesterol level or height. The subchromosomal regions where the spelling difference is co-inherited with the disorder. Now, this doesn't mean we have the genes yet. It means we have spelling differences near the genes. And to find the genes themselves, we need many more of these SNPs, and it's here that these DNA chips come into play. Because by analyzing the population in a highly parallel fashion, that can now score 100,000 of these SNPs simultaneously on the same DNA

sample, we can perform the experiment I just told you about and ask which of these SNPs, as they've come to be known as, are associated with which disease?

25. Studies of isolated populations can find rare single-gene disorders (41:11)

This pursuit is greatly advanced by the fact that on an island such as Kosrae, with very few founders, there's much less gene variation to begin with. So we need actually fewer of these SNPs to get an enormous amount of information. There's another advantage, however, of studying an isolated population of this sort. And that's that in a small population with a small number of founders, you sometimes see rare genetic disorders at high frequency. Why would this be? Well, there are fewer founders. The pedigree--look up here. The pedigree begins with a very small number of individuals. So if this individual were to carry a mutation in a single gene, what you might find is many generations downstream their descendants would find one another and intermarry and now that single-- that recessive gene would be expressed in the descendants. And we've seen that already in one rather surprising case for a rare disorder known as beta-sitosterolemia. This is a disorder described by Helen Hobbs, another Hughes investigator, that leads people to inappropriately absorb what are known as plant sterols. When you eat a plant, the plant sterols-- like cholesterol, but not exactly so-- are not absorbed. They're taken up by the intestinal cells, but they're immediately pumped back out into the intestine by a transporter protein known as the ABCG8 transporter. When this transporter is ablated by a mutation, the plant sterols get absorbed, they act like cholesterol, and these people get atherosclerosis. We were screening the Kosrae population for plant sterols for other reasons entirely, and this was the work of our colleague Jan Breslow. And lo and behold, some of the patients had this disease. And moreover, the patients who had it were brothers. Well, this sounds like some of the individuals may, in fact, have this disorder. So we'll quickly go and sequence the gene, and guess what. The two brothers have a mutation in this ABCG8 transporter. We go back to the pedigree, and what do we see? Unbeknownst even to them-- these are the 3-- the 2 affected siblings. Unbeknownst even to them, their maternal great-grandmothers were sisters.

26. Mapping rare genetic markers by analyzing homozygous individuals (43:28)

Well, what does this mean? It means that by using all these SNPs on the island and associating with their disease, we may get entry points into simple forms of these-- of diseases, single genetic disorders that can be studied in a parallel way to the approaches I just developed for multiple, or what are known as polygenic disorders. But this kind of result suggests a really nice strategy that I think is completely feasible when the genetic studies are done. How would this work? Well, let's imagine we use these DNA chips on everyone on the island and we could identify those individuals that are homozygous for a particular set of genes at a given chromosomal region. And that's what this represents. These are individuals who, for a given gene, are homozygous one type, homozygous the other type, and heterozygous. If we compare this group to this group for the different phenotypic traits that we have catalogued, and see a difference, then we might be able to infer that there's a single gene difference in this segment that contributes to the trait under study. Let me put it a different way. Imagine we had done this analysis and found that some individuals had one sequence there at ABCG8, and another individual had a different sequence there. We compare those individuals and find one of these groups had very high plant sterol levels. We might be able to infer that somewhere on this chromosomal region is a gene that controls plant sterols. And by developing this kind of strategy at greater length, the thinking is that we may be able to identify other genes.

27. Developing a genetic framework to understand how leptin works (45:05)

Now, the idea here is this. Maybe by taking an approach of this sort, working within a population, and using some of the features of it to advance genetic studies, we'll be able to identify other genetic variants

that account for differences in weight, perhaps not to the same degree as is evident in this child, but that contributes nonetheless. And the challenge, then, is gonna be to take those genes as we identify them and to put them into this biological pathway, to ask what are the genes that contribute to differences in weight? Where in the pathway do they act? Do they act in the fat cell? Do they act in the brain? Do they act in the target organs that Dr. Evans told you about? Fat, muscle, liver. Once we do this, we'll then have an opportunity to further ask how do environmental factors or even psychological factors modulate that circuit? And if there's anything I want to leave you with today it is the following. And that is my belief that the only way we're gonna really make progress in this field is by looking at it in this fashion: that is by developing a molecular and genetic framework as a starting point to understand how other factors, in turn, modulate it. And so that's the objective here. And I think that will allow us to advance beyond sort of simple recommendations that are-- that have not been proven to work with any consistency in humans, such as this. You might hear all the time someone say, "They should eat once a day, and walk as long as possible." Anybody want to guess who said this? Yeah, that's right. Hippocrates. 2,000 years ago. So, this wisdom, this recommendation you'll hear is 2,000 years old. And I think you might all want to ask yourselves why is that recommendation any more likely to work today than it was then? In my belief, it's unlikely to work any better, and that a higher level of understanding is gonna be necessary. Here's another set of recommendations from the classic textbook of the 1800s. "The principal remedies are un-nutritive food," basically low-caloric intake, "and exercise." 1800s. No different than what you might hear today. So I think that as an alternative to this, I'd like to propose that we think about approaching this problem the way we would any other biological problem. Define the molecular components of the pathways that regulate weight. Define the molecular alterations in those pathways that lead to obesity. This also provides an opportunity to understand how environmental factors: diet, lifestyle, hormones, other factors, influence those pathways. And from this will come rational therapies, less empiric therapies.

28. It's important not to blame and stigmatize the obese (47:47)

The other thing I'd actually ask you to keep in mind is this: while we might identify this as a health problem and seek to treat it, I think it's very important here not to blame the victim. I think biology is telling us that people don't have complete control over what they weigh, that there's a robust biological system that to a very large extent drives differences in weight. And this is illustrated-- the fact that this isn't always adhered to is illustrated all over the lay press. During the recent election, Jonathan Alter, a *Newsweek* correspondent, commented on a radio show that Bill Richardson was not a viable candidate because he was overweight and thought to lack discipline. A U.S. Representative, William Sensenbrenner, said to the obese, "Look in the mirror because you're the one to blame." In Stockholm, a Swedish court ordered a 5-year-old child removed from her parents because they couldn't stop her from eating. You know that a similar case took place in London, England? A child was about to be removed from her parents, and it was found that that child actually had a mutation in the leptin receptor. It's a fact that obese people make less money and are less likely to be promoted than their counterparts. And this has led an opera singer who was fired for being too heavy, Deborah Voight, to say that "This attitude towards the heavy people is the last bastion of open discrimination in our society." And so I want to close with an opinion and then take a poll that's perhaps a little bit loaded and simply say that there's a tendency in our culture to blame the victim. I think that there are many reasons to do science, but one of them is to learn more about who we are and what makes our differences evident. And I think that's the case clearly for obesity. So let's close with a poll. Do you think it's fair to stigmatize the obese? OK. Well, you're young, and I think that changing views is gonna have to start with people of your generation, because other views become deeply inculcated. But I think that it's always worth keeping in mind that image of the child from England whenever you see someone who is, perhaps, in your estimation overweight. And finally, I want to let you know that a talk like this is not just about me. There are a large number of people who did the research, collaboratively in my group, who I didn't have

time to mention. I'd like to thank them and all of you and would be happy to take a few additional questions.

29. Q&A: Disadvantages of studying an isolated population? (50:36)

OK.

[STUDENT:] What are the disadvantages of studying an isolated population? And keeping those in mind, do you think that these disadvantages affect the validity of your study?

[DR. FRIEDMAN:] That's actually a very subtle and good question. There are good things about an isolated population and bad things from a genetics point of view. From a genetic point of view, there's less complexity. And in a time where things are so complicated, and there are so many genes and so many possibilities, reducing complexity is a good thing. I mean, the ultimate of that, in a way, is the mice. The mice--those obese mice are very genetically uncomplex, and that gave us some opportunities. On the other hand, you don't get to sample the broad palate of human variation. But you have to remember, this is a worldwide effort, and the notion here is that by understanding the genes that are relevant on Kosrae, it gets put into a worldwide database of other variants, and then time will give us a rather broad picture, I think, of what the genes that cause obesity are.

30. Q&A: Does the research provide any benefit to the Kosraeans? (51:41)

[STUDENT:] Is there any special benefits for the Kosrae people of this research?

[DR. FRIEDMAN:] So the question was, is there any benefit to the Kosraeans. Well, I was careful to emphasize to you that this was a collaboration with the population. And we worked with them to try to make sure that they could reap some benefits from the study. The main one is this. On that population--in that population, treating diseases when they develop is often a lot more difficult than it is to prevent them. And so by working with them, their medical staff, and their nurses, they were able to assemble databases that allow them to decide who's at the most risk and try to intervene a little earlier, at a time where it's perhaps easier to treat the condition. The Kosrae population are also our partners in other ways, including any commercial opportunities that might come out of this research.

31. Q&A: How often do you need to inject leptin? (52:38)

Someone in the back?

[STUDENT:] How many injections of leptin does a person need before they start to see results? And how long after the treatment is stopped would you start to see weight gain again?

[DR. FRIEDMAN:] OK, so if you want to use leptin as a therapeutic, you actually have to inject it with standard leptin once a day. And it has to be an injection, because if you were to ingest it, it would get degraded in your stomach and wouldn't work. There are newer versions you can make using molecular biology tools these days that make hormones longer lived. And there are actually some formulations, I think, that can last a week or more. And, again, they're being explored. The other thing you have to remember is, if you stop the leptin, weight goes back to where it started. So the injections would have to continue. I want to emphasize now that this response, however, is not evident in everybody. Some people respond. Some don't. And that's what drives a lot of the research I've been telling you about.

32. Q&A: Can anorexia be caused by leptin oversensitivity? (53:40)

[STUDENT:] I understand that with morbid obesity there can be leptin resistance, but is the opposite possible, like maybe with people who are anorexic, they have leptin oversensitivity? Because how else-- how could a person starve themselves without the, um... biological drive to eat overcoming that?

[DR. FRIEDMAN:] So you're asking-- one side of the coin is obesity. You're asking about extreme leanness. So, you're right. The causes of extreme leanness could be that you're hypersensitive to leptin. It could also be that you overproduce leptin, and there are examples of that, actually, in some animal strains. But with respect specifically to anorexia, I want to remind you that feeding for all of us is some amalgam of higher cognitive or conscious wishes and basic drive. A lot of what drives anorexia are these higher cognitive inputs that somehow are able to shut off the drive to appetite. But the situation with respect to leptin is a little more interesting than that. It turns out that anorectics make more leptin in some cases than you would predict based on the amount of fat they have. In addition, when girls with anorexia nervosa are re-fed, their leptin level goes up more briskly than would be the case for the average person. So my analysis, or the analysis of the people who've done these studies, is that hypersecretion of leptin is a permissive factor for the development of anorexia. It doesn't cause it, but it may allow those individuals who have the psychological drive to eat less to actually pull it off. The individuals who might not have that feature just aren't able to pull it off. I think, unfortunately, we have time for only one more question.

33. Q&A: Is there a connection between depression and leptin? (55:29)

OK. Let's see, who hasn't asked one yet? You.

[STUDENT:] Do you believe there could be a connection between depression and leptin activity in the body?

[DR. FRIEDMAN:] So the question is connections between depression and leptin. Well, all these centers talk to each other. And there's no question that depression can actually affect food intake. In real depression, actually, food intake goes down. So that's yet another case. We have emotional factors integrating with the basic drive to eat, and for some period of time, depressed indiv-- that overrides the drive to eat. So I think that is yet another case where the challenge is gonna be to understand how these different inputs are cobbled together into a signal. But I want to emphasize again what I said yesterday. There's no correlation that I know of among the obese in general of a particular behavioral trait. I think in the same way as there's no correlations that I know of between behavioral traits and differences in height. So I think we're out of time. I want to thank you again for all your attention.

34. Closing remarks by HHMI President Dr. Thomas Cech (56:44)

[DR. CECH:] Jeff, that was a great way to close our series. I want to thank everyone who contributed to these wonderful Holiday Lectures. The audience, of course, the production staff, and especially our 2 speakers. Now, already planned for next year is a series of Holiday Lectures on the important topic of evolution. The lectures will be presented by 2 more of our HHMI investigators. Sean Carroll, from the University of Wisconsin, in Madison and David Kingsley from Stanford University. Until then, from all of us, the very best holiday season to all of you.