Lee E. Farr Lecture, Jeffrey S. Flier, Dean of the Faculty of Medicine, Harvard University

It is a distinct pleasure and honor to be asked to give the Farr Lecture, which has a tradition of excellent speakers and is especially meaningful for its focus on medical students' research. My own research career began when I was a medical student, and the state of medical student research at HMS, the school that I now lead, is something to which I have given much attention. Research is an aspect of medical education that has been prominent at Yale for more than a century. Harvard has always had many students who do research, but as of this year it is now a requirement for every entering student to complete a scholarly project, and this is an exciting initiative for the School.

This lecture will not focus in great detail on the most recent work in my laboratory. Rather, my goal is to use some research vignettes to illustrate my own approach to research over the years. I will begin with the start of my own research during medical school, how I continued this at the NIH and then through a long career on the HMS faculty at Beth Israel and then Beth Israel Deaconess Medical Center. I won't speak much about being the dean of HMS, but I will speak about family, and the bigger picture of "what it's all about."

I entered the Mount Sinai School of Medicine in 1968, in the first entering class of the school. I was one of 36 entering students. One of the reasons I decided to go to Mount Sinai was that I thought being part of this new school would be an adventure. I also was impressed with the people named as chairs of the major departments. As it turned out, working with two of them in particular influenced me greatly.

The chair of biochemistry, P.G. Katsoyannis, led a group that was the first to synthesize insulin through peptide synthesis and then show it to be biologically active. He and his colleagues made an enormous number of insulin analogues with varying properties that represented an important body of work. I got to know him and I became interested in insulin during my first few months at Mount Sinai.

The chairman of medicine was Solomon Berson. He became my personal hero. He and his partner, Rosalyn Yalow, developed the technique of radioimmunoassay, and though he unfortunately died prematurely during my senior year of medical school, Dr. Yalow went on to win the Nobel Prize in 1977 for their joint discovery, which had many practical implications, notably providing the first ability to quantitate levels of insulin, growth hormone and many other molecules. So I worked with the person who first synthesized insulin and the person who first measured it. I was clearly destined for a career studying insulin.

I had other mentors. Dr. Kurt Hirschhorn was an immunologist and the head of genetics and I spent most afternoons working in his lab, and authored my first paper with him. He thought that I might go into immunology, but I turned to endocrinology instead. The head of endocrinology was Dr. Dorothy Krieger and she was also a very important mentor to me. She came back to the field after having taken time away to raise children, and she became one of the leaders in understanding ACTH and pro-opiomelanocortin. She was also a mentor to my wife, who was about four years my junior in medical school.

As you can see I had some great mentors at Mount Sinai and they fostered in me a desire to become either an endocrinologist or an immunologist or some fusion of the two. Shortly before he died, Dr. Berson recommended that I work with Dr. Jesse Roth, the head of the diabetes branch at NIH who had been Dr. Berson's first student. At that time, the Roth group was leading the world in the identification and characterization of cell surface receptors. At that time, the structure of such receptors was unknown, either biochemically or genetically. Were they on the membrane? Were they inside the cell? What kind of molecules were they? How did they generate signals? Jesse Roth's group had terrific science and many smart people, and the atmosphere was quite electric.

I was very fortunate in my research at NIH, but during my first six months of research literally nothing worked. I had begun to think that I would leave for some further clinical training when I had one opportunity to pursue a good idea and everything just sort of fell into place. A few months later that idea evolved in a paper in the journal *Science* [1] in which we showed that some patients with extreme insulin resistance had autoantibodies against their insulin receptor. The paper also showed for the first time that the receptor we identified with this antibody was truly critical for insulin's action. We were able to show that the patient's own cells—removed and studied ex vivo, had reduced insulin binding, and we could reproduce this by exposing normal cells to serum and eventually to purified immunoglobulin. I also thought for one intense weekend that perhaps similar antibodies might be the cause of type 2 diabetes. So I located serum from patients with type 2 diabetes to test, but alas, they did not seem to have these pathogenic antibodies to explain the insulin resistance that was present.

But what was truly fascinating was another group of patients that had a very similar clinical syndrome but did not have these antibodies. Many of these patients subsequently proved to have mutations in the insulin receptor gene. That said, this was the paper that got me started, and once I had made this observation I said, "This is what I am going to do for the rest of my life." And then the question was, "Where would I do it?"

I had the chance to choose possible faculty positions at Beth Israel in Boston, Columbia and Yale, all of which were conducting very interesting research taking place in field of diabetes. The person who recruited me to Beth Israel Hospital, more than anyone else, was Franklin Epstein. Some people in this audience knew him exceptionally well. [2, 3] He was a wonderful person, a leading nephrologist, and a great physician, famous among other things for his Saturday rounds in which he would round with an intern and a student. No one who ever saw him in that context would ever forget how he conducted these rounds.

In my time on the HMS faculty I pursued a variety of research directions. I gradually added an academic administration and leadership role to my responsibilities.

The theme of this lecture is to say a few things about the concept of hormone resistance, because at this point I am trying to understand why is it that I kept on coming back to this subject in my work. The simplest answer is, "Because it exists!" It seems to be,

surprisingly, the key to the pathophysiology of many diseases, including diabetes and obesity, and by studying insulin resistance and other forms of resistance, we have identified key facts regarding hormone action, physiology and pathophysiology that might not have otherwise been discovered. I've studied three different molecules related to resistance. They are insulin, leptin and FGF21.

In the area of insulin, my research began with rare human syndromes of extreme insulin resistance, as I just discussed. These disorders were so-called "experiments of nature" and they were both immune and genetic. I also worked on common syndromes, including obesity and non-insulin-dependent diabetes mellitus.

When leptin was discovered we were interested in aspects of leptin biology in rare mouse syndromes and common syndromes. Most recently I've done some work with my wife, Terry (Eleftheria Maratos-Flier), on FGF21.

Insulin:

After that paper in *Science* [1], we published a paper in the *New England Journal of Medicine* describing the clinical syndromes of insulin resistance with the skin lesion, acanthosis nigricans, which is a cutaneous marker for this severe resistance. There were three patients who we referred to as "type B" patients who had receptor autoantibodies, and there were three other patients who did not have them. So we learned that there were two new syndromes [4].

The immune syndrome provided the best evidence that this receptor was truly the key receptor for insulin action and then it provided, for a period a years, a unique tool. Using it, we studied the receptor, explored aspects of the mechanism of action, undertook partial purification of the receptor, and used it to develop an immunoassay for the receptor. We also showed that monovalent antibodies were antagonists and that bivalent antibodies were agonists, and we did that early on in the field, while that common principle of receptor dimerization and crosslinking was still unknown.

When I came to Boston in 1978, among the things that I decided to do was to gather the patients who did not have these autoantibodies, suspecting they would be the basis for a new line of discovery. I began to collect these patients clinically and create cell lines from them. It was not until 1985 that the insulin receptor was cloned—considered a major breakthrough at the time—it made the cover of *Nature* [5]. With that information we looked at the patients we had and discovered insulin receptor mutations in several. We characterized them, and for about five to seven years that was a significant portion of my work.

At various points I thought that we would learn far more about the nature of insulin signaling by working with these different mutants, but it just did not work out that way. We learned a great deal, but we reached a point where "the well ran dry." There were few other molecules related to insulin signaling that we could study along with the receptor.

So where do we stand 23 years after the discovery of the genetic syndromes and 36 years after the autoantibodies?

We know that a syndrome of severe inherited insulin resistance with acanthosis nigricans (type A) exists. It turns out, in retrospect, that only about 15% have identified genetic etiology despite strong efforts to find the molecular explanation now with deep sequencing. Almost all of those identified mutations are of the insulin receptor. The insulin receptor substrates have not been established as a cause. There are a couple of extremely rare, single case families in which resistance appears to be due to dominant negative Akt2 [6], but we still don't have an adequate explanation for most patients with severe inherited insulin resistance, and I never would have predicted that to be true so many years after this research began.

And what about the case of insulin resistance in type 2 diabetes, an extremely important and prevalent disease? Insulin resistance is common, develops early in the course of the disorder and appears to be strongly influenced by genetics. Amazingly, the genetic etiology of insulin resistance in type 2 diabetes remains largely unknown. After millions spent on sequencing of candidate genes and on genome-wide association studies, the canonical signaling pathways seem to be exonerated and few, I believe, predicted that outcome. So what are the causative genes? We don't know. What about leptin?

Leptin:

I had been interested, even as a fellow at the NIH, in the ob/ob mouse model. I made a number of abortive attempts to do research related to them, but I decided that someday I would work on these mice because there had to be something profoundly important to explain this recessive obesity. Then in December of 1994, a paper by Jeff Friedman [7], who has given an earlier Farr Lecture and is a friend of mine, reported on the cloning of the ob gene, demonstrating that it encoded a protein mainly expressed in fat. This protein is essentially absent in the ob/ob mouse due to a nonsense mutation. This discovery changed everyone's thinking about the field of energy balance.

As an aside, seven or eight years before that paper I worked on a molecule called adipsin in collaboration with Bruce Spiegelman, and for a while we were convinced that adipsin could be the missing fat cell–secreted protein whose absence could cause obesity. We worked very hard to see if that could possibly be true. A few months after that we published two back-to-back papers in *Science*. The one that reported my portion of the work showed that there was severely impaired adipsin expression in genetic and acquired obesity [8]. We then spent considerable time working with a company that we co-founded to give recombinant adipsin to ob/ob mice to see if we could cure obesity. The bottom line was that we did not have any effect on obesity and to this day we do not know what exactly is going on with this adipocyte-secreted protein, which we now know as "complement factor D." It still remains an open question. The only lasting positive from that work is a tremendous collaboration and friendship with Bruce that lasts to this day. I also became psychologically prepared for the discovery of leptin when it was discovered by Jeff Friedman.

In 1995 leptin was known in the field as an anti-obesity hormone. The physiological feedback loop was thought to be that as you get fatter your fat cells enlarge and leptin production goes up—which it does—and then this somehow acts on the brain. There was evidence that it had a very powerful action on the brain, and the consequence of acting on the brain would be to reduce food intake and increase energy expenditure, causing weight loss in an elegant feedback loop. That's the way it was imagined to work.

In fact, that construct was consistent, not only with the initial mouse data, but with exciting human data. The first ob/ob human treated with leptin showed a dramatic effect of leptin therapy [9]. Within 24 hours of receiving leptin, the individual treated was eating a normal amount of food and remarkable weight loss followed. These studies were conducted by Sadaf Farooqi and Stephen O'Rahilly. Steve had been a post doc in my lab at Beth Israel working on the genetics of insulin resistance a few years earlier.

My lab devised a way to quantitate leptin and we measured it in fat and lean mice, and we showed that there was an extremely clear positive relationship between total body lipid and leptin levels. Levels were greater at any given body fat in female mice than in male mice, a result that holds true in every other subsequent study. We wrote a paper that claimed that leptin levels reflect body lipid content in mice, and that this provided evidence for diet-induced resistance to leptin action; this is the earliest of 3,000 articles on PubMed if one searches "leptin resistance" [10]. On the human side—in the *New England Journal* paper that followed—you could also see a positive relationship between leptin levels in blood and body fat, and once again it seemed that my research was gravitating toward the phenomenon of resistance.

This led to one of the great "aha!" moments of my career. In 1995, I was teaching Harvard medical students in their basic metabolism course. One of the things that I taught was the physiology of insulin, and its having two roles. When you eat, it is important that insulin levels go up to prevent hyperglycemia, and when you are not eating, it's extremely critical that insulin levels go down. If they don't go down, you will die of hypoglycemia. So the fall in insulin during food restriction is as important—if not more important—than the rise. That is when I got to thinking that perhaps the same concept might be true for leptin. Perhaps falling leptin was a starvation signal. The idea would be that as you starve, the leptin levels go down—which we showed to be the case—and that would be a signal to the brain to increase food intake and decrease energy expenditure. Falling leptin might also be an endocrine regulator because we know that when you starve reproduction is suppressed and various other endocrine axes are suppressed.

So we did an experiment to test that hypothesis. I had a wonderful endocrine fellow at the time, Rex Ahima, who had trained in neuroendocrinology before he came to our endocrine program. I had tried to sell this project to three different fellows before Rex. They had all been nice, smart people, but none of them were motivated to take on this experiment. When I mentioned this project to Rex Ahima, he was immediately excited and had excellent ideas about how to approach the critical experiment. So we took some normal mice and we fasted them, with or without replacing leptin. We found that fasting

alone caused a long delay in estrous cycling, but when we gave leptin during the fast that delay of estrus did not occur. And then we also did studies with thyroid and other endocrine axes and, though not as dramatic, there was a similar story with leptin limiting the effect. And the last line of our paper was, "given the high prevalence of apparent leptin resistance in obese rodents and humans the physiologic response to decreasing leptin concentration with starvation may be the dominant role of this hormone."

When we submitted this paper to *Nature* originally it was rejected, on the basis of comments from two reviewers. Ironically, the first reviewer said that "it could not possibly be true," and the other one said, "everyone already knows that it's true." In the end the paper was published, and it has been cited more than any I've written, about 2,500 at the moment [11]. I'm also happy to say that Rex was recently made a full professor of medicine at Penn and he is an outstanding investigator of whom I am very proud.

We were interested in discovering the mechanisms for leptin resistance. We gave leptin to mice who were either on a typical low-fat diet or on a high-fat diet. We took out the hypothalamus after leptin administration and looked to see if leptin was activating signals in the hypothalamus of animals with the high-fat diet, and it was not. That indicated that there was leptin resistance at the level of signaling caused by high-fat diets in these obese mice. But what was the molecular basis of this leptin resistance? There was an issue of *Nature* in which three papers came out describing the suppressors of the cytokine-signaling family of proteins [12]. Since leptin is a member of the broad cytokine family of proteins, we made probes for all the SOCS-family proteins that we knew of at the time and found that only one, SOCS3, was activated by leptin and was activated precisely in the medial basal hypothalamus where leptin has important actions. We ultimately showed that it is in those cells that have leptin receptors that SOCS3 mRNA is induced, and then we showed that if you co-express SOCS3 with a leptin-responsive system, you block leptin signaling. So now we had a molecule that was induced by leptin, and that would cause leptin resistance, and we spent five years working on that, trying to understand it. The key question was, would SOCS3 deficiency actually protect against obesity?

We were able to rapidly obtain some SOCS3 knockout mice. In the homozygous form, SOCS deficiency is embryonic lethal, and so we studied heterozygous knockout mice and we found what we were hoping to find, that is, at a very low dose of leptin the mice with only one SOCS3 allele had a better response to leptin administration than the wild type mice and these haplo-insufficient mice also had a greater loss of triglyceride mass in response to leptin. In further studies we asked, "Are SOCS3 haplo-insufficient mice protected against obesity?" And the answer was yes, they are. So, whereas a normal mouse on a high-fat, high-sugar diet will gain weight, the SOCS3 haplo-insufficient mouse gained less weight. They also did not increase food intake or show a rise in glucose as did the wild-type mouse.

And then, even more interestingly, it turned out that SOCS3 is also an antagonist of insulin signaling. So SOCS3 is actually a very good candidate to be one of the major mediators of both leptin and insulin resistance. The problem with leptin resistance being localized primarily to the brain is that we cannot readily sample the hypothalamus of

humans. So we cannot readily prove that this mechanism occurs in the medial basal hypothalamus of humans.

So leptin resistance characterizes typical obesity in humans. It most often is acquired and rarely is genetic. It's selective, so that even within the central nervous system, leptin's reproductive signal remains largely intact while the effect on body weight is diminished. This may be evolutionarily advantageous in that leptin's primary role may be in the transition from the fed to the starved state as leptin levels decrease, while leptin also confers limited protection against excessive energy storage as leptin levels rise. That's the hypothesis that I am fond of, but if true it would limit the therapeutic response to leptin, and as you probably know, there has been little success in leptin's therapeutic application despite the efforts of several companies.

To summarize, the genetic causes of leptin resistance are limited to rare leptin-receptor mutations that cause severe congenital obesity, and I believe that identifying the molecular mechanisms responsible for leptin resistance in typical obesity should be a high priority in obesity and diabetes research.

FGF21:

Though this may seem an odd segue, I sense there may be some working couples here who may benefit from this, so I would like to answer the question of what it is like to actually collaborate with your wife as a scientist for 34 years. Terry and I had always been advised that we should never work together, but we worked together on many different topics and have authored 29 papers together on a variety of topics, and we have actually found that our work has been synergistic, with each of us making significant contributions. As my administrative responsibilities have increased, my primary scientific contribution has decreased, and I will close this lecture with some remarks about the work related to FGF21 that has been predominantly hers.

FGF21 is a new and exciting metabolic hormone. It is a member of the FGF gene family; it is dominantly, but not exclusively, expressed in the liver. It acts through a subset of FGF receptors. It requires a co-receptor, beta klotho.

FGF21 gained scientific attention after researchers at Eli Lilly published a paper showing both in vitro and in vivo stimulation, by FGF21, of glucose uptake into fat cells by inducing glucose-transporter synthesis [13]. FGF21 is an interesting candidate for the treatment of non-insulin-dependent diabetes mellitus.

We entered the field as a result of Terry's studies that aimed to understand the molecular physiology of the very-low-carbohydrate, high-fat, so-called "ketogenic" diet that produces weight loss in a manner that she felt required molecular explanation. We all know about the Atkins diet, much debated and much discussed, and we know that it works, but no one had done an Atkins-like mouse model to try to understand energy balance and the molecular basis of the diet, so that's what Terry did with four groups of normal lab mice.

One group was fed regular chow, one the typical high-fat, high-sucrose diet, and one the ketogenic diet, which is a specially made diet that includes high-fat and low-carbohydrate foods. The fourth group was fed normal chow but was calorically restricted such that it would lose the same amount of weight as the group fed the ketogenic diet. The first three groups, ketogenic-diet group included, ate exactly the same number of calories; the calorie-restricted group ate 30% fewer calories [14].

She found that though the mice on the ketogenic diet ate as many calories as the mice eating a high-fat diet or regular chow, they dramatically lost weight while appearing to be otherwise healthy. They lost as much weight as mice eating normal chow but 30% fewer calories. This was a striking observation and indicated that the consumption of the ketogenic diet induces a unique metabolic state and weight loss with unchanged caloric intake, and this has now been repeated three or four times and many physiological correlates have been investigated and published. These ketogenic-diet mice have very low insulin levels, ketosis, increased energy expenditure, increased insulin sensitivity, and a mild fatty liver, but not a fatty liver of the variety that would be worrisome.

So then the question was: What is the molecular explanation? We did not feel that there was an adequate explanation based on changes in the levels of known hormones. So we performed transcriptional analysis by gene arrays on the mildly fatty liver, and we found that changes in the levels of one gene were striking. That gene was FGF21.

We found that FGF21 expression was markedly and selectively induced in the liver by ketogenic diets. After we found that FGF21 expression in the liver was physiologically regulated by simple starvation, we made an adenoviral anti-sense to inject into the circulation and knock down FGF21 in the liver, and when we put those mice on a ketogenic diet, we saw a massive increase of fat in the liver. FGF21 appeared to be necessary for the adaptive increased burning of fat.

Then we were fortunate to collaborate with Eli Lilly, which three years earlier had made FGF21 knockout mice. As we knew, if we put the wild-type mice on a ketogenic diet, they lost weight. If we put the FGF21 knockout mice on ketogenic diet, however, they actually gained weight! They also had increased food intake, increased body fat, and all the hallmarks of hepatic steatosis. In this way we had found that FGF21 is an essential molecular mediator of the weight loss associated with a ketogenic diet. This was quite exciting to us.

We have gone on more recently to human studies and found that people with non-alcoholic fatty liver disease (NAFLD) have higher levels of FGF21 in their blood. To recapitulate, we now know that FGF21 has a role in lipid metabolism and this knowledge arose from studies of the ketogenic diet in the manner that I have described. Suppression of FGF21 in the liver using adenovirus, or systemically through gene knockouts, has major phenotypes, as I've described. In recent work, we show that obese humans with NAFLD have paradoxically increased expression levels of FGF21. One would think, given the studies of FGF21 knockout mice above, that high FGF21 levels should be

protective against steatosis. These patients have high levels of FGF21, but they also have fatty liver disease. This new finding brings us back again to a theme that has been recurring throughout my career: Is there also resistance to FGF21? The answer seems to be that yes, indeed, these patients are FGF21 resistant [15].

The study of this novel hepatic hormone is very exciting. We know that its expression in liver is regulated by the composition and volume of the diet, and it has actions related to lipid oxidation and adipocyte glucose uptake. There are many other issues currently under investigation in our lab and elsewhere, and now it seems that FGF21 also has an action in the brain. It is also a regulator of brown adipose tissue thermogenesis. Terry and Bruce Spiegelman have just published a paper showing that FGF21 induces brown-fat activation in white-fat depots and promotes weight loss [16]. There also seem to be mechanisms for FGF21 action to limit hepatic lipid accumulation and toxicity, fibrosis and inflammation, and we are trying to understand how this occurs and to understand the mechanism for FGF21 resistance. The current state is: FGF21 action and resistance may be important factors in the pathophysiology of obesity, type 2 diabetes, and NAFLD. And it is a potential novel therapy for diabetes and NAFLD, and this is being explored by several pharmaceutical companies.

Hormone resistance, in summary:

Hormone resistance seems to be at the core of the pathophysiology of obesity and type 2 diabetes and insulin, leptin and FGF21 are certainly involved. Well-delineated genetic causes exist, at least for insulin and leptin resistance, but these are rare, and the genetic and molecular etiology in the vast majority of patients with common syndromes remains uncertain despite much effort. I find this very disappointing given the advances in fundamental understanding in the field, especially for insulin signaling, over the past 30 years. We have illuminated the black box such that we know an extraordinary amount about insulin signaling; we know dozens of molecules that are modulated up and down and are phosphorylated and dephosphorylated, but we still don't know the genetic cause for insulin resistance in type 2 diabetes. This does suggest, however, that major breakthroughs remain to be made, so I would counsel investigators to stay in this extremely important field. This disease is as important as it ever was, and many of the big questions have yet to be answered.

Allow me to end with a few reflections. The first: medicine is and will remain an extraordinary profession. Research is under assault, as its funding sources are being threatened, but I do believe that it is still a wonderful career choice. Second, I would say that for anyone wishing to do research over the course of a career, you should take advantage of the diversity—and the surprises—that will emerge over a lifetime and a career. It may seem at the beginning that it will be a simple path. One may think, "I'm going to do this, and then I'm going to keep doing it," but it almost never works out that way. I know people, friends of mine, who have been extremely successful in that way, they just go deeper and deeper on the same problem, and that is great if you can do it. But I also know many people who have moved from one important problem to another, and

even from one field to another, and they can also be very, very successful. More often than not there will be surprises rather than a straightforward path.

Third, whatever your area—whether it is biomedical bench science, or social science, or policy—remain alert for ideas, connections and opportunities that suggest a new direction. They are out there, but you should expect some negative response, because you will always get some negative responses to your best ideas, and our job is to not be discouraged inappropriately.

Fourth, it is really important to have mentors and role models, and I think your program here at Yale has been built upon that thesis for over 100 years. The only thing that I would counsel is this: do not seek to imitate your mentors. I know people who walk like, talk like, and have body tics like their mentors. Body tics are not usually a good thing to emulate. Take elements, but do not take the whole package. And then the final point—though it might sound a little sappy—in the end, over a lifetime, happiness is the key, and this may not always correlate with professional success. You may be in many situations where you will be needing to make choices that balance your personal happiness and that of your family with your profession, and there are people who make the wrong decisions every day when they are confronted by these choices, but striking the proper balance is what life is all about.

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