A tale of two hormones

Jeffrey M Friedman

"Der Mensch denkt, Gott lenkt." ("Man proposes, God disposes.")

—German proverb

A little more than a century ago, an arc of research began that culminated in the identification of insulin by four scientists working in Toronto. With astonishing speed, this landmark discovery became a life-saving treatment for thousands of people with diabetes around the world. In time, insulin was established as the most important anabolic hormone and found its place in the pantheon of medicine's greatest discoveries.

Fifteen years ago, leptin was identified as another metabolic hormone with major catabolic effects. This story, however, is not about leptin or insulin. Rather, it is about the path to discovery for each of them and about the path to discoveries in general. It is a story of discoveries made and not made. It is about how I found my way into the world of Israel Kleiner, who, but for the mysterious winds that shape our paths, might have been the man who discovered insulin almost a century ago.

I have had the privilege of listening to numerous first-hand accounts from many great scientists describing their moments of discovery. And over the course of my career, I, too, have experienced this thrill. But even before the discovery of leptin, I was fascinated by the experiences of people who have made important discoveries. My interest in great discoveries led me to *The Discovery of Insulin*, by Michael Bliss[1]. This enthralling work tells the tale of the 1922 discovery of insulin. In gripping fashion, it traces the progression of ideas that led to one of the key achievements of the twentieth century and arguably the first instance in which science provided a new lifesaving medicine for the morbidly ill.

Before the discovery of insulin, a diagnosis of diabetes, particularly in children, was often equivalent to a death sentence[1]. The only available treatment was a starvation diet advocated by Frederick Madison Allen, a physician working at the Rockefeller Institute for Medical Research (now Rockefeller University) and a leading authority on diabetes[1–5]. Allen was the first to realize that diabetes was a general disorder of metabolism and that acidosis and death could be forestalled if caloric intake was restricted. When acidosis developed, calories were further reduced, and, for many, diabetes was a race between starvation and acidosis, the ultimate result of either condition often being death. The discovery of insulin by Frederick Banting, Charles Best, James Bertram Collip and John James Macleod changed the treatment of diabetes forever by providing a hormonal treatment from the pancreas that could rapidly restore bedridden, cachectic and moribund children and young adults to healthy lives.

One of the many scientists who tested the ability of pancreatic extracts to treat diabetes before the discovery of insulin was Israel Kleiner (**Fig. 1**), a biochemist who also worked at the Rockefeller Institute between 1910 and 1919. In a study published in 1919, two years before Banting and Best published their first paper, Kleiner conclusively showed that extracts of pancreas but not other tissues could lower blood glucose in diabetic dogs[6]. Referring to Kleiner's work, Bliss wrote, "of all the publications before the work at Toronto, it was the most convincing"[7], and later opined that "his controls had been impressive; his follow-up discussion was a beautiful piece of scientific writing"[7].

Then, Kleiner's work abruptly ceased. While history has correctly bestowed the credit for discovering insulin on the Toronto group, Bliss wrote of Kleiner, "in 1919 he was closer to success than any of them, and made no claims at all"[8]. Kleiner never publicly discussed why his research stopped so precipitously, other than writing four decades later "why we did not continue and isolate the antidiabetic factor is a long story and has no place in the present discussion"[9].

Kleiner's story had great personal resonance for me. Here was another Jewish scientist, the grandson of immigrants, working a century earlier at the same institution as me. But on the cusp of isolating the most important hormone ever discovered, he walked away from it. How did Kleiner come to study diabetes? Why did his studies cease so abruptly? Did Kleiner and his colleagues fully understand the implications of his research? What was the personal impact of his having missed the opportunity of a lifetime? Kleiner's work and career also raise a general question: what are the elements of a discovery? These were the questions I set out to answer.

Israel Kleiner's magnificent paper

In 1889, Josef von Mering and Oskar Minkowski observed that removing a dog's pancreas resulted in polyuria, polydipsia and diabetes[10]. After Eugene Opie noted that the islets of Langerhans in the pancreas were often destroyed in humans with diabetes, the crucial question became how this structure controlled sugar metabolism[11]. One possibility was that the islets of Langerhans produced a hormone, referred to then as an 'internal secretion', that regulated glucose concentrations in the blood. Beginning with Georg Ludwig Zuelzer in the early 1900s[12], a number of scientists began to test the possibility that the pancreas might make a hormone that controls blood sugar levels, although definitive evidence of this was lacking. Thus, although Joseph Pratt and Macleod had concluded that individuals with diabetes were missing an internal secretion from the pancreas and that a pancreatic extract could be used to treat diabetes[13,14], this view was not universal. In 1913, Allen wrote, "though pancreas feeding may have at a least a digestive value in some cases of diabetes, injections of pancreatic preparations have proved both useless and harmful. The failure began with Minkowski and continued to the present without an exception"[15]. Forty-two years later, in a letter to Kleiner, Allen acknowledged that it was Kleiner's pioneering discovery that

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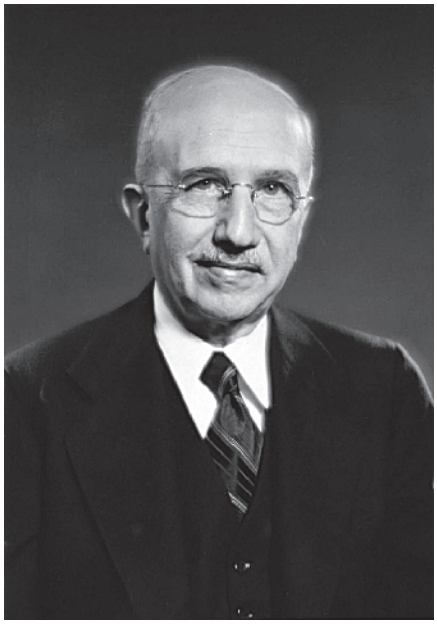

Figure 1 Photograph of Israel Kleiner as he neared retirement as a professor of biochemistry at the New York Medical College.

pancreatic extracts lowered blood glucose that had helped to change his opinion, as he wrote, "I can corroborate Joseph H. Pratt's statement that you announced a pancreatic extract with sugar-reducing properties something like eight years before the announcement by Banting and Best"[16].

The controversy about whether diabetes results from a deficiency of a hormone made by the pancreas existed largely because the available tools were limited. There were two key problems. First, it was possible to measure sugar in the urine, but not the plasma, of animals with diabetes, and the imprecision of measuring urinary glucose made it difficult for investigators to conclusively ascertain whether the pancreas extract was improving glucose metabolism[9]. Second, the extracts were quite crude and had side effects, including fever and inflammation, making them unsuitable for human use[17]. These difficulties impeded Zuelzer's efforts to treat patients with diabetes. Banting, Best, Macleod and, particularly, Collip solved the second problem. Israel Kleiner confirmed the first.

Israel Kleiner was born in New Haven, Connecticut in 1885. His grandfather emigrated from Frankfurt, his grandmother from Alsace. His father was a tailor and had hopes that his son would follow in his path. Israel had other plans (R. Glanz, A. Glanz and K. Glanz (Kleiner's daughter and grandsons, respectively), personal communication).

After completing his undergraduate studies at Yale University in New Haven, Kleiner entered the graduate school at Yale, then called the Sheffield Scientific School, and did research in the laboratory of Lafayette Mendel[18]. Mendel was one of the intellectual giants at the school and is now considered to be the father of nutrition research in the US. Mendel and his colleagues had developed an apparatus that allowed them to selectively deprive an animal of specific nutrients and used it to show that animals require essential amino acids that cannot be synthesized endogenously[19]. Among many other contributions, he also reported the consequences of vitamin A deficiency[19].

Kleiner's thesis was entitled *Studies in Intermediary Metabolism*, and his research focused on pyrimidines, with the ultimate goal of understanding more about the function of nucleic acids[18]. After teaching at Tulane University in New Orleans for a year, he applied to the laboratory of Samuel Meltzer at the Rockefeller Institute, expressing an interest in returning to research[20]. By 1910, the Rockefeller Institute had already established a reputation as one of the leading non-university research institutes in the world and one of the few places in the US that provided funding for research[21]. Simon Flexner, the director, had assembled a group of highly distinguished international scientists whose mission was to address problems of human health. In 1904, Samuel Meltzer was the first physiologist appointed at the Institute[22]. Meltzer was a highly distinguished member of the National Academy of Sciences, and his most notable achievement is the development of the respirator, or 'insufflation device', as he referred to it[23].

In Meltzer's laboratory, Kleiner initiated studies of the disposition of glucose after intravenous injection into normal and diabetic (pancreatectomized) dogs[24,25]. In 1914, Kleiner and Meltzer reported that 1.5 hours after the injection of a glucose bolus, the plasma glucose concentration in a diabetic dog was more than three times that found in a normal dog[24]. In another study, Kleiner and Meltzer also reported that extracts of pancreas could prevent the abnormal rise of plasma glucose after glucose injections into pancreatectomized dogs and that these extracts could also correct the hyperglycemia of diabetic dogs at baseline[26]. In retrospect, these studies were the first to show that insulin can correct the abnormal glucose tolerance of a diabetic animal.

Kleiner and Meltzer concluded that glycemia after a pancreatectomy was not due to an overproduction of sugar and raised the possibility that "the removal of the pancreas causes a decrease of consumption of glucose by some of the body tissues"[24], later writing, "in the presence of the pancreas the circulation rids itself easily of the intravenously introduced dextrose; but that it is unable to do it satisfactorily in the absence of the pancreas"[26]. This distinction is important because, starting with the brilliant work of Claude Bernard showing that the liver can synthesize glucose[27,28], the prevailing view was that diabetes primarily results from glucose overproduction. We now know that both overproduction and decreased consumption contribute to the disease, and Kleiner and Meltzer's results were the first to show that insulin regulates glucose use.

Although the complete data set was not published until 1919, Kleiner's and Meltzer's preliminary report was the first to show a beneficial effect of a pancreatic extract on blood glucose levels in diabetic dogs[6]. The only prior report measuring blood glucose after injection of pancreatic extracts showed an increase rather than a decrease in blood glucose levels[29]. The implications of Kleiner's and Meltzer's findings, published seven years before Banting's and Best's first paper, were reported in a prominent article in the *New York Times* on August 19, 1915 under the headline "Find Diabetes Cause, Now Seek a Remedy: Rockefeller Institute Doctors Say Disease is Due to a Failure of the Pancreas"[26,30].

However, efforts to seek a remedy were soon interrupted. The world was at war. The research enterprise at Rockefeller was disrupted and, regarding to this period, Kleiner later wrote, "in the meantime, the first World War had come about and interfered with much of this work"[9]. Kleiner did not take up his studies of the effects of pancreatic extracts until 1918, with Meltzer writing in his January 1919 report to Flexner[31], "Kleiner took up again our former studies of the neutralizing effect of intravenous injection of pancreas extracts upon the hyperglycemia and glycosuria produced by depancreatization."

By that time, research at the Institute had largely returned to normal[32], but not for Kleiner. Meltzer had diabetes and decided to retire, writing at one point to Flexner, "there is not much difference in the fate of an old fashioned and a modern diabetic. Confusion and humbug reigned then and reign still"[33]. With Meltzer's retirement, a decision was made to disband Meltzer's Laboratory of Pharmacology and Physiology, and his laboratory members needed to find new positions. The only position Kleiner could find was at the New York Homeopathic College, and he moved there in September 1919 (ref. 34). However, before moving, Kleiner completed his studies on diabetes and published a paper, "The Action of Intravenous Injections of Pancreas Emulsions in Experimental Diabetes," of which he was

the sole author. The paper was submitted in September 1919 and published two months later[6], approximately two and a half years before Banting and Best's first publication[35].

The paper is a masterpiece. In it, Kleiner presented a comprehensive data set comparing the effects of injections of pancreatic and submandibular gland extracts on plasma glucose levels in pancreatectomized dogs. The choice of submandibular gland extract is noteworthy; because the glands are also an exocrine organ with high levels of digestive enzymes, the extract is a perfect control for a pancreas extract. In addition, to minimize immune reactions, Kleiner prepared the extracts from the organs of the same dog that would later receive it.

The studies were all performed under precisely the same conditions and were well controlled, and the results were dramatic and unequivocal even for modern standards of analysis. Pancreatic, but not submandibular, extracts lowered blood glucose levels. Papers of this era were generally presented in a narrative form, describing each experiment that was done in sequence, and the data in this paper were in this format. Statistical analyses were not typically performed in this and other contemporaneous papers. Nevertheless, Kleiner summarized his data in a table in a clear and precise manner, making it straightforward to now graph these data and perform statistical analyses. (In the paper, Kleiner only reported the raw data for each dog.)

The reanalyzed data (**Fig. 2**) show that baseline glucose levels (mg dP–1) were nearly identical (pancreatic extract (PE): 0.28 ± 0.012, submandibular extract (SE): 0.31 ± 0.033; $P = 0.38$). By contrast, glucose levels at the end of the infusion (PE: 0.21 ± 0.014, SE: 0.31 ± 0.022) and 95 minutes after the infusion (PE: 0.18 ± 0.013, SE: 0.34 ± 0.029) were significantly different for pancreatic versus submandibular extracts ($P = 0.008$ and $P = 0.005$, respectively). Comparisons of blood glucose in diabetic dogs before, during and after infusion of a pancreatic extract were even more significant. These plasma glucose levels were baseline 0.28 ± 0.012 SE versus 0.21 ± 0.014 SE at the end of the infusion, $P < 0.005$, and baseline 0.28 ± 0.012 SE versus 0.18 ± 0.013 SE 95 minutes after the end of the infusion, $P = 0.0005$.

Kleiner was fully aware of the implications of these findings (as were others, in time)[36–38]. The introduction of the paper stated that the demonstration of a beneficial effect of a pancreas preparation when given to a diabetic dog would "support the internal secretion hypothesis of diabetes" and "suggest a possible therapeutic application." In the discussion he wrote,

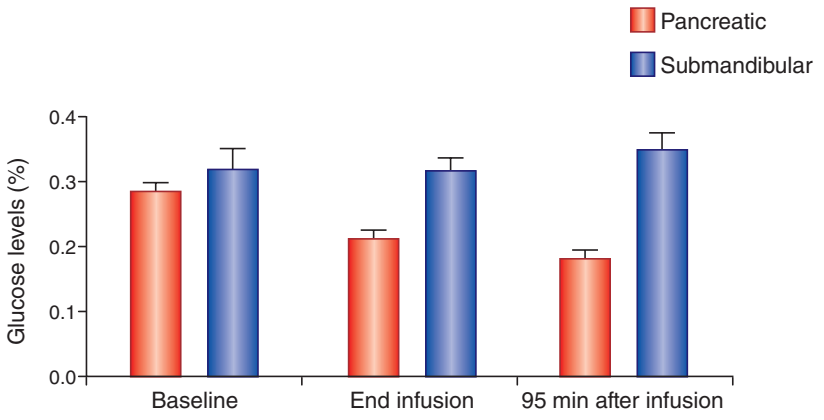

Figure 2 Kleiner's key experiment. The graph shows the effect of pancreatic versus submandibular extracts on glucose levels in pancreatectomized dogs. *$P = 0.008$, **$P = 0.005$. Data reanalyzed from ref. 6.

"the fact that these pancreas emulsions lower blood sugar in experimental diabetes without causing marked toxic effects indicates a possible therapeutic application to human beings." He wondered whether an "emulsion of the pancreas from another species" would have the same effect, foreshadowing the use of extracts prepared from animals to treat humans. He closed by stating that the "effective agent or agents, their purification, concentration and identification are suggested as promising fields for further work."

Indeed they were, but not by him, as there were no opportunities for Kleiner to do research at the New York Homeopathic College (D. Lehr (Professor of Pharmacology at New York Medical College from 1941 to 1980), personal communication). The institution did not have animal facilities at that time and, when Kleiner eventually resumed his research career, he had to conduct his studies on the effect of insulin on glucose transit from the bloodstream at the Cold Spring Harbor Laboratory (R. Glanz, A. Glanz and K. Glanz, personal communication)[39,40].

Insulin was discovered three years later. In the summer of 1921, Banting and Best, working in the laboratory of Macleod, began their studies on the effect of a pancreatic extract on diabetic dogs. In their first paper on this topic, published in February 1922, they reported on 71 injections of pancreatic extracts into six dogs[35]. (In the discussion of their paper, Banting and Best refer to 75 injections into 10 dogs, but the results section only shows the above data.) There were only two trials using extracts from other tissues, one from liver and one from spleen.

Of the trials with interpretable data, 37 of 63 injections were suggested to have an effect on blood glucose, although, in many cases, the effect was small. In these studies, 25 sets of conditions were used, differing with respect to how the extracts were made (acid or base extraction), whether whole pancreas or pancreas after pancreatic duct ligation was used and how long the time interval was between the preparation of the extract and its injection. Owing to the highly variable protocols and the fact that rigorous quantitative data are not presented, it is not possible to perform statistics on the data or even draw a firm conclusion on whether their pancreatic extract had an effect. In short, the results of this study, published seven years after Kleiner's and Meltzer's original report and nearly three years after Kleiner's definitive paper, are inconclusive.

Fortunately, this paper was only the beginning of their group's work. Macleod next suggested that Banting and Best begin working with Collip, a biochemist, to purify the factor. First, by alcohol precipitation and later isoelectric focusing, they prepared an extract free of toxins and, less than ten months from the publication of Banting's and Best's initial paper, the first injection of insulin was administered to a human subject with diabetes[41,42]. Within two years, insulin was the new standard of care for diabetes. In August of 1923, Banting and Macleod were awarded the Nobel Prize in Physiology or Medicine. (Controversy followed when Banting announced that he would share his award with Best and Macleod announced he would share his with Collip.)

In short, although the credit for the discovery of insulin and its development as a treatment for human disease belongs to Banting, Best, Collip and Macleod, they were not the first to prove the existence of a pancreatic hormone that regulates glucose—this achievement is Kleiner's. Even Banting and Best acknowledged Kleiner's primacy in their own paper, where they wrote that injections of pancreatic extracts lower blood glucose, "thus confirming Kleiner"[32]. Kleiner's research predates by

several years similar studies by Nicolae Paulesco, who would nonetheless make strident claims that it was he who discovered insulin[1,17,43,44].

Kleiner was the scientist who, building on decades of research by others, established that the pancreas contains an internal secretion that can be used to treat diabetes. On the occasion of Kleiner's seventieth birthday, Donald Van Slyke, Kleiner's former colleague at the Rockefeller Institute and one of the fathers of clinical chemistry wrote, "I recall the early days at the Rockefeller Institute when you demonstrated the effect of pancreas extracts in decreasing blood glucose concentration and urinary glucose excretion and pointed out clearly the probable therapeutic application of pancreatic extract in human diabetes. After your paper in the 1919 *Journal of Biological Chemistry*, one may say that the next steps, in separating insulin from the extract and applying it to treatment of diabetes, were inevitable. A call to a teaching position interrupted your own studies, and the next step was soon made in Toronto, but the honor of clearly showing the way remains yours"[45].

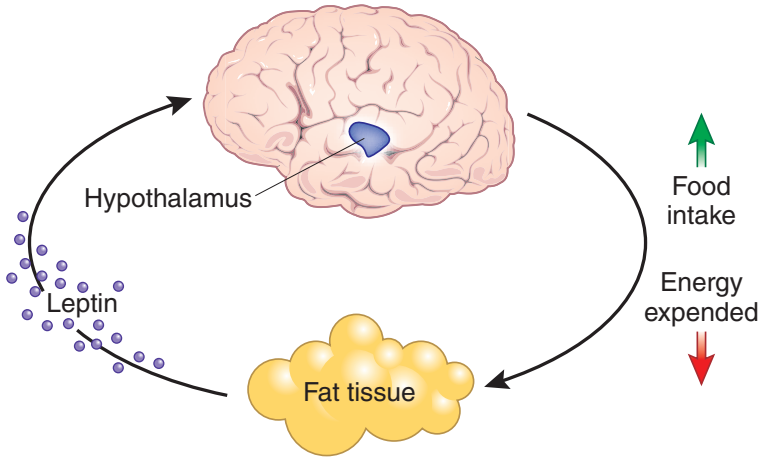

Figure 3 Leptin and the regulation of energy balance. Leptin is the afferent signal in a negative feedback loop that maintains homeostatic control of adipose mass. It circulates in the blood and acts on the brain to regulate food intake. When fat mass falls, plasma leptin concentrations fall too, stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing appetite until weight is lost. This system maintains homeostatic control of adipose tissue mass. Leptin acts on a receptor localized in the hypothalamus, and elsewhere in brain, to regulate energy balance and other systems[64,65]. Leptin thus conveys nutritional information to specific neural populations in the brain, which in turn regulate most, and perhaps all, other physiological systems. This homeostatic system enables mammalian organisms to maintain optimal levels of stored energy (fat) under a wide range of environmental conditions.

The path to discovery

For many discoveries, Pasteur's famous aphorism applies, that "in the fields of observation, chance favors only the prepared mind." However, many other discoveries are not the result of serendipitous observation and rather are 'in the air'. Discoveries of this sort are often made nearly simultaneously and referred to as 'multiples'[46,47]. With reference to this frequent concurrence of important discoveries, Robert Merton paraphrased Francis Bacon, writing, "once the right path is followed, discoveries in limitless number will arise from the growing stock of human knowledge"[47]. Such discoveries can often be characterized in the following way.

First, as a result of a set of observations, a question is posed. In the case of insulin, the key observation was Minkowski's finding in 1889 that diabetes occurs after the removal of the pancreas. In this instance, chance favored Minkowski's prepared mind. The question then became whether there was a hormone made by the pancreas that lowers blood glucose.

The second element is a technical advance (in the hands of a scientist with the technical expertise to implement it) that provides a means for addressing the question in a new way. In Kleiner's case, this was the development of methods that made it practical to measure glucose in small blood samples[9,48,49]. It was the limitation of measuring urine glucose that made it so difficult to establish the importance of a pancreatic factor before Kleiner's study. In the case of Banting, Best, Collip and Macleod, the technical advance was the development of biochemical methods, together with Collip's competence as a biochemist, that made possible their purification of insulin.

The third element is good fortune, or at least the absence of ill fortune, which provides a scientist in the right place at the right time with the opportunity to complete his or her work. Although it can never be known whether Kleiner would have succeeded in purifying insulin or even if he would have embarked on this course, he clearly lacked a suitable place to continue his studies. In 1919, there were few institutions other than the Rockefeller Institute where a scientist, particularly a Jewish scientist, had access to the facilities and funding necessary to do research. Although formal quotas for Jewish students—*numerus clausus*—did not begin until the 1920s, there were few positions available for Jews on a university faculty then, and for decades after (D. Lehr, personal communication; J. Darnell (Rockefeller University), personal communication)[50]. At the Sheffield School (the forerunner of Yale Medical School) the only Jew on the faculty was Lafayette Mendel. And, despite his great achievements, even Mendel was becoming less comfortable in his position during same period in which the Kleiner story unfolded[51].

Thus, with regard to this final requirement for making key scientific discoveries, one might consider inverting Pasteur's quote by suggesting that, for those making discoveries, "the prepared mind is favored by chance."

The discovery of leptin

The same elements played a part in the discovery of leptin in my laboratory. A set of relevant hypotheses and questions were in the air. Decades before our work, in the 1950s, Gordon Kennedy proposed that adipose tissue mass is regulated by an endocrine system[52]. Building on Albert Hetherington's and Stephen Ranson's studies from the 1930s showing that lesions in the hypothalamus can cause obesity in rats, G.R. Hervey used parabiosis to suggest that an endocrine factor that acts on the hypothalamus controls this putative homeostatic system[53,54].

The identification and characterization of obese (*ob/ob*) mice by George Snell and his colleagues provided further support for the hypothesis that body weight is under physiological control; they showed that an autosomal recessive mutation on mouse chromosome 6 resulted in massive obesity and hyperphagia[55,56]. In the 1970s, Doug Coleman at the Jackson Laboratory used parabiosis to characterize *ob/ob* mice and diabetic (*db/db*) mice—a second mutant model that Coleman identified that also developed severe obesity. On the basis of these studies, Coleman predicted that *ob/ob* mice lack a blood-borne factor that regulates body weight and that *db/db* mice lack its receptor[57].

Thus, studies from multiple laboratories over the course of several decades suggested that body weight is regulated by an endocrine

Figure 4 *Savin Rock*, by Israel Kleiner. Savin Rock was an amusement park close to Kleiner's childhood home.

loop, that the *ob* gene encodes the key hormone in this pathway and that this hormone acts on a receptor, encoded by the *db* locus, located in hypothalamic centers that are known to regulate weight.

This was the working hypothesis of my laboratory when we set out to clone *ob* and *db*. However, similarly to Allen's skepticism about the existence of an antidiabetic hormone in the pancreas, the view that an endocrine system might regulate body weight was not widely shared (D. Coleman (Jackson Laboratory), personal communication). Indeed, many researchers at the time questioned the existence of a physiological system that regulates body weight and the relevance of the *ob* and *db* genes for human physiology, arguing that many of the features of the mutant mice were different from those of obese humans.

Determining who was correct required that the endocrine factor proposed by Kennedy and Hervey be identified and that the *ob* and *db* genes be cloned. However, the biochemical methods available at that time were not suitable for this purpose. For those who were interested in identifiying the *ob* protein and preceded us, and for reasons that became fully evident only after the genes were cloned, the relevant factors are present in amounts too small to allow their identification by a conventional feeding bioassay[58]. In addition, we now know that the leptin does not act acutely to suppress feeding, therefore requiring longer periods of monitoring and possibly even multiple doses to observe a robust effect[58].

Fortunately for my group, a methodology was developed in the early 1980s that allowed investigators to identify mutant genes based on their position on a genetic map[59,60]. This task was intrinsically difficult owing to the limitations of available methodologies for working with large segments of DNA that were megabases in length. It was not until more than a decade later that this task became routine. While difficult, the task of positional cloning did not seem impossible, and it was this methodology that we employed when in 1986 we set out to clone the *ob* gene. Eight years later, my laboratory reported the identification of *ob* in mouse and human[61].

The *ob* gene encodes leptin, an adipocyte hormone that functions as an afferent signal in a negative feedback loop that regulates food intake and body weight[58,62–65]. This homeostatic system enables mammals to maintain optimal levels of stored energy (fat) under a wide range of environmental conditions (**Fig. 3**). The identification of leptin confirmed that body weight and feeding behavior are regulated by a robust physiological system and not, as many believed, controlled primarily by willpower. These findings also established adipose tissue as an endocrine organ. Numerous reviews of this subject are available to those who wish to delve deeper[66–68].

Discovering a previously unknown hormone and establishing its physiological function was the greatest professional pleasure that I have known. At 5 a.m. one morning in May

1994, I developed an X-ray film that showed alterations in the expression of a fat-specific gene in the two available mouse lines carrying the *ob* mutation (Fig. 3a in ref. 61). This single experiment confirmed that we had indeed cloned the *ob* gene and that it was under feedback control, a finding that strongly suggested that Coleman's hypothesis that *ob* encodes a novel hormone was correct. The elation of peering into the depths of nature and being the first to see something new is impossible to describe. This article and the award that led me to write it are an echo of the jubilation of that moment.

Fifteen years after the discovery of leptin, I remain mindful of the good fortune that made possible its discovery in my laboratory. Unlike Kleiner, I had an opportunity to conduct my research in an environment that was optimal for this purpose. As an investigator of the Howard Hughes Medical Institute at Rockefeller University and with support from the US National Institutes of Health, I had adequate resources. And, furthermore, I benefited from the talents of numerous colleagues and laboratory members.

I am also mindful of the absence of ill fortune that could have led to a different outcome. During the eight years my laboratory was inching its way down the chromosome toward *ob* at a glacial speed, hundreds of investigators around the world were using a set of newly available tools to place newly cloned genes on the mouse genetic map. If a gene happened to map to a site on the chromosome near the position of a mutation, it was straightforward to establish whether that gene was causal by sequencing mutant and wild-type DNA. In several cases, mutant genes were cloned in this manner, rendering for naught the years of effort by other investigators who had taken a positional-cloning approach[69]. Throughout the seemingly endless period during which we were searching for the *ob* gene, I lived in constant fear that I would one day receive a phone call informing me that someone else had gotten to *ob* first. Were this to happen, I often wondered, would I have been a less able or insightful scientist for having embarked on the course I had chosen?

One last ingredient

There is one additional element that, although not guaranteeing success, often has a prominent role in the path to discovery. This is the fierceness of one's determination—the single-minded insistence that a body of work be seen through to its logical conclusion. It is worth considering the extent to which this is an essential (though not sufficient) trait. Banting had this attribute. Although he did not have the grounding in research of either Macleod or Collip, without whose expertise he would not have gotten very far, he attacked his objective to find a pancreatic factor with a fierceness born of desperation and, as much as anyone, was responsible for driving an idea that was decades old into a new therapy for desperately ill patients in less than two years[70].

In contrast, even though Kleiner and Meltzer were convinced that the pancreas synthesized an antidiabetic factor in 1915, there is no evidence between 1916 and 1919 that they had any plans to purify the factor. Instead, they worked on a set of tangentially relevant projects: the effect of morphine and magnesium sulfate on glucose metabolism and the mechanism by which the factor stimulated the transit of glucose from the blood. The extent to which the onset of the war or other factors shaped their focus is not known. But it is not until Kleiner's paper of 1919 that it is clear that he fully understood both the basic and potential clinical importance of his findings. In 1918, Meltzer wrote to Flexner, "Kleiner has to pay the penalty for being a gentleman and of a modest retired disposition"[71]. This description was still relevant in 1955 when Elliot Joslin, founder of the Joslin Clinic, on the occasion of Kleiner's seventieth birthday wrote of how much he appreciated Kleiner's "reticence in never calling attention in public to (his) close association with the discovery of insulin"[72].

There is one final irony. Whereas Kleiner by all accounts lived a happy, calm and comfortable life (R. Glanz, A. Glanz and K. Glanz, personal communication), Banting did not. Banting's combativeness, fierceness and acquisitiveness appeared not to diminish with age[73]. Following his untimely death in an airplane accident in 1941, *Time* titled his obituary "Spark-Plug Man" and described him as "a stubborn man of strong feelings, sudden temper, trenchant speech"[74]. Banting was never satisfied. In September 1923, only weeks after his having received the Nobel Prize, the following item appeared in *Time* with the headline "Greater than Insulin": "Dr. F. G. Banting, discoverer of insulin, would shortly announce a new discovery of even greater importance than the world-famed diabetes treatment," adding "Dr. Banting had something so good we couldn't believe it." There is no further record of this "amazing" discovery[75].

Kleiner grew old surrounded by his wife, two children and five grandchildren. In addition to the pride and satisfaction he took from his early work on diabetes, teaching and writing a well-reviewed biochemistry textbook, he painted (**Fig. 4**) and, to the great amusement of his family, wrote doggerel (R. Glanz, A. Glanz and K. Glanz, personal communication).

This raises one final question. If you were forced to make a choice between a contented life and the thrill of having made a great discovery, which would you choose? Many people I ask choose the former. I know what I would choose.

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