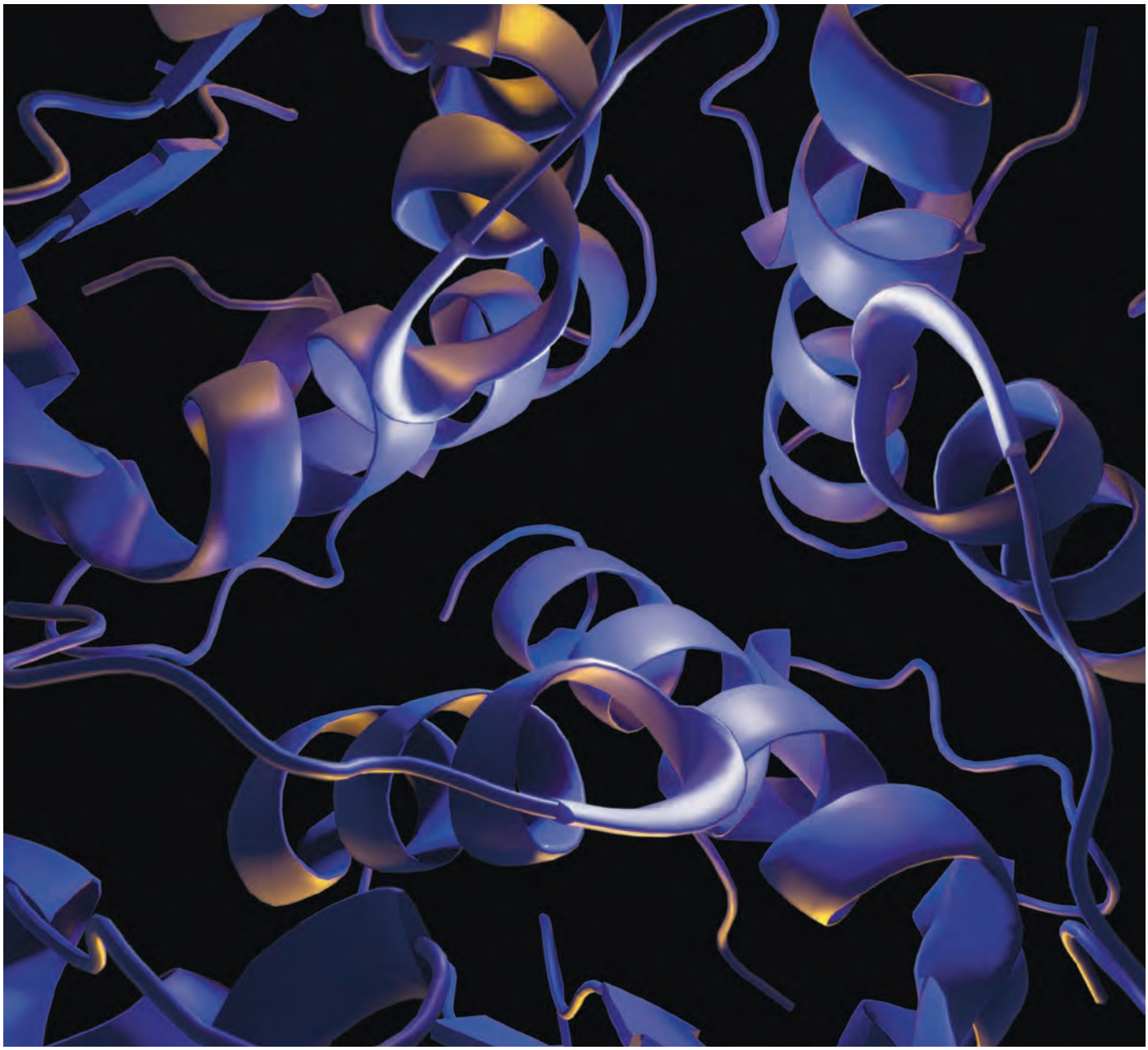
nature

milestones

Diabetes



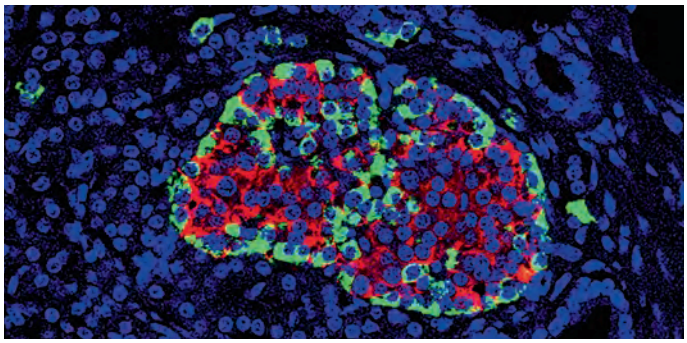
Produced by
Nature Reviews Endocrinology

With support from

AstraZeneca

Medtronic

novo nordisk®



A key role for islets **p.S4**

Contents

Diabetes complications **p.S7**

Visit the interactive timeline online
The *Nature Milestones in Diabetes* collection contains all of the articles included herein along with details of other important events and multimedia components that are presented in an interactive timeline. Visit the content online using the QR code or URL provided on page S3.

Contributing journals
BMC, Nature Communications, Nature Immunology, Nature Medicine, Nature Reviews Disease Primers, Nature Reviews Drug Discovery, Nature Reviews Endocrinology, Nature Reviews Immunology, Nature Reviews Molecular Cell Biology, Nature Reviews Neurology, Nature Reviews Nephrology, Nature Reviews Rheumatology

Citing the Milestones
Nature Milestones in Diabetes includes Milestone articles written by our editors and an online collection of previously published material. To cite the full project, please use *Nature Milestones: Diabetes* https://www.nature.com/collections/diabetes-milestones (2021). Should you wish to cite any of the individual Milestones, please list Author, A. Title. *Nature Milestones: Diabetes* <Article URL> (2021). To cite articles from the Collection, please use the original citation, which can be found online.

Personal subscriptions
subscriptions@springernature.com

Customer services
www.nature.com/help

nature
milestones

Diabetes

For more on *Nature Milestones in Diabetes*, visit www.nature.com/collections/diabetes-milestones

Editorial Office
Springer Nature,
The Campus, 4 Crinan Street,
London N1 9XW, UK
Tel: +44 (0)20 7833 4000

Coordinating Editors
Claire Greenhill, Anna Kriebs, Deepitha Maennich, Kirsty Minton, Jennifer Sargent, Shimona Starling

Copy Editor
Rebecca Hill

Production
Simon Fenwick, Susan Gray, Nick Bruni

Design & Web Development
Chris Ryan

Multimedia
Dan Fox, Geoff Marsh

Publisher
Rebecca Jones

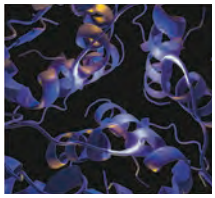
VP, Publishing
Richard Hughes

Marketing
Kimberly Petit, Fiona Kyprianou

Sponsorship
David Bagshaw, Marlene Stewart, Claudia Danci

Editor-In-Chief, Nature Publications
Magdalena Skipper

Executive Editor
Mina Razzak



On the cover
Close up view of an insulin hexamer, the storage form of insulin. Credit: Chris Ryan/Springer Nature Limited

Frederick Banting declared that "insulin is not a cure for diabetes; it is a treatment" in his 1923 Nobel lecture. The year 2021 marks 100 years since the discovery of insulin, which revolutionized the management of patients with type 1 diabetes. The past 100 years have seen seismic shifts in our understanding of the pathogenesis of the different types of diabetes, leading to advances in patient care. In this *Nature Milestones in Diabetes*, we highlight some of these key discoveries, which lay a path to the elusive goal of finding a cure for diabetes.

Following the discovery and early use of insulin by Frederick Banting, Charles Best, James Bertram Collip and John Macleod (Milestone 1), there are now a range of insulin analogues that can be used to treat patients. Technological advances have also led to the use of closed-loop systems for insulin delivery (Milestone 21). Insulin has been at the forefront of scientific discovery – it was the first protein to be sequenced, the first human protein to be chemically synthesized and the first recombinant protein to be produced in bacteria, for example.

However, the high cost of insulin analogues and the scarcity of more affordable human insulin, coupled with other barriers to access such as storage issues, have serious ramifications for many patients. Such inequities are non-trivial and urgently need to be addressed, particularly in low-income and middle-income countries. Here, however, we focus on the scientific achievements that have driven increased understanding of diabetes and have led to therapeutic advances.

Indeed, the past few years have seen an expansion of therapeutic options for patients with type 2 diabetes, with the advent of SGLT2 inhibitors, GLP1 receptor agonists and DPP4 inhibitors (Milestones 9, 22 and 24). In immunology, new discoveries are enabling the development of immunotherapies for type 1 diabetes (Milestones 17 and 20).

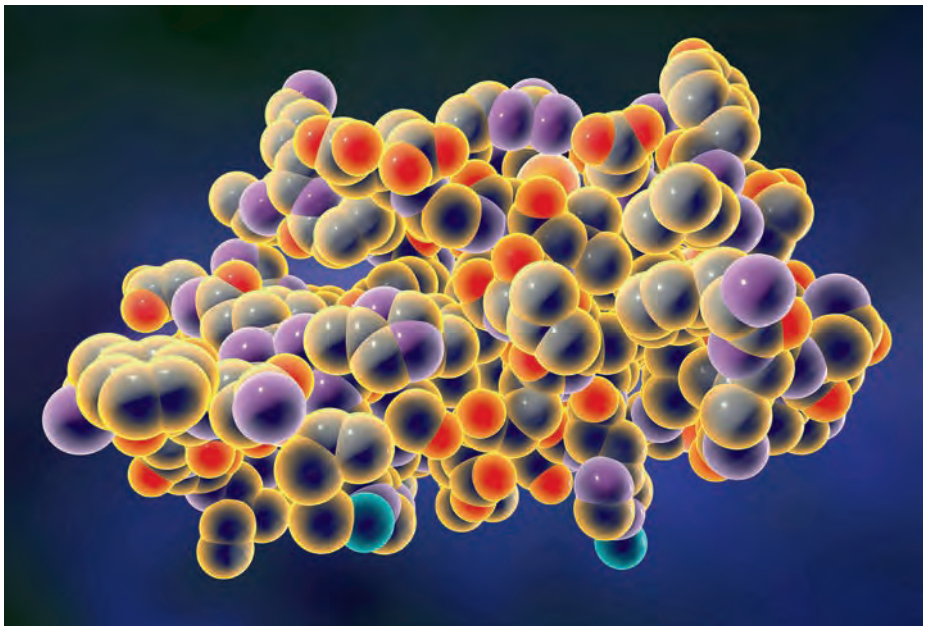
Each Milestone and the papers that we have highlighted here represent the culmination of years of work from teams of researchers, each building on the work of their predecessors and colleagues. Notably, these Milestones are not an exhaustive list, and we acknowledge the many important contributions to the field that have not been included in the Timeline. We thank the researchers and clinicians who have advised us on this project or agreed to be interviewed. We are pleased to acknowledge financial support from AstraZeneca, Medtronic and Novo Nordisk. As always, responsibility for the editorial content remains with Springer Nature.

Claire Greenhill Chief Editor, *Nature Reviews Endocrinology*
Anna Kriebs Senior Editor, *Nature Communications*
Deepitha Maennich Associate Editor, *Nature Reviews Disease Primers*
Kirsty Minton Senior Editor, *Nature Reviews Immunology*
Jennifer Sargent Senior Editor, *Nature Medicine*
Shimona Starling Senior Editor, *Nature Reviews Endocrinology*

Produced by
nature reviews endocrinology
www.nature.com/nrendo

With support from
AstraZeneca
Medtronic
Novo Nordisk

Milestone 1



CREDIT: KATERYNA KON / SCIENCE PHOTO LIBRARY

A history of insulin: initial discovery to first use in the treatment of T1D

In 1921, Frederick Banting and Charles Best designed a series of experiments to identify how the pancreas was involved in carbohydrate metabolism. Their work, published in 1922, built on findings from studies over the previous 40–50 years showing that the pancreas, and more specifically the islets of Langerhans, was key for carbohydrate metabolism and might be involved in the pathogenesis of the disease we now know as type 1 diabetes (T1D). This work led to the identification of insulin.

Together with John Macleod, Banting and Best hypothesized that carbohydrate metabolism was either controlled by the blood being modified while passing through the pancreas, or by the islets of Langerhans producing an internal secretion. Banting and Best began by removing the pancreases from several dogs, which then went on to display signs of diabetes, including increased blood and urine levels of glucose. The dogs were then injected with different pancreatic extracts, after which blood and urine levels of glucose fell and their overall health improved. Banting and Best noted that the degree of the effect varied depending on how concentrated the extract was. They also found that extracts containing pancreatic juices (containing digestive enzymes) negated the positive effects of the pancreatic extract on glucose levels. These results demonstrate that a pancreatic secretion was involved in carbohydrate metabolism.

"As Banting noted in his Nobel Prize lecture, insulin is not a cure for T1D; it is instead a treatment"

Following these findings, the researchers started work to purify the extract. Early use of this pancreatic extract in patients was unsuccessful, owing to its high protein content. James Bertram Collip then joined the team in order to help purify the pancreatic extract further, to give insulin. The researchers went on to devise a method to produce larger quantities of insulin, so that they could start to administer it to patients.

Work began at Toronto General Hospital, Canada, to administer insulin to a small number of patients with T1D, under close supervision. Leonard Thompson (14 years old) was, famously, one of these early patients – and was potentially the first patient to be administered with insulin. Similarly to the earlier animal work, blood and urine levels of glucose dropped following administration of insulin in these patients. The patients also reported complete resolution of the subjective symptoms associated with T1D.

Reporting on the case of Leonard Thompson, the researchers noted his low weight, lethargy and high blood and urine levels of glucose. Following daily administration of insulin, Leonard quickly began to feel better and was more alert, and his blood and urine levels of glucose reduced. On withdrawal of treatment for 2 days, his symptoms returned. In their 1922 paper in the *Canadian Medical Association Journal*, the researchers note that "These results taken together have been such as to leave no doubt that in these extracts we have a therapeutic measure of unquestionable value in the treatment of certain phases of the disease in man."

The researchers noted that their early findings were positive enough to warrant further work to refine the treatment regimen and improve patient outcomes. The following decades saw rapid advances in the use of insulin to treat T1D, such as the discovery in the 1930s that the action of insulin can be prolonged with the addition of protamine.

Banting and Macleod were awarded the Nobel Prize in Physiology or Medicine for their work in identifying insulin. Recognizing the key involvement of Best and Collip, Banting and Macleod decided to share the prize money with their two colleagues. As Banting noted in his Nobel Prize lecture, insulin is not a cure for T1D; it is instead a treatment that enables people with T1D to metabolize carbohydrates. T1D research has advanced considerably in the 100 years since Banting and Best's seminal paper, but a cure has remained elusive. It is to be hoped that a cure will be found in the next few years.

Claire Greenhill Chief Editor, *Nature Reviews Endocrinology*

Milestone study

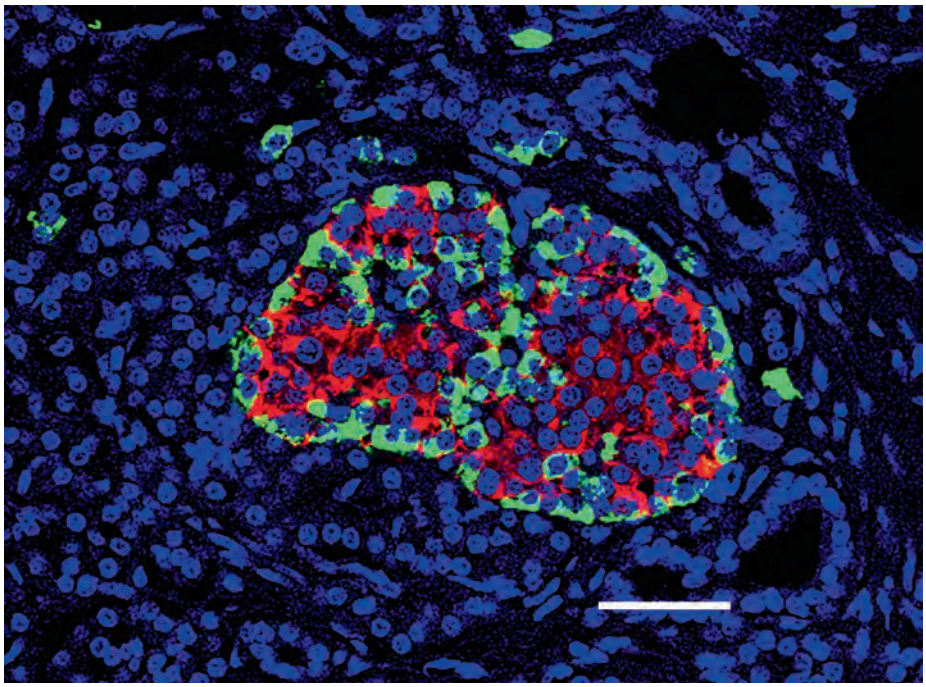
Banting, F. G. & Best, C. H. The internal secretion of the pancreas. *J. Lab. Clin. Med.* **7**, 465–480 (1922)

Further reading

Please visit the online article for a full list of further reading.

Milestone 2

Islet pathology in diabetes



An adult human islet. Red colour shows insulin staining, green shows glucagon and blue shows nuclei. Scale bar 50 μm.

CREDIT: SUSAN BONNER-WEIR / JOSLIN DIABETES CENTER

The discovery of insulin in 1921 (Milestone 1) revolutionized the treatment of people with diabetes. However, the pathophysiology of diabetes remained uncertain for many decades. In 1965, important histological findings were published that revealed the pathological anatomy of the pancreas in early-onset (prior to age 31 years) diabetes, which we now know as type 1 diabetes (T1D).

The classic view was that T1D was caused by an absolute deficiency of insulin secretion owing to a severe inadequacy of insulin-secreting tissue. The advent of histological techniques that could identify insulin-secreting β-cells, as well as α-cells, within pancreatic islets enabled this early hypothesis to be tested. In a 1965 study, researchers examined pancreatic tissue samples obtained at autopsy from 56 patients with early-onset diabetes, as well as 26 samples from young people without diabetes. Notable differences were seen between patients who died within 6 months of diagnosis (acute disease) and those who had the disease for longer than 1 year (chronic disease).

The findings from this study informed a new hypothesis that β-cells are under the influence of an 'extrapancreatic diabetogenic factor' that causes a progressive deterioration in T1D. For example, patients with acute disease showed decreased numbers of β-cells (<10% of normal values); however, the remaining β-cells showed signs of hyperactivity. Furthermore, 68% of patients with acute disease had peri-islet and intra-islet inflammatory infiltrates. By contrast, these infiltrates were never seen in patients with chronic early-onset diabetes, and the majority of these patients had a complete absence of β-cells. At disease onset, three types of islet were found — atrophic without β-cells, with β-cells and inflammatory infiltrates, and normal looking without infiltrates — thereby suggesting ongoing β-cell destruction.

"The findings supported the model of immune-mediated β-cell destruction in T1D"

The findings of this study contrasted with previous histological findings from patients who had diabetes diagnosed at an older age. These patients, who had what we now call type 2 diabetes (T2D), typically showed a moderate decrease in islet tissue and β-cell mass that was 40–50% of normal values.

Further insights into the pathogenesis of T1D were made in a 1984 autopsy study of pancreatic tissue from 11 children with T1D, 9 of whom had died within 24 h of their initial presentation (recent-onset). In the children with recent-onset T1D, two populations of islets were present: small insulin-deficient islets and large islets containing β-cells. Notably, eight of these children had inflammation of their pancreatic islets, which affected 18% of insulin-containing islets but only 1% of insulin-deficient islets. The findings supported the model of immune-mediated β-cell destruction in T1D.

A case report published in 1985 of a 12-year-old girl who died as a result of recent-onset T1D showed that many of the cells infiltrating the pancreas were cytotoxic or regulatory T cells, some of which were activated. The affected islets also showed upregulated expression of HLA class I molecules, which present peptides to T cells. Taken together, these findings and others helped inform our understanding of the autoimmune mechanisms of β-cell loss in T1D.

The pathogenesis of T2D is different, being characterized by tissue insulin resistance as well as impaired insulin secretion. T2D is associated with obesity and older age, although a large proportion of individuals with obesity do not develop T2D. A comprehensive 2003 autopsy study of pancreatic tissue from 124 people with obesity and/or T2D or prediabetes or of lean individuals with or without T2D helped inform our understanding of islet pathology in T2D. The study confirmed that levels of β-cells were decreased in T2D and the underlying mechanism was proposed to be increased β-cell apoptosis.

These early studies of individuals who died have helped inform us of the pathological processes occurring in pancreatic islets during diabetes and ultimately have led to the design of treatments that will improve patient outcomes.

Shimona Starling Senior Editor,
Nature Reviews Endocrinology

Milestone study

Gepts, W. Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* **14**, 619–633 (1965)

Further reading

Please visit the online article for a full list of further reading.

Milestone 3

The genetic underpinnings of T1D

By the 1970s, researchers recognized that type 1 diabetes (T1D) was strongly driven by genetic factors. Their studies suggested a polygenic pattern of inheritance; however, the genes associated with disease development were not known. Defining the underlying genetic factors was not a straightforward task in the absence of a map of the human genome and the technologies available today.

Understanding that the development of T1D had an immune component (Milestones 7 and 8) pointed the way to identifying the first genes associated with the disease. The HLA system had been shown to be genetically linked to antigen-specific, cell-mediated immunity. HLA-mediated immunity was associated with the susceptibility to viral infections, as well as autoimmune diseases.

In 1974, Nerup et al., writing in *The Lancet*, clarified previous contradictory reports by demonstrating that HL types A8 and W15 were found more frequently on white blood cells from individuals with T1D and were associated with anti-pancreatic, cell-mediated immunity. Although how HLA variants controlled disease development was not known, the researchers hypothesized that these flavours of the histocompatibility complex somehow triggered the autoimmune reaction that destroyed pancreatic cells.

"HL types A8 and W15 were found more frequently on white blood cells from individuals with T1D"

Later, genome-wide studies shed light on other genomic regions associated with T1D. In 2009, in an untargeted analysis, Barrett et al. listed single-nucleotide polymorphisms in the genomes of individuals from the UK with and without T1D and combined these data with other similar studies. The researchers found additional genomic locations, many linked to immune functions, that had a possible relevance to T1D.

Another notable discovery followed in 2015 when Onengut-Gumuscu et al. compared the genetic profile of 15 autoimmune diseases and demonstrated that T1D was genetically similar to other diseases characterized by the occurrence of autoantibodies.

Further studies untangled the relative importance of genetic variants to T1D risk and pinpointed the exact locations that constituted important disease alleles. It became evident that variants within the HLA locus represent half of the genetic risk in T1D. In particular, positions coding for amino acids within the region of the HLA proteins where antigenic peptides are bound and presented to immune cells, the so-called antigen-binding grooves, strongly conferred risk. Hu et al. found that three amino acid positions in the antigen-binding grooves of HLA genes together showed a notable effect. The demonstration that the critical locations for amino acid variations in HLA proteins were on the face of the protein that immune cells interacted with helped explain the occurrence of autoimmunity.

While the HLA genes were strongly associated with the occurrence of autoimmunity, it was less clear whether they were also driving the progression to clinical T1D. In fact, the progression from producing autoantibodies to insulin insufficiency is variable and the genetic risks were unclear. Beyerlein et al. clarified that genetic risk scores could indicate the rate of progression to clinical T1D and that the most prominent HLA genotypes were not the main drivers in this equation.

Based on the recognition that an integrated genetic risk score would capture the likelihood to develop the disease more accurately than focusing on the main HLA genotypes alone, Sharp et al. developed an updated genetic risk score for T1D in 2019 to predict future T1D in infants.

Although studies of T1D genetics over the past 50 years have advanced our understanding of important genetic risk loci and informed research on disease mechanisms, they are not broadly useful. Previous studies have mainly focused on individuals with European ancestry, and thus genetic risk scores based on this research fail to accurately capture genetic susceptibility for the majority of the population. Onengut-Gumuscu et al. (2019) demonstrated the improved performance of an ancestry-specific T1D risk score that included HLA alleles from individuals with African ancestry. Inclusion of ancestry-specific disease-associated variants is needed to accurately calculate genetic risk.

Anna Kriebs Senior Editor,
Nature Communications

CREDIT: OSAKAWAYNESTUDIOS

Milestone study

Nerup, J. et al. HL-A antigens and diabetes mellitus. *Lancet* **304**, 864–866 (1974)

Further reading

Please visit the online article for a full list of further reading.

Milestone 4

Animal models of T1D

Animal models of type 1 diabetes (T1D) enable the study of mechanisms underlying its pathogenesis and the development of therapeutic interventions. One of the most widely used models of the disease is the spontaneous diabetic Wistar rat, commonly referred to as the BB rat, which was first described in 1974 and then more extensively reported on in 1978 by Nakhooda and colleagues.

This group performed a longitudinal study of 51 weanlings from insulin-treated diabetic male and non-diabetic female parents with the specific aim of tracking the evolution from normoglycaemia to overt diabetes. In order to characterize the disease onset and early development, they monitored daily food and water intake, as well as body weight, and collected urine and blood samples. These samples were collected biweekly as diabetes was left untreated until the animals' condition mandated sacrifice, at which point the pancreas was removed for histological staining.

Changes in analysed parameters were referenced to the onset of overt diabetes, defined as the first day of detected glycosuria. They found that 9 of 51 animals became overtly diabetic, but the time of onset varied considerably (40–87 days), as did incidence between litters (9–50%). They also noticed that the onset of glycosuria was accompanied by rapid weight loss. Concurrently, the researchers measured a sharp increase in plasma levels of glucose and glucagon, as well as a decrease in insulin levels, for all 9 overtly diabetic animals. This dysregulation of glucose homeostasis was also detected via significant increases in plasma free fatty acid and total blood ketone concentrations.

Furthermore, oral glucose tolerance tests showed abnormal glycaemic responses in 6 of 9 rats compared with age-matched non-littermate controls. Finally, histological staining revealed that islets of select diabetic rats were smaller, less numerous and composed mostly of non β-cells; however, end-stage morphologies differed considerably.

One of the main take-home messages of this study was the recognition that disease onset and progression are rapid, occurring on the timescale of hours to days, but also heterogeneous. Many other groups would use the BB rat model in the coming decades to study T1D, including in the context of genetics, environmental factors and autoimmunity.

This article would certainly not be complete without mentioning the non-obese diabetic (NOD) mouse model, which was first described by Makino and colleagues in 1980. Along with the BB rat, this mouse model has proven useful for preclinical T1D research and is associated with several advantages linked to its better-defined genome, greater availability of lab reagents and imaging tools, and lower maintenance costs.

"two animal models of T1D have advanced our understanding of disease pathophysiology … insights gained cannot be directly applied to humans"

The first NOD mouse was generated during routine breeding of CTS mice, and discovered owing to its abnormal polyuria and glycosuria, accompanied by rapid weight loss. Although this first mouse died within 1 month, the researchers were able to establish the NOD strain via selective breeding, specifically by using its offspring to generate eight mating pairs tested for spontaneous diabetes and reproductive ability – a huge effort that involved more than 1,500 mice in total.

It is important to point out that, although other groups had previously generated hereditary diabetic mouse strains suitable for the study of maturity-onset diabetes or juvenile-onset diabetes induced by a virus or chemical, the NOD mouse was the first spontaneous non-obese diabetic strain.

Researchers tracked body weight and water intake, as well as urine volume, food consumption, plasma and urine glucose levels, and plasma cholesterol over time, defining the onset of diabetes based on a commercial urine glucose test. Interestingly, they found much higher incidence rates in females (80%) than in males (10%), the latter also featuring later disease onset. Histological analyses revealed lymphocyte infiltration into pancreatic islets, as well as a reduction in the number of β-cells and size of islets.

These two animal models of T1D have advanced our understanding of disease pathophysiology. Still, clinical translation of therapeutics has remained challenging, as curative success is dependent on treatment dose, timing and other parameters. Researchers in the field now agree that, although useful, these remain mere models and insights gained cannot be directly applied to humans. In fact, clinical trials have shown that numerous therapeutic interventions that were effective in animals later proved ineffective for patients with diabetes. Much work has focused on this issue and more remains to be done for the development of bona fide models to guide translation and more accurately predict therapeutic outcomes in humans.

Aline Lueckgen Associate Editor, *Nature Communications*

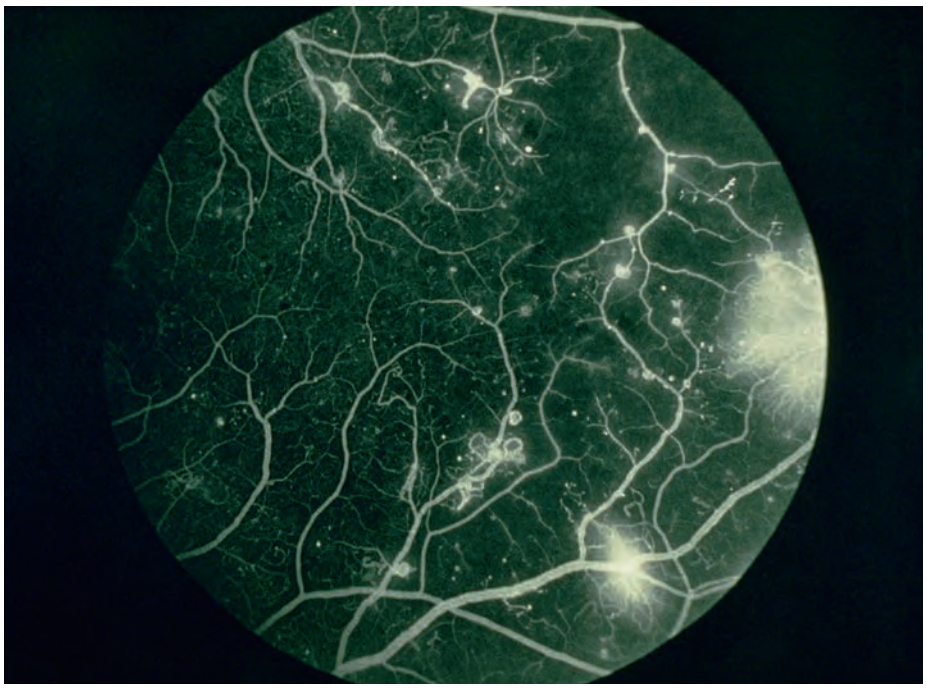
CREDIT: ROBERT PICKETT

Milestone studies

Nakhooda, A. F. et al. The spontaneously diabetic Wistar rat (the "BB" rat). *Diabetologia* **14**, 199–207 (1978) | Makino, S. et al. Breeding of a non-obese, diabetic strain of mice. *Exp. Anim.* **29**, 1–13 (1980)

Milestone 5

A pioneering study of diabetes complications



CREDIT: LESTER V. BERGMAN

By the middle of the twentieth century, the link between diabetes and degenerative conditions such as neuropathy, retinopathy and nephropathy was already well established. However, the precise relationship between these complications and glycaemic control was not fully appreciated until the publication of a ground-breaking longitudinal study, which was initiated by the Belgian physician Jean Pirart in 1947 and continued for over three decades.

"In 1947, I had the good fortune to take up practice at the Cesar de Paepe Clinic in Brussels, which treated hundreds of diabetic patients," explained Pirart in a key paper charting the first 25 years of the study, which was first published in French in 1977 and was translated into English in 1978. "The faithful attendance of these patients and the quality of their files over the preceding years gave me the idea to attempt a longitudinal study, which I have been able to follow for more than 30 years because of my long association with the clinic."

The 1977 paper included data from 4,398 patients with diabetes, each of whom underwent a series of examinations, including blood tests and neurovascular evaluations, at least once a year for a period of up to 25 years. In total, around 21,000 such examinations were conducted over the first 25 years of the study. At each assessment, the patients were rated according to their level of glycaemic control (good, fair or poor).

In light of the frequent co-occurrence of neuropathy, retinopathy and nephropathy, Pirart adopted the practice of referring to these manifestations as a 'diabetic triopathy', as originally proposed by Root and colleagues in 1954. The risk of developing one or more components of this triopathy correlated robustly with long duration of diabetes and poor glycaemic control, particularly in the year leading up to the examination. By contrast, the degree of glycaemic control seemed to have little influence on other vascular manifestations, such as coronary and peripheral atherosclerosis.

"Owing to a meticulous approach and a high level of patient engagement, Pirart's study was unprecedented in terms of size and duration"

Owing to a meticulous approach and a high level of patient engagement, Pirart's study was unprecedented in terms of size and duration. Earlier studies often relied on single measurements taken at baseline to determine diabetes severity, but the longitudinal assessments performed by Pirart and his colleagues reflected the true dynamic nature of glycaemic control status and raised the prospect of modifying this status to reduce the risk of degenerative complications.

Pirart acknowledged that his data could not prove the existence of a causal link between long-term hyperglycaemia and the diabetic triopathy. However, his work laid the foundations for further investigations, such as the UK Prospective Diabetes Study, which set out to determine whether improved glycaemic control could prevent the development of complications from type 2 diabetes. Researchers are also exploring the mechanisms underlying glucose-mediated vascular damage, including defects in the mitochondrial electron transport chain.

"Our study ... conclusively proves that diabetic triopathy and not atherosclerosis is a function of the duration and intensity of diabetes and more precisely of hyperglycaemia," concluded Pirart in his paper. "Of course, this can be lessened by treatment. This fact should encourage physicians to strive toward normoglycaemia in diabetes therapy, while at the same time recommending various methods of hygiene which could slow down the development of atheromatosis and of hypertension."

Heather Wood Chief Editor, *Nature Reviews Neurology*

Milestone studies

Pirart, J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Part 1. *Diabetes Care* **1**, 168–188 (1978) | Pirart, J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Part 2. *Diabetes Care* **1**, 252–263 (1978)

Further reading

Please visit the online article for a full list of further reading.

Milestone 6

Insulin gets an upgrade

After the discovery of insulin in 1921 (Milestone 1), the use of porcine or bovine insulin to control blood glucose levels in individuals with diabetes became widespread and saved many lives. However, insulin derived from animal pancreases had several limitations, including erratic effects on glucose levels and allergic reactions, both resulting from the production of insulin antibodies by the patient's immune system. This immunogenicity was thought to be the result of contamination of insulin with other pancreatic substances and small differences in amino acid composition between human and animal insulin. Purified animal insulins were developed and reduced the occurrence of allergic reactions, but further improvement was needed.

In the 1970s, advances in DNA synthesis and recombinant DNA technology raised the possibility that bacteria could be genetically altered to produce human insulin. A major step towards this goal was made in 1977 when, for the first time, a functional polypeptide was generated from a chemically synthesized gene. Itakura et al. synthesized the gene for the hormone somatostatin and incorporated it into a plasmid. The plasmid was inserted into *Escherichia coli* and the resulting strain produced a polypeptide containing the somatostatin amino acid sequence. Cyanogen bromide was used to cleave somatostatin from this larger protein in vitro. Somatostatin is just 14 amino acids long and was chosen in part because of its size — the time-consuming nature of chemical gene synthesis was considered a barrier to the synthesis of longer polypeptides such as the 51-amino-acid insulin.

"generation of fully synthetic human insulin was a major step forwards"

Nevertheless, just 2 years later, Goeddel et al. reported the first successful generation of fully synthetic human insulin. Native human insulin consists of two amino acid chains — the A chain and the B chain — that are linked by two disulphide bonds. The team chemically synthesized the DNA fragments encoding the two chains and used plasmids to express the A-chain gene in one strain of *E. coli* and the B-chain gene in another.

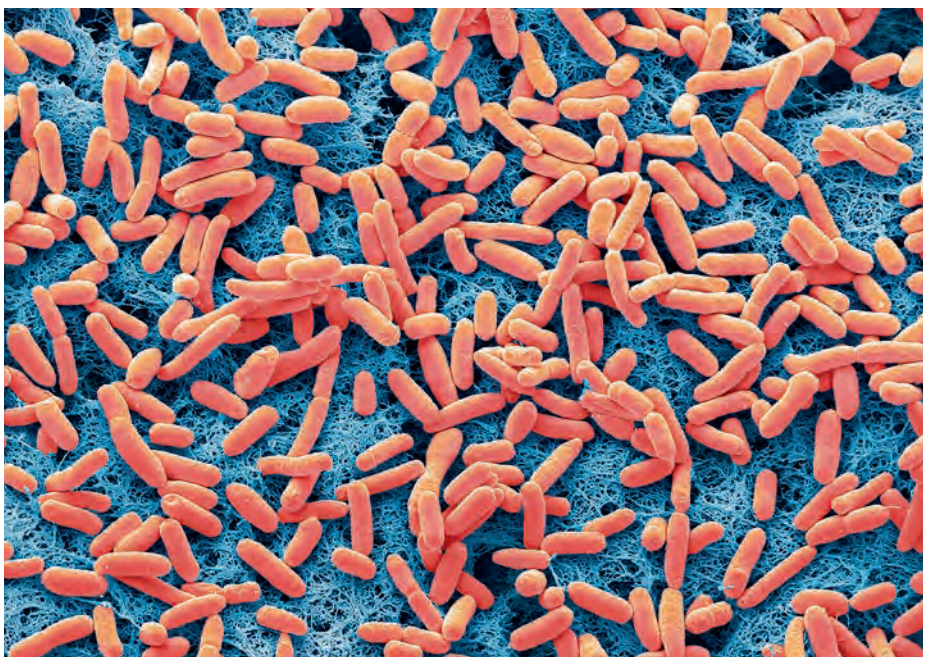
Similar to the somatostatin experiment, the team designed the plasmids so that the insulin chains would be produced as part of a large, relatively inactive protein. After purifying the *E. coli* products, they cleaved the A and B chains from this precursor protein using cyanogen bromide treatment and joined the two chains together using an existing method. The resulting product was tested using several approaches, including the relatively new technique of reversed-phase high-performance liquid chromatography, and behaved in the same way as human insulin.

The next big step was to establish the safety and efficacy of this new insulin product in humans. In 1980, Keen et al. published the results of a study investigating the effect of synthetic insulin in 17 healthy men. No adverse reactions to cutaneous, subcutaneous or intravenous administration of the synthetic insulin were identified. Furthermore, the synthetic insulin depressed blood glucose levels to a similar degree and with a similar trajectory to highly purified porcine insulin. These positive results indicated that synthetic insulin could provide a viable alternative to animal insulin and in 1982 synthetic insulin became the first genetically engineered product to be approved by the FDA. Despite the novelty of the product, review and approval took just 5 months, at a time when the average approval time for new drugs was more than 2 years. From 1986, the original method was replaced with one that used a plasmid containing the gene for human proinsulin.

Evidence soon indicated that the new synthetic human insulin was indeed less immunogenic than animal-derived insulin. In 1983, Fineberg et al. published the results of a study in 221 individuals with diabetes who had undergone 12 months of insulin treatment. Participants treated with synthetic human insulin had lower levels of insulin antibodies in blood than participants treated with porcine insulin.

The generation of fully synthetic human insulin was a major step forwards in the diabetes field and an important breakthrough in medical biotechnology, paving the way for the FDA approval of many more therapeutic recombinant proteins — more than 100 to date.

Sarah Lemprière Associate Editor, *Nature Reviews Neurology*



CREDIT: STEVE GSCHMEISSNER / SCIENCE PHOTO LIBRARY

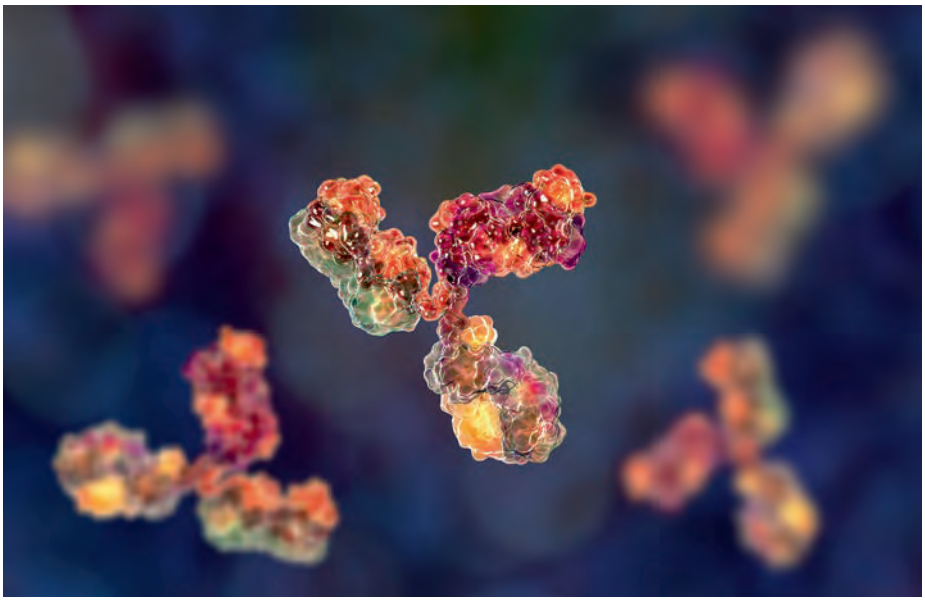
Milestone studies

Goeddel, D. V. et al. Expression in *Escherichia coli* of chemically synthesized genes for human insulin. *Proc. Natl Acad. Sci. USA* **76**, 106–110 (1979) | Keen, H. et al. Human insulin produced by recombinant DNA technology: safety and hypoglycaemic potency in healthy men. *Lancet* **316**, 398–401 (1980)

Further reading

Please visit the online article for a full list of further reading.

Milestone 7



Autoantibodies emerge on the scene

By the 1960s, various lines of evidence were converging on the idea that type 1 diabetes (T1D, better known at the time as juvenile diabetes) is an autoimmune-mediated condition. Some patients with T1D showed signs of insulitis, an immune-mediated lesion that could also be induced experimentally in animals (Milestones 8 and 19), new animal models of spontaneous autoimmune diabetes were emerging (Milestone 4) and there was a growing recognition that specific HLA alleles were associated with this condition (Milestone 3). However, an important step forwards came with the discovery of islet cell antibodies (ICAs).

This discovery had previously been fraught with difficulties owing to technological limitations of the time and the fact that, little known to researchers, such autoantibodies typically peak during the early stage of disease but subsequently disappear from the circulation as the disease progresses. Nevertheless, in 1974, within the space of 2 months, two independent groups of researchers in the UK reported the presence of ICAs in patients with multiple endocrine or autoimmune disorders, including juvenile diabetes. Notably, other research found that these antibodies were present before the onset of disease. Before long it was well recognized that ICAs were a characteristic feature of juvenile diabetes, providing the final impetus needed for shifting the field towards accepting T1D as an autoimmune condition.

> **"these antibodies were present before the onset of disease"**

The finding that ICAs were present during a so-called prodrome period raised hope of a window of opportunity for therapeutic interventions. That is, that disease could be prevented or delayed in some patients. This concept spurred on research in T1D prevention and ushered in an era of autoantibody testing that continues to this day. Indeed, the ICA immunofluorescence test remained a cornerstone of diabetes research for the next 30 years. Although testing for ICAs proved useful in identifying those individuals likely to progress to diabetes, assays for ICAs lacked sensitivity and were difficult to standardize owing to the heterogeneity of the samples used as substrate. Thus prompting the search for autoantibody targets: the autoantigens.

An important breakthrough came in 1982, with the identification of the first autoantigen: a ~64-kDa isoform of glutamic acid decarboxylase (GAD65). Using immunoprecipitation experiments, Baekkeskov and colleagues identified autoantibodies to this islet protein in the sera of 8 of 10 children with newly diagnosed T1D that were not present in sera of healthy children. As with ICAs, these islet autoantibodies were predictive of T1D. Although the role of GAD65 in pancreatic islet β-cells and in T1D pathogenesis remains elusive, these findings provided the basis for testing autoantigen-specific therapies, still under investigation, and the development of standardized islet autoantibody assays currently in use.

The search for autoantigens continued and a plethora of autoantibody targets have been identified. Subsequent studies found that the use of islet autoantibody combinations could improve diabetes prediction. Today, assays that measure various islet autoantibodies, including autoantibodies to GAD65, insulinoma-associated protein 2, zinc transporter 8 and insulin, are in common use for both predicting and confirming a diagnosis of T1D. Indeed, in 2015, the American Diabetes Association, the Juvenile Diabetes Research Foundation and the Endocrine Society introduced a new system for staging T1D that begins with individuals with no symptoms but who are positive for two or more islet autoantibodies (that is, individuals in the preclinical stages of disease).

It is now known that monitoring at-risk individuals can reduce the incidence of diabetic ketoacidosis and the mortality and morbidity associated with the clinical onset of T1D. Hence, early diagnosis is critical, particularly given the increasing incidence of the disease over the past decade. However, the majority of individuals who are diagnosed with T1D have no family history of the disease. The use of autoantibody screening holds promise not only for recruiting islet autoantibody-positive first-degree relatives into prevention trials, but also for population-based screening. Current efforts aimed at improving the performance, cost and availability of islet autoantibody assays are ongoing in the hope that routine measurements of islet autoantibodies in children and young adults will soon become a reality.

Jessica McHugh Senior Editor,
Nature Reviews Rheumatology

Milestone study

Baekkeskov, S. et al. Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* **298**, 167–169 (1982)

Further reading

Please visit the online article for a full list of further reading.

Milestone 8

Cytokines directly implicated in T1D

The discovery of islet cell-specific antibodies in patients with type 1 diabetes (T1D) (Milestone 7) and the subsequent revelation that these antibodies could be present years before disease onset turned the prevalent theory that an initial viral insult precipitated T1D on its head. Histological analysis of pancreatic tissue from patients with acute T1D in the 1960s (Milestone 2) had revealed immune cell infiltrates in affected pancreatic islets, but whether the immune cells represented a primary immune response to an infection or a response secondary to islet cell damage or death was unknown. A more detailed immunohistochemical analysis of islets published in 1985 (Bottazzo et al.) pinpointed T cells as the most abundant infiltrating cell type, as well as showing the deposition of islet cell-specific antibodies and high levels of expression of MHC class I and class II molecules on surviving β-cells. When coupled with results from transplantation studies in identical twins, in which insulitis and β-cell death occurred in the affected twin within weeks of partial pancreatic transplantation from an unaffected twin, these findings were strongly suggestive of an autoimmune pathology for T1D.

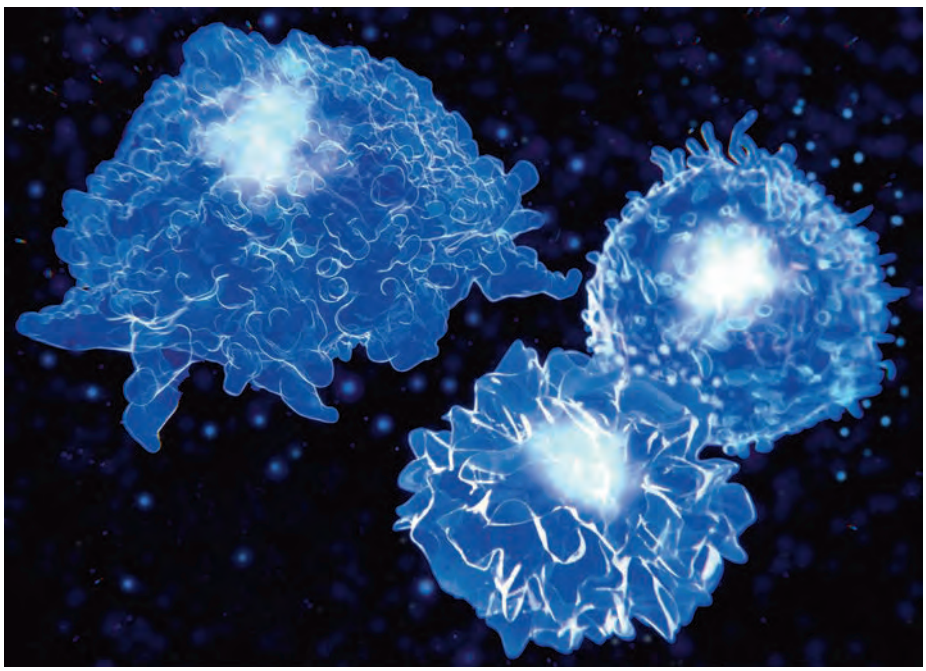
Several theories abounded as to how the immune system had turned on itself to destroy β-cells. One idea was that an environmental insult of some sort (possibly a virus) damaged β-cells, causing them to release proteins that could be picked up by antigen-presenting cells such as macrophages and used to stimulate self-reactive T helper cells that, in turn, stimulated B cells to produce antibodies and activate cytotoxic T cells. An alternative theory was that an environmental factor (possibly a cytokine such as IFNγ) caused β-cells to overexpress MHC class II molecules on their cell surface and act as antigen-presenting cells themselves. Both of these theories centred on MHC class II molecule expression and antigen presentation, firmly implicating the adaptive immune system in T1D but relegating innate immunity to the side lines.

The idea that innate immunity had, at best, a supporting role in the pathogenesis of T1D came to an abrupt end in 1986 with the publication of a study in *Science* that showed a direct role for the cytokine IL-1 in mediating β-cell death. IL-1 is predominantly produced by innate immune cells such as macrophages and monocytes and can activate a whole range of cells, including T cells and B cells. The study by Bendtzen et al. showed that supernatant from polyclonally activated blood mononuclear cells could inhibit the production of insulin by isolated pancreatic islets in vitro, and that this effect could be reversed by the addition of IL-1-specific antibodies and restored again by removal of the antibodies using an acid wash. These findings suggested that, in the absence of self-antigen recognition, a cytokine was able to produce a cytotoxic effect on a non-immune cell — something that had been only rarely reported at the time.

Bendtzen et al. went on to evaluate the effects of other cytokines on insulin production by islet cells, concluding that IFNγ was unlikely to be toxic and that, although tumour necrosis factor (TNF) reduced insulin release, it did not alter the amount of insulin within the β-cells and therefore had limited effects. By contrast, both naturally occurring and laboratory-produced IL-1 (specifically the *p*I 7 type of IL-1 that is now known as IL-1β) were able to reduce both released and intracellular insulin, indicating that this cytokine was toxic to β-cells. These results suggested a new theory in which circulating IL-1 (and potentially other cytokines) causes damage to β-cells, thereby triggering an autoimmune response that perpetuates damage.

Initial attempts to stop the autoimmune response using the immunosuppressant cyclosporine (Feutren et al., 1986) showed some promise, but any beneficial effects soon wore off when treatment was stopped, and cyclosporine could not prevent insulitis from developing in patients with T1D following pancreas transplantation. Targeting individual cytokines offered a more selective approach but would take several decades to develop and test. Unfortunately, IL-1 inhibition has not shown efficacy in clinical trials for T1D, but targeting TNF has proved more successful and is still being explored as a potential therapy, alongside other immunotherapies (Milestones 17 and 20).

Joanna Clarke Senior Editor, *Nature Reviews Rheumatology*



CREDIT: JUAN GAERTNER / SCIENCE PHOTO LIBRARY

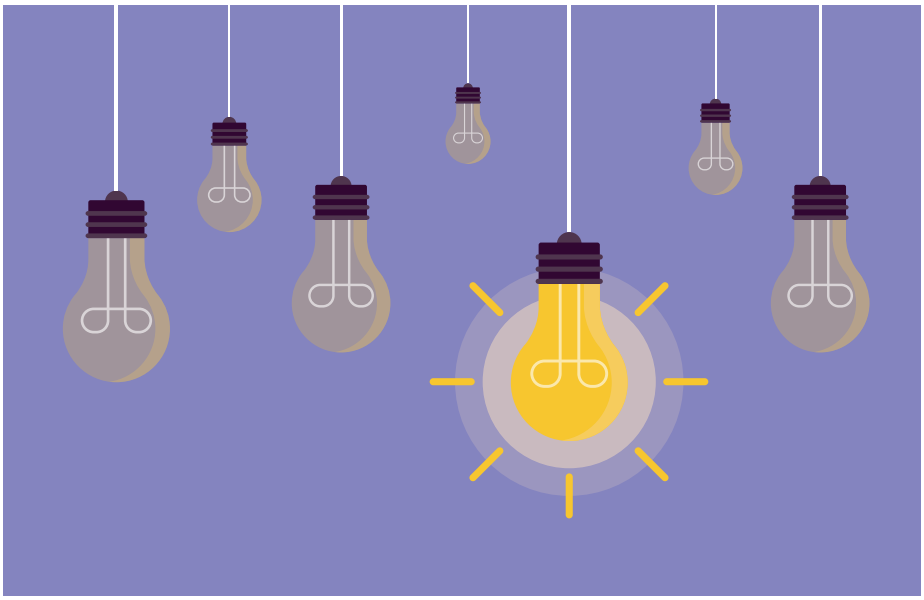
Milestone study

Bendtzen, K. et al. Cytotoxicity of human *p*I 7 interleukin-1 for pancreatic islets of Langerhans. *Science* **232**, 1545–1547 (1986)

Further reading

Please visit the online article for a full list of further reading.

Milestone 9



Illuminating the incretin effect

The idea that the intestine produces factors following nutrient ingestion that stimulate the release of substances from the pancreas to modulate blood glucose levels first emerged in the early 1900s, and, in 1932, Jean La Barre coined the name 'incretin'. The 1960s witnessed formal proof of the existence of a gut hormonal mechanism that stimulated insulin secretion — plasma insulin levels following oral intake of glucose were shown to be greater than those observed when glucose was given intravenously, referred to as the 'incretin effect' — and the search for incretins began.

The first significant incretin to be identified was the duodenal glucose-dependent insulinotropic polypeptide, GIP. In 1973, Dupre et al. demonstrated that infusion of GIP in combination with glucose into healthy humans led to potentiation of insulin secretion and improvement of glucose tolerance. However, 10 years later, Ebert et al. reported that incretin activity was preserved after removal of GIP from rat gut extracts, indicating that GIP was probably not the exclusive incretin.

Attention then turned to glucagon. In addition to the pancreas, the glucagon gene is expressed in the intestine. Although the glucagon gene encodes a proglucagon that exhibits tissue-selective processing, glucagon-like peptides (GLPs) were found to be liberated in both tissues. Furthermore, the intestine contained GLP1 in at least two forms, 31 and 37 residues long (GLP1(1–37) and GLP1(7–37)).

"In 1987, key papers from the laboratories of Habener and Holst implicated GLP1 as a regulator of insulin secretion"

In 1987, key papers from the laboratories of Habener and Holst implicated GLP1 as a regulator of insulin secretion. Drucker et al. synthesized GLP1(1–37) and GLP1(7–37) and examined their effects on cAMP formation (which promotes insulin secretion), insulin mRNA levels and insulin release in a rat islet insulinoma cell line. GLP1(7–37) was found to be a more potent insulinotropic peptide than GLP1(1–37), exerting a greater increase in cAMP levels, insulin mRNA transcripts and insulin release. Further, Mojsov et al. studied the effects of synthetic GLP1 on insulin secretion in the isolated perfused rat pancreas. Low concentrations of GLP1(7–37) potently stimulated insulin secretion, while even high concentrations of GLP1(1–37) had no effect. Providing yet further evidence of the insulinotropic activity of GLP1, Holst et al. isolated naturally occurring GLP1 from pig small intestinal mucosa, which corresponded to proglucagon 78–107. This isolated natural peptide, as well as a synthetic proglucagon 78–107 fragment, dose-dependently and potently increased insulin secretion in the perfused pig pancreas.

Together, these studies laid the foundations for further exploration of the role of intestinal GLP1 in glucose homeostasis. In addition to augmentation of glucose-stimulated insulin secretion, GLP1 is now known to exert multiple effects, including inhibition of pancreatic glucagon secretion, gastric emptying and food intake, while stimulating β-cell proliferation.

In type 2 diabetes, which is characterized by hyperglycaemia resulting from defects in insulin secretion and action, the incretin effect is typically diminished. Although the secretion of GLP1 may be reduced and GIP secretion is largely normal, the effect of GLP1 is preserved whereas the effect of GIP is severely impaired. This preserved effect of GLP1 inspired attempts to treat type 2 diabetes with GLP1.

However, the clinical utility of the GLP1 peptide itself is limited by an extremely short half-life, due to rapid degradation by dipeptidyl peptidase 4 (DPP4) in human serum, as noted by Mentlein et al. and Kieffer et al. in 1993 and 1995, respectively. To address this, two solutions emerged that have given rise to two major classes of antidiabetic drugs: inhibitors of DPP4 and analogues of GLP1 protected against DPP4-mediated degradation (Milestone 22). Today, incretin hormone-based treatments represent widely and successfully used therapeutic agents in the management of type 2 diabetes.

Sarah Crunkhorn Senior Editor, *Nature Reviews Drug Discovery*

CREDIT: TOMMY

Milestone studies

Drucker, D. J. et al. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl Acad. Sci. USA* **84**, 3434–3438 (1987) | Mojsov, S., Weir, G. C. & Habener, J. F. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J. Clin. Invest.* **79**, 616–619 (1987) | Holst, J. J. et al. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. *FEBS Lett.* **211**, 169–174 (1987)

Further reading

Please visit the online article for a full list of further reading.

Milestone 10

CREDIT: KATYATYA

GLUT4 traffic control

Insulin is essential for glucose homeostasis and one of its key functions is to drive glucose uptake into adipocytes and myocytes. Insulin's role in this process became apparent in 1921 and the concept was refined well into the 1950s, with several studies demonstrating accelerated transport of glucose into these tissues in response to insulin. Yet, the underlying mechanisms remained elusive for nearly three decades.

The way towards understanding insulin-stimulated glucose uptake has been paved by studies between the late 1970s and 1980. These studies demonstrated that in adipocytes, insulin increases the number of functional glucose transporters in the plasma membrane. Furthermore, this activity was shown to be mediated by the translocation of the yet unknown 'transport system' from the intracellular stores associated with the membrane fraction, known as low-density microsomes.

By the end of the 1980s, several glucose transporters had been identified in different tissues and cell types, including erythrocytes, liver, kidney, intestine and brain. However, it was unclear whether insulin-stimulated glucose transport specific for adipocytes and myocytes was mediated by one of these known systems that is differently regulated, or whether it relied on a unique type of transporter that was selectively expressed in these tissues. The key answer to this question was published in *Nature* in 1988 by James and colleagues. In this work, the authors isolated the microsomal membrane fraction from rat adipocytes and raised a monoclonal antibody that would specifically recognize the putative insulin-stimulated glucose transporter. This antibody selectively labelled muscle and adipose tissues and did not react with cells that did not show insulin-stimulated glucose transport. This study provided clear evidence that the insulin-stimulated glucose transporter in muscle and adipose tissue is molecularly distinct from the previously reported glucose transport systems. The following year, the gene encoding this unique transporter was cloned and its chromosomal location mapped by several different laboratories. In reflection of its similarity to GLUT1–3 glucose transporters, which had already been cloned, the protein was termed GLUT4.

> **"the insulin-stimulated glucose transporter in muscle and adipose tissue is molecularly distinct"**

Identification of GLUT4 as a unique glucose transporter whose subcellular localization is regulated by insulin raised questions about the physiological implications of these trafficking events. Decreased responsiveness of cells to insulin will inevitably lead to diminished glucose uptake from the bloodstream and hyperglycaemia, which are hallmarks of type 2 diabetes. Various mechanisms contribute to this glucose uptake defect, and aberrant trafficking of GLUT4 in skeletal muscle is one of them. Hence, modulation of GLUT4 subcellular localization has emerged as a potential therapeutic strategy against insulin resistance in type 2 diabetes.

Exploring the therapeutic potential of modulating GLUT4 trafficking requires a thorough understanding of the molecular machineries and signalling networks implicated in translocating GLUT4 from the intracellular pool to the plasma membrane upon cell stimulation with insulin. Work across the years from multiple groups has revealed that GLUT4 undergoes endosomal recycling, with fast endocytic rates that favour the retention of the majority of GLUT4 molecules in intracellular membranous compartments. When insulin is present, it binds to its receptor on the surface, inducing PI3K–AKT signalling. One of the substrates of AKT in adipocytes and myocytes is a GTPase-activating protein termed TBC1D4 (also known as AS160), which regulates the activity of RAB GTPases — key mediators of membrane dynamics — thereby linking insulin signal reception to GLUT4 trafficking.

Despite this progress, many gaps still remain in our understanding of the molecular underpinnings of insulin-stimulated glucose uptake. Further dissection of the molecular machineries governing GLUT4 intracellular trafficking, their regulation and their links to insulin signalling will be required to translate these findings into practical interventions.

Paulina Strzyz Senior Editor,
Nature Reviews Molecular Cell Biology

Milestone study

James, D. E. et al. Insulin-regulatable tissues express a unique insulin-sensitive glucose transport protein. *Nature* **333**, 183–185 (1988)

Further reading

Please visit the online article for a full list of further reading.

Milestone 11

The discovery of monogenic diabetes

The genetics of diabetes was a highly debated topic in the 1970s (Milestone 3) and studies proposed many possible modes of inheritance. Early reports from Tattersall provided evidence for the genetic heterogeneity of diabetes. He described the presence of a form of non-insulin-dependent diabetes, known as maturity-onset diabetes of the young (MODY), characterized by early onset (<25 years of age) and an autosomal-dominant mode of inheritance. The molecular basis underlying glucose intolerance in MODY remained unknown for many years.

In 1992, laying the groundwork for future studies, Froguel and colleagues performed genetic linkage analysis in 16 French families who displayed clinical characteristics of MODY to map and identify the genes causing their diabetes. The researchers investigated candidate genes involved in glucose transport, such as *SLC2A2* (encoding GLUT2), *GCK* (encoding glucokinase, the key enzyme in glucose metabolism) and a candidate region (adenosine deaminase, *ADA*) on the long arm of chromosome 20 that co-segregated with MODY.

In this first study, 14 of the families showed significant genetic linkage between the *GCK* locus on chromosome 7p and diabetes. Different families also showed varying linkage to *ADA*, highlighting the heterogeneity of MODY. Subsequently, two independent teams in France (Froguel and colleagues) and in the UK (Hattersley, Turner et al.) reported that mutations in *GCK* were the cause of hyperglycaemia in a large proportion of French and UK MODY families.

In 1996, in two seminal studies, Yamagata and colleagues used linkage analysis and identified that mutations in *HNF1A* and *HNF4A* (encoding transcription factors originally described in the liver) resulted in severe progressive insulin secretory defects and hyperglycaemia associated with microvascular complications. These studies represent a landmark in the field, as they shed light on the importance of transcription factors for pancreas development and function. Furthermore, patients with this genetic aetiology were shown to respond optimally to oral sulfonylureas, providing an early example of precision medicine.

Adding to the repertoire of monogenic diabetes, in the mid-2000s, different groups investigated the cause of another form of monogenic diabetes, which presents in the first 6 months of life, called neonatal diabetes. Gloyn and colleagues discovered that heterozygous activating mutations in *KCNJ11* (encoding ATP-sensitive potassium channel subunit Kir6.2) were the major cause. Certain *KCNJ11* mutations may also be associated with developmental delay, muscle weakness and epilepsy. Furthermore, moderately activating mutations in *KCNJ11* caused transient neonatal diabetes, highlighting the multiple phenotypes associated with this gene. Subsequent studies identified *ABCC8* (encoding sulfonylurea receptor) and *INS* (encoding insulin) as additional genetic causes. With the advances in molecular genetics, MODY-related mutations have been identified in different genes, including *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *INS*, *NEUROD1*, *PDX1*, *ABCC8*, *KCNJ11*, *CEL* and *RFX6*.

A correct diagnosis of MODY or neonatal diabetes can have profound implications for treatment and prognosis. Importantly, studies focusing on the clinical care of patients with MODY reported that the aetiology of hyperglycaemia affected the treatment outcomes. For instance, sulfonylurea therapy was found to be safe and more effective than insulin therapy in patients with *KCNJ11* mutations and future studies also confirmed its long-term efficacy and safety.

"the knowledge of monogenic diabetes has evolved from simple clinical characteristics to well-defined molecular genetics"

Since the 1970s, the knowledge of monogenic diabetes has evolved from simple clinical characteristics to well-defined molecular genetics. Despite such advances, >80% of patients are not diagnosed via genetic testing, probably owing to costs and unavailability of tests in certain settings. The field has moved towards next-generation sequencing, which provides a highly sensitive method for simultaneous analysis of all monogenic diabetes genes. Precision medicine has taken momentum; a systematic approach integrating advances in biology and technology and implementing universal screening programmes in a cost-effective manner is imperative to achieve the best possible outcomes for all patients.

Deepitha Maennich Associate Editor, *Nature Reviews Disease Primers*

Milestone studies

Froguel, P. et al. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* **356**, 162–164 (1992) | Hattersley, A. T. et al. Linkage of type 2 diabetes to the glucokinase gene. *Lancet* **339**, 1307–1310 (1992)

Further reading

Please visit the online article for a full list of further reading.

CREDIT: YAY MEDIA AS / ALAMY STOCK VECTOR

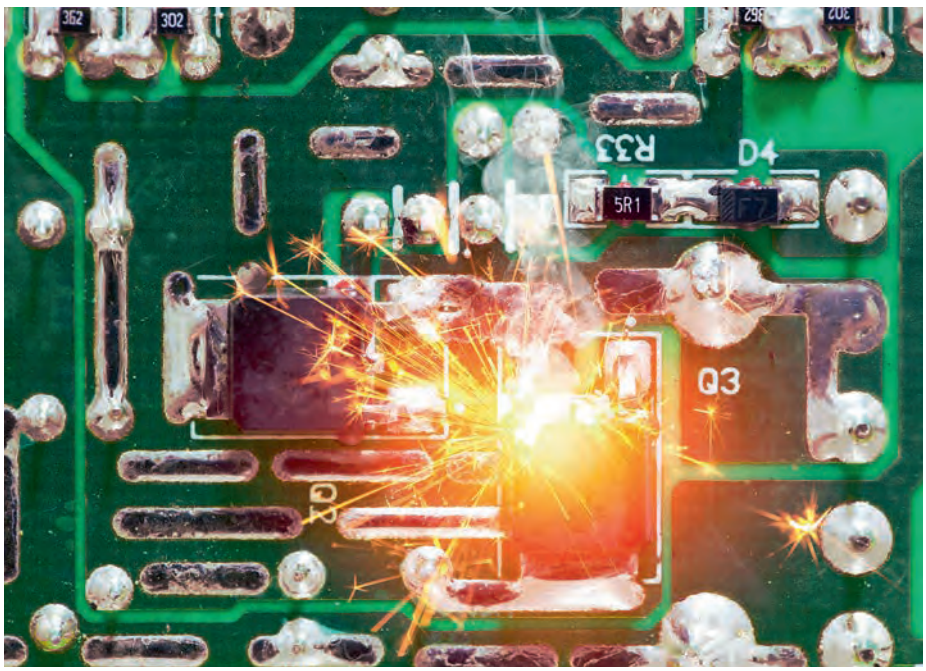
Milestone 12

TNF short-circuits the insulin receptor

By the 1990s, it was well established that the immune system's destruction of pancreatic β-cells that produce insulin results in type 1 diabetes (Milestones 7 and 8). However, the mechanisms responsible for the development of type 2 diabetes, which was linked to insulin resistance, were still unclear. What was known at the time was that insulin resistance was seen in numerous disease conditions, including chronic infections and cancer, and was ubiquitously linked with obesity.

A major breakthrough in understanding the mechanistic basis of insulin resistance — and why this is observed in such a diverse range of conditions — came with the publication of several key studies in the 1990s by Gökhan Hotamisligil and co-workers in the laboratory of Bruce Spiegelman. In two papers published in 1993 and 1995, these scientists found that the pro-inflammatory cytokine tumour necrosis factor (TNF) is upregulated in the adipose tissues of obese animals and humans with obesity and that blocking TNF improves insulin sensitivity in animal models of obesity. These observations were followed by a 1996 study that identified the molecular basis of TNF-driven insulin resistance. Productive signalling through the insulin receptor involves its autophosphorylation in response to insulin binding and the subsequent tyrosine phosphorylation of insulin receptor substrate 1 (IRS1). Hotamisligil et al. found that TNF disrupts this process by inducing serine phosphorylation of IRS1, which converts IRS1 into an inhibitor of the insulin receptor and prevents productive signalling following insulin binding. They further showed that this inhibitory form of IRS1 was present in the adipose tissue and muscle of obese animals and was responsible for insulin resistance in these tissues.

"type 2 diabetes and obesity itself are chronic inflammatory diseases in their own right, and not simply metabolic disorders"



These findings helped to explain why numerous chronic diseases were associated with insulin resistance. Moreover, the work of Hotamisligil, Spiegelman and others contributed to the eventual appreciation that type 2 diabetes and obesity itself are chronic inflammatory diseases in their own right, and not simply metabolic disorders. Subsequent research, including two key studies published in 2003 from the laboratories of Hong Chen and Anthony Ferrante, showed that obesity induces immune cell changes in adipose tissue that affect insulin sensitivity. These authors found that macrophages are increased in adipose tissue during obesity and produce TNF and other inflammatory mediators that promote insulin resistance. Shortly after this, it was shown that specifically disrupting NF-κB signalling in myeloid cells could alleviate obesity-induced insulin resistance. Many other immune cell types have since been studied in obesity and, in general, obesity is linked with activation of pro-inflammatory immune cell responses and the suppression of anti-inflammatory ones.

A key question that has still not been fully resolved is how obesity initiates inflammation in the first place. Current thinking in the field is that chronic nutrient overload can result in endoplasmic reticulum (ER) stress, which may fuel tissue inflammation by promoting cell death or the production of pro-inflammatory mediators that are linked with the ER stress response.

Yvonne Bordon Senior Editor, *Nature Reviews Immunology*

Milestone studies

Hotamisligil, G. S., Shargill, N. S. & Spiegelman, B. M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259**, 87–91 (1993) | Hotamisligil, G. S. et al. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* **95**, 2409–2415 (1995) | Hotamisligil, G. S. et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. *Science* **271**, 665–670 (1996)

Further reading

Please visit the online article for a full list of further reading.

Milestone 13

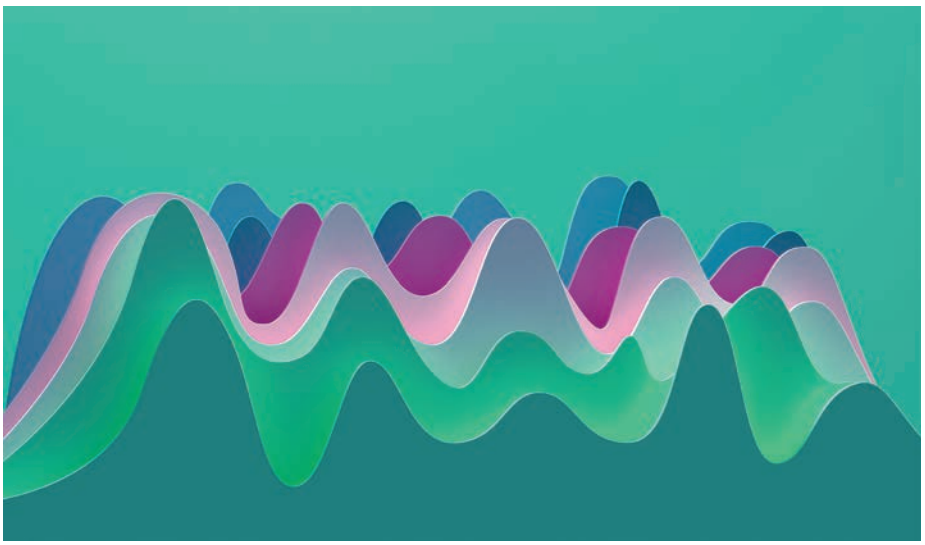
Findings from DCCT — glycaemic control prevents diabetes complications

The complications from type 1, 'insulin-dependent', diabetes include microvascular, neurological and macrovascular sequelae that cause substantial morbidity and mortality (Milestone 5). In 1983, the Diabetes Control and Complications Trial (DCCT) started recruiting patients to examine whether intensive treatment — in which insulin is administered with an external pump or by three or more daily injections of insulin with the goal of achieving glucose control as close to the non-diabetic range as safely possible — could improve outcomes for patients with type 1 diabetes. Compared with standard treatment at the time (one or two daily injections), intensive treatment reduced the occurrence of retinopathy, neuropathy and nephropathy by a range of 35% to >70%. The publication of these results in 1993 marked a new era in diabetes management.

The DCCT was a multicentre, randomized trial that examined patients with type 1 diabetes from two categories: those with no clinical evidence of retinopathy (the 726-patient primary prevention cohort) and those with early retinopathy (the 715-patient secondary intervention cohort). Patients aged 13–39 years were randomly assigned to receive either intensive or standard therapy. The choice of insulin doses in the intensive therapy arm was guided by frequent self-monitoring of blood glucose levels. Patients were followed up for a median of 6.5 years, and the primary outcome was retinopathy.

In the primary prevention cohort, the cumulative incidence of retinopathy was approximately 50% lower in the intensive therapy group than in the standard therapy group. In the secondary intervention cohort, there was an increase in retinopathy in the first year in patients who received intensive therapy. However, from 36 months onwards, patients who received intensive therapy had a 54% lower risk of progression of existing retinopathy than those who received standard therapy. Other end points, including nephropathy and neuropathy, were substantially lower in the intensive therapy group than in the standard therapy group in both cohorts.

The most common adverse event was severe hypoglycaemia, including instances of coma or seizure, which was approximately three times higher in the intensive therapy group than in the standard therapy group. The benefits, however, substantially outweighed the risks, and the study authors recommended intensive therapy for most patients with type 1 diabetes.



"intensive treatment reduced the occurrence of retinopathy, neuropathy and nephropathy by a range of 35% to >70%"

At the conclusion of the DCCT, patients in the standard therapy group were offered intensive therapy and most were enrolled in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, a long-term observational study.

During the EDIC study, the original intensive therapy group continued to have decreased incidence and slower progression of existing retinopathy, nephropathy and neuropathy. More severe microvascular outcomes, cardiovascular disease and mortality were also less common in this group than in the original standard therapy group, which switched to intensive therapy in the EDIC study. The continued separation in outcomes occurred despite the convergence of glycaemic levels between the two original treatment groups. These results suggest that intensive therapy is most effective when started early and that benefits persist throughout therapy. The likelihood of worsening complications, including developing the first signs of complications, strongly correlated with levels of HbA_{1c}, a marker of chronic blood glucose levels.

This seminal work, begun in the DCCT and continued in the EDIC study, established that maintaining blood glucose levels at near-normal values is key to avoiding the complications of diabetes.

Megan Cully Senior Editor,
Nature Reviews Drug Discovery

Milestone study

The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**, 977–986 (1993)

Further reading

Please visit the online article for a full list of further reading.

Milestone 14

Role of bariatric surgery in T2D

A landmark study published in 1995 introduced the world to the idea that bariatric surgery could 'cure' type 2 diabetes (T2D). In 91% of patients with obesity and either non-insulin-dependent diabetes (now known more commonly as T2D) or impaired glucose tolerance, a gastric bypass operation normalized levels of glucose, insulin and HbA_{1c} for decades. This breakthrough study was important given that then, as now, alternative approaches to T2D therapy (including diet, exercise, insulin and oral agents) had proved inadequate in maintaining long-term euglycaemia.

In the study by Pories et al., 608 patients with a BMI ≥35 kg/m^2 with complications of obesity or a BMI ≥40 kg/m^2 without complications underwent a gastric bypass. The operation was found to produce significant and durable weight loss. However, more striking was the control of T2D that surgery afforded. Of the 608 patients at baseline, 330 had either non-insulin-dependent diabetes or impaired glucose tolerance. Of 298 of these patients with adequate follow-up, 271 (91%) maintained normal values of fasting blood glucose and HbA_{1c} for the 14 years of follow-up. Ten patients did not return to euglycaemia because of technical failures of the surgery (staple line breakdowns). Interestingly, patients whose surgery was intact but who did not return to euglycaemia (17 patients) were generally older (48.0 versus 40.7 years) and had diabetes of longer duration (4.6 versus 1.6 years).

> **"alternative approaches to T2D therapy … had proved inadequate"**

Although the study by Pories et al. is now considered pivotal in the discovery of an operation as a means of treatment for T2D, it took several years for randomized studies to confirm the efficacy of bariatric surgery in the long-term control of T2D. The *New England Journal of Medicine* published two such studies in 2012. Schauer et al. compared intensive medical therapy alone versus medical therapy plus Roux-en-Y gastric bypass (RYGB) or sleeve gastrectomy in 150 patients with uncontrolled T2D. After 12 months, bariatric surgery achieved glycaemic control in significantly more patients than medical therapy alone. Meanwhile, Mingrone et al. randomly assigned 60 patients to receive medical therapy or to undergo either gastric bypass or biliopancreatic diversion. At 2 years, remission of T2D occurred in no patients in the medical therapy group, 75% of patients in the gastric bypass group and 95% in the biliopancreatic diversion group. In 2021, the 10-year follow-up data showed that 25% and 50% of patients remained in remission in the gastric bypass group and biliopancreatic diversion group, respectively. These studies have paved the way for further acceptance of bariatric surgery as a treatment for T2D.

But how and why does bariatric surgery work to treat T2D? Back in 1995, Pories et al. described the normalization of glucose metabolism as occurring "with surprising speed, even before there was significant weight loss" and before a reduction in the mass of adipocytes. Instead, they speculated that reduction of caloric intake had an important role. They also tentatively suggested that changes in incretin stimulation might also contribute (such as increases in the levels of glucagon-like peptide 1; GLP1 (Milestone 9)).

Since then, various studies have further investigated the mechanisms of action of bariatric surgery for remission of T2D. In 2006, patients who had undergone RYGB were found to have increased postprandial levels of insulin and GLP1, possibly contributing to improvements in glycaemic control. This finding was further confirmed in 2010. A study in 2013 demonstrated that the Roux limb in RYGB-treated rats undergoes reprogramming of intestinal glucose metabolism, contributing to glycaemic control. In 2014, vertical sleeve gastrectomy (an alternative bariatric surgical procedure that also results in remission of T2D) was found to exert its positive effects on glycaemic control via increased levels of circulating bile acids and changes to gut microbial communities.

More information is clearly needed on the mechanisms behind the positive effects of bariatric surgery on T2D remission. In turn, this knowledge might help in the development of further agents to treat T2D. Meanwhile, bariatric surgery for T2D (now known as metabolic surgery) continues to gain further acceptance as an effective treatment option for this increasingly common disease.

Isobel Leake, Senior Editor, *Nature Reviews*

Milestone study

Pories, W. J. et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Ann. Surg.* **222**, 339–352 (1995)

Further reading

Please visit the online article for a full list of further reading.

CREDIT: HRAUN

Milestone 15

Better living (not) through chemistry

Obesity and overweight are the major risk factors for the development of type 2 diabetes (T2D). The explosion in T2D prevalence over the past half century has paralleled that of the obesity epidemic, thought to be driven by changes in lifestyles associated with increased availability of calorie-dense foods, as well as decreased physical activity and the emergence of urban obesogenic environments. Left unchecked, by 2050, as many as 750 million people will have T2D worldwide.

While new therapeutics aimed at tackling excess adiposity and elevated HbA_{1c} levels are highly effective for managing T2D, the best strategy to reduce the global burden is undoubtedly prevention — in part through identification and early treatment of individuals at high risk of developing T2D.

Prediabetes is defined by an elevated level of blood glucose that does not meet the threshold for a definitive T2D diagnosis and it is considered an intermediary stage towards development of clinical T2D. Generally characterized by a higher than normal BMI, the debate regarding whether and how to treat individuals with prediabetes with pharmaceutical interventions is contentious. However, the concept that prediabetes is reversible and therefore T2D is preventable in at-risk populations (and more broadly) is widely accepted by the medical community — and the evidence is strong.

In 1986, the first large-scale, randomized controlled trial aimed at reducing incidence of T2D through behavioural interventions — healthy diet and/or exercise — was initiated in Da Qing, China. Investigators leading the Da Qing IGT and Diabetes Study screened >100,000 men and women, enrolling 577 individuals with impaired glucose tolerance, irrespective of BMI. Participants were randomized to either a no-intervention control group or one of three interventional groups: diet only, exercise only, or diet-plus-exercise, with regular follow-up visits with a physician over the course of the study. At 6 years, the cumulative incidence of T2D was 67.7% in the control group compared with 43.8% (diet only), 41.1% (exercise only) and 46% (diet-plus-exercise) in the interventional groups, demonstrating a statistically significant reduction in T2D incidence associated with diet and/or exercise over the course of the study.

"evidence supporting prevention and remission of T2D provides a strong case for expanding support for dietary and physical interventions in clinical practice"

A post hoc 30-year follow-up of the Da Qing study, including 540 of the original participants, compared outcomes between the control group and the interventional groups combined. Participants in the healthy diet and/or exercise group had a median delay in onset of T2D of almost 4 years, fewer cardiovascular events and lower incidence of microvascular complications and reduced all-cause and cardiovascular deaths.

Clinical trials in other settings have affirmed these beneficial effects in individuals with impaired glucose tolerance. The Diabetes Prevention Program (DPP) in the USA found that a lifestyle modification intervention was more effective than metformin for reducing T2D incidence in a diverse, at-risk population; and the Finnish Diabetes Prevention Study reported similar findings. Long-term follow-up over 15 years and 7 years, respectively, demonstrated sustained effects of the diet/exercise interventions and, in the case of DDP, demonstrated cost-effectiveness.

Extending the work of prevention studies, in 2018, the DiRECT trial showed that T2D in patients with a recent diagnosis (<6 years) was reversed in 46% of participants in the low-calorie diet intervention group. These findings were confirmed in the DAIDEM-I trial, reporting significant reductions in weight, and with diabetes remission observed in 61% of participants in the low-calorie diet intervention group, in a population from the Middle East and North Africa region with a diagnosis of T2D within the past 3 years.

The evidence supporting prevention and remission of T2D provides a strong case for expanding support for dietary and physical interventions in clinical practice. However, further research is urgently needed to understand patient acceptability and ways to prevent weight regain when normal diets resume after the initial phase, and to determine the optimal settings in which to deliver the interventions.

Jennifer Sargent Senior Editor, *Nature Medicine*

CREDIT: TOM WANG / ALAMY STOCK PHOTO

Milestone study

Pan, X.-R. et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. *Diabetes Care* **20**, 537–544 (1997)

Further reading

Please visit the online article for a full list of further reading.

Milestone 16

Genetics of T2D

CREDIT: KTSDESIGN/ SCIENCE PHOTO LIBRARY

Type 2 diabetes (T2D) is a complex and multifactorial disease. Notable risk factors include poor diet, obesity, low physical activity levels and older age. However, twin studies published in the 1980s suggested that genetic factors can contribute to T2D risk, as monozygotic twins showed greater concordance for T2D than dizygotic twins.

Genetic risk factors for diseases are typically assessed by genome-wide association studies (GWAS), which analyse the genomes of many people for the presence of genetic markers that can predict disease. Of note, many factors can cause variable results in GWAS, including multiple hypothesis testing and publication bias, among others. For example, prior to 2000, many genetic associations were reported for T2D but none was confirmed in multiple populations, using comprehensive controls.

In 2000, a ground-breaking paper was published that evaluated 16 previously identified genetic associations to T2D. A multi-layered, family-based study design was used to control for population stratification. Associations were first tested in 333 Scandinavian parent–offspring trios. Alleles that showed a nominal association were tested for replication in three additional study populations of European ancestry. Notably, only one association was confirmed: the common Pro12Ala polymorphism in peroxisome proliferator-activated receptor-γ (PPARγ).

"The knowledge gleaned from these genetic factors has been used to inform mechanisms of disease"

The researchers found a small (1.25-fold) but significant ($P = 0.002$) increase in T2D risk associated with the more common proline allele (occurring at ~85% frequency). Although the effect size is modest, the high frequency of the risk allele translates into a large risk at the population level. As much as 25% of T2D in the general population might be influenced by this allele. However, the study did not confirm whether PPARγ-Pro12Ala or a variant in linkage disequilibrium was the causal variant.

In further association studies, chromosome 10q was reported to have linkage with T2D in Icelandic and Mexican American populations. To further investigate this association, a 2006 study used genotyping of microsatellite markers. The probable gene associated with T2D risk was identified as *TCF7L2*, which encodes a transcription factor implicated in blood glucose homeostasis.

Encouragingly, in 2007, three independent GWAS of different European populations were published in a single issue of *Science*, with overlapping findings. All three studies identified T2D susceptibility loci in or near *CDKAL1*, *CDKN2A* and *CDKN2B*, *IGF2BP2*, *HHEX* and *SLC30A8*. Later work identified protein-truncating mutations in *SLC30A8* as the first loss-of-function mutations that are protective against T2D. This gene encodes ZnT8, which is highly expressed in insulin granules, and loss of its function increases insulin secretion. These important studies provided in vivo validation of ZnT8 as a drug target in T2D.

As the field advanced, findings from GWAS were further interrogated to elucidate the mechanistic basis for observed associations. For example, 2014 and 2015 studies on obesity and T2D-associated variants in *FTO* found that this gene forms long-range functional connections with the transcription factor *IRX3* and could be involved in a pathway for adipocyte thermogenesis regulation.

In 2019, a GWAS was published of T2D in sub-Saharan African individuals, an understudied group. This paper identified a new T2D risk locus specific for African populations at *ZRANB3*. Also of note, a 2020 meta-analysis of GWAS T2D risk in East Asian populations analysed data from 77,418 individuals with T2D and 356,122 control individuals. The analysis identified 61 loci that are newly implicated in T2D risk. It is important that further investigations are carried out in understudied populations in order to realize the true breadth of genetic risk of T2D.

To date, GWAS have identified >550 signals associated with the risk of T2D. The knowledge gleaned from these genetic factors has been used to inform our understanding of mechanisms of disease. In the future, precision medicine approaches might also use genetic information to predict which people will respond best to different therapies.

Shimona Starling Senior Editor, *Nature Reviews Endocrinology*

Milestone study

Altshuler, D. et al. The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat. Genet.* **26**, 76–80 (2000)

Further reading

Please visit the online article for a full list of further reading.

Milestone 17

Anti-CD3: the agonist and the ecstasy

Studies published in the 1960s and 1970s began to solidify the concept that human type 1 diabetes (T1D) is an autoimmune disease (Milestone 2). This was an important conceptual advance because it offered hope of treating the cause of the disease rather than simply controlling symptoms via the provision of insulin (Milestone 1). Initial treatments focused on well-established immunosuppressive drugs such as corticosteroids and cyclosporine. However, the broad immunosuppression caused by these drugs, their associated toxicities and the need for their prolonged use in predominantly young patients ruled them out as a viable therapy for T1D.

An important breakthrough happened in 1985 — namely, the first clinical approval of a monoclonal antibody (mAb), Orthoclone OKT3. This drug was initially used for managing rejection of kidney, heart and liver transplants. Orthoclone OKT3 targets the CD3 molecule that is present exclusively on T cells and depletes them from the circulation, which makes the drug an effective immunomodulator. Unfortunately, its use was frequently accompanied by a severe cytokine release syndrome stemming from its strong initial activation of T cells, which led to its eventual withdrawal from the clinic. Although Orthoclone OKT3 could not fully realize its clinical potential, it did show CD3 to be a rational drug target for treating autoimmune diseases such as T1D.

The 1990s saw key technological advances in the development of modified CD3-specific mAbs that did not stimulate T cells as potently, which meant that they circumvented the harmful cytokine release syndrome that had dogged earlier approaches. Initial pre-clinical studies in mouse models of T1D were encouraging. For example, a 1994 paper by Lucienne Chatenoud, Jean-Francois Bach and colleagues showed that short-term anti-CD3 treatment in recently diagnosed non-obese diabetic mice induced durable remission of disease. Importantly, treated mice seemed to have an otherwise intact immune response, suggesting that only the diabetogenic T cells were targeted.

This new class of CD3-specific mAbs only weakly and transiently depleted their target cells so the mechanisms by which they modulated diabetogenic T cells were rather elusive; however, some of the first clues were offered by a 1997 paper from the Jeffrey Bluestone lab. Binding of CD3 by modified mAbs seemed to trigger only a partial agonist signal, which led to a lasting state of T cell non-responsiveness. Numerous subsequent studies have expanded on these mechanistic findings and among other things have shown that the CD3-specific mAbs primarily exert their effects on activated cells. This would explain the relative selectivity of anti-CD3 therapy for diabetogenic T cells while leaving the rest of the immune response intact. Later evidence also suggested that the partial agonist signalling of CD3-specific mAbs enhanced the function and/or proliferation of regulatory T cells, which then exerted a dominant suppression on autoreactive cells.

These encouraging animal studies eventually led to the first clinical trial of a partial agonist CD3-specific mAb in T1D. In 2002, Kevan Herold et al. published the results of a small clinical trial of patients recently diagnosed with T1D who received an escalating dose over 2 weeks of the partial agonist CD3-specific mAb teplizumab. There was no evidence of the severe systemic inflammation seen with Orthoclone OKT3, with the most common side effects generally being a transient lymphopenia, rash and mild fever. Most importantly, the patients receiving the mAb showed slower deterioration of β-cell function over 12 months. Another intriguing aspect of these findings was that there seemed to be a durable effect on the immune response after a single short course of mAb at a relatively low dose, which suggested that it might be possible to 'rewire' the immune response into a tolerant state. This initial clinical trial was followed in 2005 by a larger phase II study from Lucienne Chatenoud and colleagues using another distinct CD3-specific mAb, otelixizumab, in new-onset T1D. This study also showed that a brief course of mAb improved preservation of β-cell function, this time over an 18-month follow-up. CD3-specific mAbs continue to be actively studied in human T1D. For example, Kevan Herold and colleagues completed the first successful prevention trial in 2019. This showed that relatives of patients with T1D at high risk for development of clinical disease who received teplizumab had slower progression to disease than the placebo group.

After some initial false starts, it now seems that anti-CD3 therapy might be starting to realize its full therapeutic potential. Future trials of CD3-specific mAbs, including those in combination with other immunomodulating drugs, could have great potential in protecting β-cell function and restoring immunological tolerance in T1D.

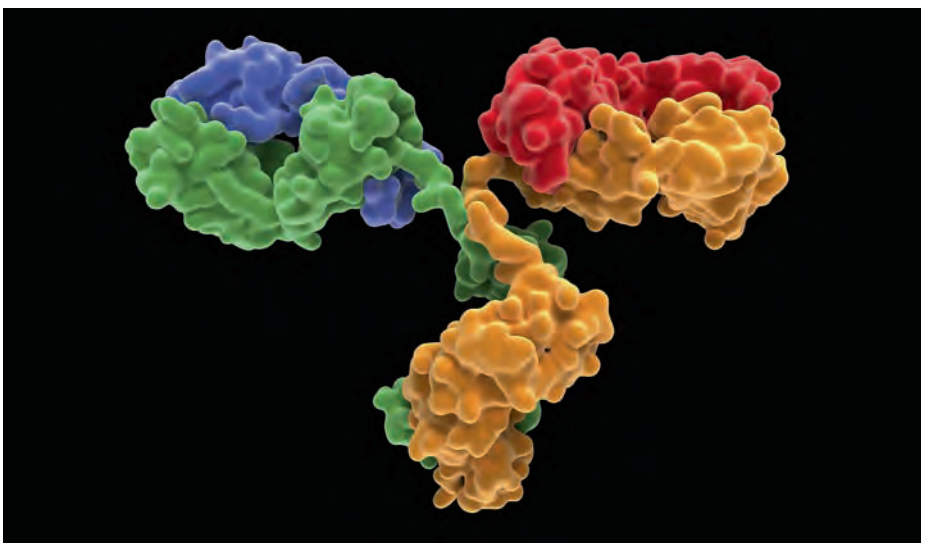
Zoltan Fehervari Senior Editor, *Nature Immunology*

Milestone study

Herold, K. C. et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N. Engl. J. Med.* **346**, 1692–1698 (2002)

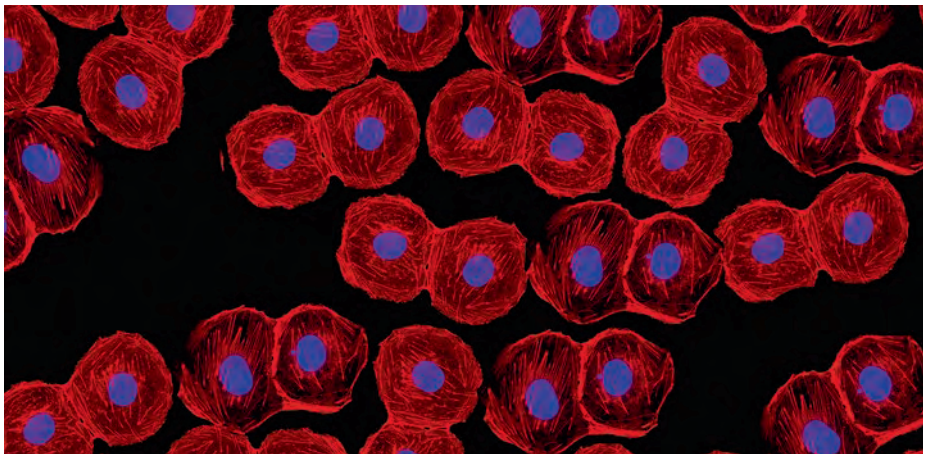
Further reading

Please visit the online article for a full list of further reading.



CREDIT: SCIENCE PHOTO LIBRARY / ALAMY STOCK PHOTO

Milestone 18



CREDIT: EDGLORIS MARYS / ALAMY STOCK PHOTO

Towards a stem cell therapy for diabetes

The isolation of the first human embryonic stem cell (hESC) lines in 1998 opened the possibility of stem cell therapies for a variety of conditions. Type 1 diabetes (T1D) is particularly suited to this approach, as transplantation of the insulin-producing pancreatic β-cells could provide long-lasting therapy or even a cure. By the early 2000s, the stem cell differentiation field was benefitting from embryo studies in model organisms that had identified the role of various signalling pathways in controlling pancreatic cell lineage specification.

In 2005, D'Amour et al. reported the production of hESC differentiation cultures containing >80% definitive endoderm cells: the embryonic progenitors of pancreatic cells. Differentiation occurred in 2D culture, using a low serum medium containing activin A, a protein that is essential for endoderm specification in the embryo.

Following this advance, in 2006, D'Amour et al. published a key paper reporting the generation of endocrine pancreatic cells, capable of secreting insulin, glucagon, somatostastin, pancreatic polypeptide and ghrelin from differentiating hESCs. Their differentiation protocol included five stages, during which hESCs went through a series of intermediate cellular states: definitive endoderm, primitive gut tube, posterior foregut, pancreatic endoderm and endocrine precursors and hormone-expressing endocrine cells. Specific growth factors were supplied at each step, in an effort to mimic embryonic pancreas development. Indeed, characterization of mRNA and proteins in the different cell states showed that they resembled the equivalent embryonic endodermal progenitors.

"These cells expressed key β-cell transcription factors"

At stage five of this study, staining by zinc-chelating agent dithizone was used to identify the pancreatic endocrine cells. Their insulin content was similar to that of primary adult human islets and the de novo synthesis of insulin was confirmed by mRNA, C-peptide and pro-insulin protein measurement. However, compared with adult islets, the C-peptide content of hESC-derived cells was inferior, indicating a reduced efficiency in processing pro-insulin. C-peptide secretion was responsive to multiple secretory stimuli, albeit only minimally responsive to glucose.

Subsequently, in 2008, the same group (Kroon et al.) succeeded in generating glucose-responsive endocrine cells in vivo, following transplantation of hESC-derived stage-four pancreatic endoderm in mice. These cells expressed key β-cell transcription factors, showed efficient processing of pro-insulin and contained mature secretory granules. Importantly, the hESC-derived endocrine cells protected the mice against hyperglycaemia.

In 2014, Rezania et al. described a seven-stage hESC differentiation protocol that generated glucose-responsive insulin-producing β-cells that reversed diabetes in mice upon transplantation. In the same year, Pagliuca et al. reported the in vitro large-scale generation of glucose-responsive, insulin-expressing β-cells derived from human pluripotent stem cells (hPSCs), by sequentially modulating key signalling pathways in a 3D culture system. These hPSC-derived β-cells were very similar to human β-cells in terms of gene expression and ultrastructure and also ameliorated hyperglycaemia in diabetic mice upon transplantation.

In 2015, Russ et al. reported a simplified suspension-based, directed differentiation protocol to generate glucose-responsive insulin-producing human β-cells from hPSCs in vitro and in vivo. The resulting cells reduced blood levels of glucose in diabetic mice in a matter of weeks, as opposed to months in previous studies.

In 2019, Nair et al. reported the generation in vitro of mature β-cells from hESCs, by allowing the immature β-like cells to form islet-like clusters, thus recapitulating endocrine cell clustering in vivo. Mature β-cells showed enhanced metabolic and structural maturation of mitochondria and hyperglycaemia was reduced within days of transplantation in diabetic mice.

In 2020, Hogrebe et al. discovered that the state of actin polymerization influences pancreatic differentiation in vitro. Thus, timed actin depolymerization during differentiation led to the generation of β-cells from hPSCs that rapidly reversed diabetes in mice and maintained normoglycaemia for 9 months.

Fifteen years after the first insulin-secreting cells were generated by differentiating hESCs in vitro, we still do not have a β-cell replacement therapy for T1D. However, thanks to the studies mentioned here, and many others, we are much closer to this goal. Apart from further optimizing the efficiency and scalability of mature β-cell production, important issues, such as shielding the transplanted stem-cell-derived β-cells from immune rejection and ensuring their purity, need to be resolved before a stem-cell-based therapy becomes a reality.

Anna Melidoni Senior Editor,
BMC Endocrine Disorders

Milestone study

D'Amour, K. A. et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392–1401 (2006)

Further reading

Please visit the online article for a full list of further reading.

Milestone 19

Islet inflammation in T2D

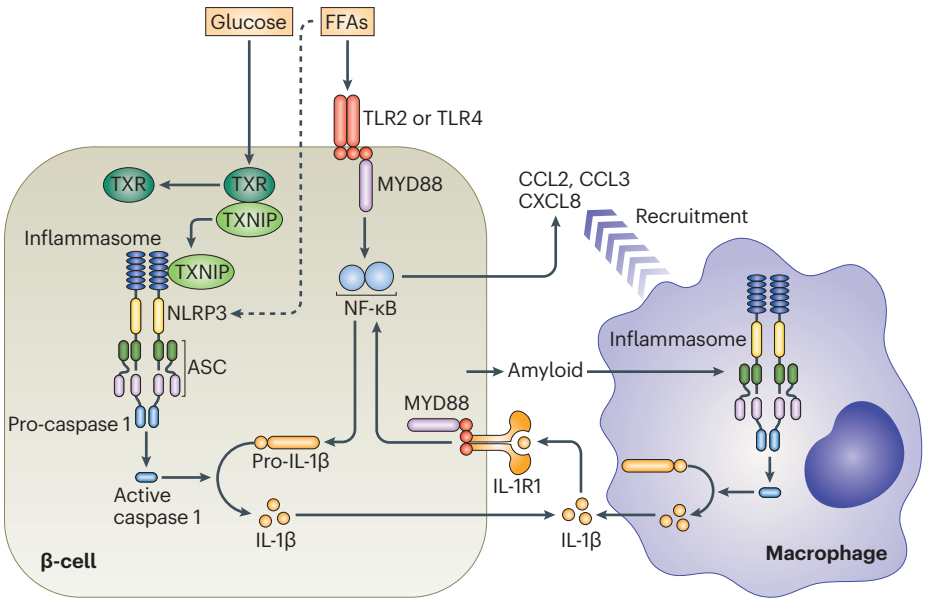
Type 1 and type 2 diabetes (T1D and T2D) converge in the later stages of disease on dysfunction of pancreatic islet β-cells and a progressive loss of insulin production. Seminal studies in the 1980s had shown the involvement of autoantibodies (Milestone 7) and islet inflammation (Milestone 8) in β-cell death in T1D. Furthermore, obesity-induced chronic inflammation was known to be a risk factor for T2D (Milestone 12). However, the general assumption was that overnutrition induces β-cell apoptosis directly and there was not thought to be a unifying mechanism of cell death in T1D and T2D.

Observations of islet macrophages in T2D were considered to be a consequence rather than a cause of β-cell death, and studies suggesting that glucose-induced IL-1β production in islets leads to β-cell apoptosis in T2D (Maedler et al., 2002) had not been replicated by others in vivo. In 2007, however, a study by Marc Donath and colleagues reported that islet-derived inflammatory factors induced by a T2D milieu of excess circulating nutrients led to macrophage infiltration of islets, thus showing that insulitis has a role in T2D as well as T1D.

Ehses et al. observed increased numbers of islet-associated CD68$^+$ macrophages in autopsy and resection samples from patients with T2D compared with nondiabetic controls. In C57BL/6J mice fed a high-fat diet, the number of CD11b$^+$ myeloid cells in islets was doubled after 8 weeks compared with standard diet controls. No apoptotic cells were detected in islets at 8 weeks, suggesting that islet inflammation precedes β-cell apoptosis. Macrophage infiltration of islets was also reported for the GK rat and *db*/*db* mouse models of T2D.

"islet-derived inflammatory factors induced by a T2D milieu of excess circulating nutrients led to macrophage infiltration of islets"

After treatment with glucose and palmitate in vitro, mouse and human islets and islet cell lines secreted increased levels of the inflammatory factors IL-6, CXCL8, G-CSF, KC (mice; the orthologue of human CXCL1) and CCL3 (human). Chemically induced apoptosis was used to rule out an indirect effect of nutrient-induced β-cell death on cytokine release. Furthermore, KC and G-CSF themselves were shown to have minimal effects on β-cell apoptosis and glucose-stimulated insulin secretion. Thus, excess nutrients were shown to directly induce the release of inflammatory factors by islet cells, which in turn had indirect effects on islet function rather than directly mediating β-cell death.



Given that CXCL8 was known to be a chemotactic factor for myeloid cells, the authors hypothesized that the factors released by pancreatic islets exposed to a T2D milieu are responsible for the macrophage infiltration of islets. Indeed, conditioned medium from human islets exposed to glucose and palmitate increased the migration of neutrophils and monocytes in vitro, which could be abrogated by neutralization of CXCL8.

The study concluded that nutrient-induced T2D involves an immune-mediated islet inflammatory process. Intriguingly, the discussion section of the paper mentioned the unpublished observation that the release of KC and G-CSF by islets could be blunted by treatment with IL-1Ra, the endogenous receptor antagonist of IL-1β. This involvement of IL-1β in type 2 insulitis was in line with the earlier results of Maedler et al. and was clarified by later studies showing that T2D is associated with inflammasome activation by thioredoxin-interacting protein (TXNIP) (Zhou et al., 2010) or by islet amyloid polypeptide (Masters et al., 2010). The findings of Ehses et al. also suggested that inflammatory mediators such as IL-1β could be a potential therapeutic target in T2D. A proof-of-concept trial by Larsen et al. (2007) in 70 patients with T2D, 34 of whom received subcutaneous IL-1Ra for 13 weeks, showed improved glycaemic control correlating with increased β-cell secretory function. The recent Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) confirmed the ability of targeting IL-1β to improve T2D in the short term, although it did not reduce the incidence of new-onset T2D in trial participants with pre-diabetes. A recent meta-analysis of 2,921 patients with T2D (Kataria et al., 2019) concluded that IL-1 blockade can significantly improve glycaemic control. Thus, although it is now well accepted that T2D is an inflammatory disease, the jury is still out on to what extent and at what stage of disease anti-inflammatory therapies might be beneficial.

Kirsty Minton Senior Editor,
Nature Reviews Immunology

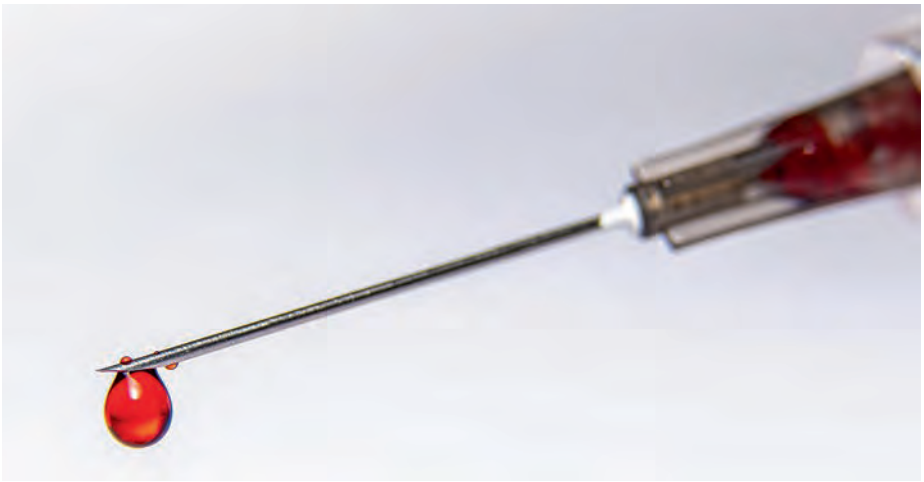
Milestone study

Ehses, J. A. et al. Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* **56**, 2356–2370 (2007)

Further reading

Please visit the online article for a full list of further reading.

Milestone 20



T_{reg} cells to the rescue: the first clinical studies

Type 1 diabetes (T1D) is an autoimmune disease in which genetically susceptible individuals, influenced by environmental and stochastic events, eventually develop pathogenic T cells that destroy the β-cells of the islets of Langerhans in the pancreas. Several immunomodulatory therapies have shown promise, including CD3-targeted antibodies (Milestone 17), LFA3Ig, thymoglobulin and bone marrow transplantation. Although none of these approaches has induced permanent immune tolerance, it emerged that their efficacy is largely due to a relative increase in regulatory T (T_{reg}) cells versus effector T cells — leading to efforts to use T_{reg} cells as 'living drugs'.

The importance of T_{reg} cells in T1D pathology had previously been established in NOD mice (Milestone 4), where the depletion of $CD4^+CD25^+$ T_{reg} cells greatly accelerates the development of T1D. Similarly, the removal of crucial co-stimulatory or proliferative signals that are necessary for T_{reg} cells, such as IL-2 or CD28, exacerbates T1D in this model. As early as 2004, it was shown that T_{reg} cells amplified ex vivo and adoptively transferred to NOD mice can tame autoreactive T cells and halt disease development.

The first studies in humans demonstrating that T_{reg} cells are impaired in T1D date back to 2005, when Tree and colleagues showed that $CD4^+CD25^+$ T cells from patients with T1D have a reduced ability to suppress T cell proliferation in vitro. Later studies showed that T_{reg} cells from patients with T1D have impaired signalling through their IL-2 receptor. Together with the mouse studies, these findings made therapy with ex vivo-expanded autologous T_{reg} cells an attractive proposition.

> **"administration of T_{reg} cells was safe and tolerable and led to a decrease in requirement for exogenous insulin"**

A big challenge was that T_{reg} cells in humans are relatively rare and the markers and methods for their isolation were yet to be worked out. In humans, isolating T_{reg} cells on the basis of CD4 and CD25 expression risks contamination with potentially autoreactive effector cells. Progress in the field was facilitated by the discovery that human T_{reg} cells can be isolated using a combination of antibodies targeted at CD4, CD25 and CD127 and that they can be expanded ex vivo on a clinical scale using beads coated with antibodies for CD3 and CD28 in the presence of recombinant IL-2.

The first clinical trial results of autologous polyclonal ex vivo-expanded T_{reg} cells in patients with T1D were published in 2012 and 2014 by Marek-Trzonkowska et al. A small trial in children with recent-onset T1D found that administration of T_{reg} cells was safe and tolerable and led to a decrease in requirement for exogenous insulin. After 1 year, the authors reported that repeated treatment was safe and that it prolonged the survival of β-cells. This follow-up also demonstrated statistically lower insulin requirements and higher C-peptide levels (which are indicative of higher insulin levels) than a matched control group.

A second trial (Bluestone et al., 2015) enrolled 14 adult patients with recent-onset T1D who were infused with T_{reg} cells. Several patients had stable C-peptide levels and insulin use for up to 2 years after therapy, although the study was not powered to determine efficacy. Infused cells were labelled with deuterium, which allowed the investigators to follow them in the circulation. They showed that their levels in the blood peaked in the first 2 weeks after injection, followed by a loss of 75% of the peak level in the circulation (owing to either cell death or extravasation to inflamed tissues) during the first 3 months, before numbers stabilized for at least a year. Phenotypic and functional data suggested that the ex vivo expansion protocol increased not only the number of T_{reg} cells but also their suppressive capacity. There was no evidence of transdifferentiation into effector cells in vivo.

Further steps to optimize adoptive therapy with T_{reg} cells may include the addition of T_{reg} cell-promoting therapies, such as low-dose IL-2, and/or strategies to deplete effector T cells, for example with LFA3Ig. Also, efforts are under way to develop islet-specific T_{reg} cells using genetic engineering, either with chimeric antigen receptors or transgenic T cell receptors. The ultimate goal is an off-the-shelf product for adoptive therapy that would forego the need to isolate and individually expand patient-specific T_{reg} cells.

Alexandra Flemming Chief Editor, *Nature Reviews Immunology*

Milestone studies

Marek-Trzonkowska, N. et al. Administration of $CD4^+CD25^{high}CD127^-$ regulatory T cells preserves β-cell function in type 1 diabetes in children. *Diabetes Care* **35**, 1817–1820 (2012) | Marek-Trzonkowska, N. et al. Therapy of type 1 diabetes with $CD4^+CD25^{high}CD127^-$ regulatory T cells prolongs survival of pancreatic islets — results of one year follow-up. *Clin. Immunol.* **153**, 23–30 (2014) | Bluestone, J. A. et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl Med.* **7**, 315ra189 (2015)

Further reading

Please visit the online article for a full list of further reading.

Milestone 21

Technology will set you free

Achieving and maintaining normoglycaemia can delay and prevent development of complications associated with type 1 diabetes. This is a significant burden for patients and requires constant monitoring of their blood glucose, and diet and activity levels, as well as precise calculation of the insulin dosages required to lower glucose levels safely to avoid life-threatening hypoglycaemic events.

As early as the fifth century BC, Ayurveda practitioners in India described what we now know as type 1 diabetes using the term Madumeha, which translates as "sweet urine". However, it was not until 1908 that Stanley Benedict developed the first glucose urine test. This test was used for around 50 years until Helen Free introduced Clinistix, which abrogated the need for cumbersome mixing of reagents and heating, and allowed for a simple dipstick measurement of glucose in urine. The Ames Reflectance Meter in the 1970s enabled the measurement of glucose in blood, paving the way for contemporary blood glucose meters. These meters were introduced in the 1980s and have progressively improved in accuracy and are now available as small, handheld devices that require only a very small drop of blood.

"emergence of smart phones and new technologies over the past two decades has culminated in the development of wearable, closed-loop artificial pancreas systems"

Continuous glucose monitors (CGMs), introduced in the late 1990s, rely on a subcutaneous sensor connected to a transmitter to provide a glucose reading every few minutes and are linked to an alarm system to alert the user when glucose levels become dangerously high or low. Advances in insulin delivery systems, including smart pens and pumps, have also accelerated with increased availability of technology. Rapid-acting insulin analogues have also been developed to increase the efficacy of delivery and stability of insulin for use in these devices (Milestone 6).

The emergence of smart phones and new technologies over the past two decades has culminated in the development of wearable, closed-loop artificial pancreas systems designed to monitor glucose and deliver hormones as required, in near real time and with reduced patient input.

The first iterations of artificial pancreas systems, termed hybrid closed-loop systems, pair a CGM device to an insulin pump and rely on an algorithm to calculate required insulin doses. Unlike fully closed-loop systems, hybrid systems perform best if the user informs the algorithm of meals and carbohydrate intake.

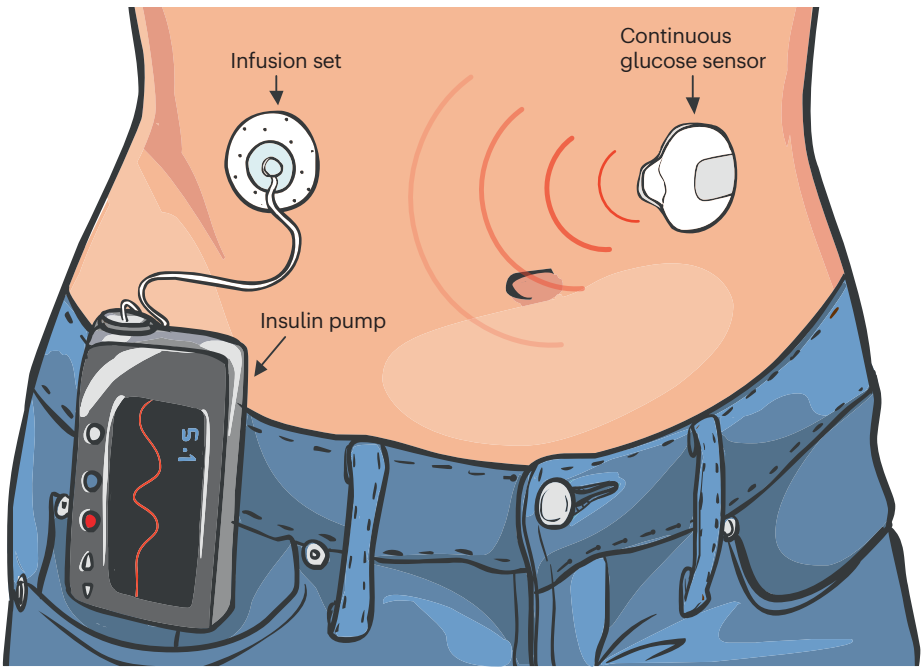
In 2014, Russell et al. reported the first outcomes of randomized crossover trials in an outpatient setting using a closed-loop bihormonal delivery system compared with insulin pump therapy. The bihormonal device delivers either insulin or glucagon as needed with only meal announcements required by the user after calibration. Trials in adolescents and adults over the 5-day intervention period demonstrated consistent reductions in mean plasma glucose levels and time in hypoglycaemia compared with the control period.

The following year, Thabit et al. showed increased time of blood levels of glucose within the target range in a randomized crossover study using an insulin-only artificial pancreas, in adults (day and night) and in children and adolescents (overnight), compared with sensor-augmented insulin pump therapy at 12 weeks.

As these devices continue to develop, larger, longer trials under truly free-living conditions have provided assurance of the safety and feasibility of closed-loop systems. Insulin-only devices have also been successfully tested in specific populations, such as pregnant women with type 1 diabetes and hospitalized patients with type 2 diabetes receiving noncritical care, further demonstrating the broad application of these devices.

At present, only a handful of hybrid closed-loop systems have been approved for marketing in Europe and the USA. Large pivotal trials are ongoing with the hope that additional closed-loop systems will soon be available to patients, freeing them of the high daily burden of managing their diabetes.

Jennifer Sargent Senior Editor,
Nature Medicine



CREDIT: HAKAN ÇORBACI / ALAMY STOCK VECTOR

Milestone studies

Russell, S. J. et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N. Engl. J. Med.* **371**, 313–325 (2014) | Thabit, H. et al. Home use of an artificial beta cell in type 1 diabetes. *N. Engl. J. Med.* **373**, 2129–2140 (2015)

Further reading

Please visit the online article for a full list of further reading.

Milestone 22

Incretin drugs for glycaemic control

Over the past 15 years, drugs targeting the incretin hormones (Milestone 9), including glucagon-like peptide 1 (GLP1), have emerged as effective anti-hyperglycaemic agents. GLP1 is produced in the intestine and the brain in response to various stimuli, including food intake and bacterial metabolites, as well as immune and neuroendocrine mediators. The glucose-dependent postprandial release of GLP1 induces insulin secretion by pancreatic β-cells and suppresses glucagon secretion from pancreatic α-cells, which regulates glycaemia. Moreover, GLP1 modulates appetite by slowing gastric emptying and inducing satiety. However, these effects are short-lived because circulating GLP1 is quickly degraded by dipeptidyl peptidase 4 (DPP4) and has a half-life ≤5 minutes in circulation. Given the physiological role of GLP1 in glycaemic control, GLP1 receptor agonists (GLP1RAs) were developed as glucose-lowering agents to treat patients with type 2 diabetes (T2D). DPP4 inhibitors that prolong the half-life of endogenous GLP1 represent another approach to therapeutically target the incretin system.

GLP1RAs are peptide drugs that were developed as analogues of either human GLP1 or exendin 4, which is a salivary protein from the Gila monster lizard with ~50% homology to human GLP1. Human GLP1-based agents include albiglutide, liraglutide, dulaglutide and semaglutide, whereas exenatide and lixisenatide are based on exendin 4. These drugs are administered by subcutaneous injection, although an oral formulation of semaglutide is also available. One of the main differences between the two types of GLP1RA is their half-lives, which are shorter for exendin-4-based compounds. Various strategies have been developed to prolong the half-life of GLP1RAs, including binding the peptides to plasma albumin or conjugating them to the crystallizable fragment of IgG to delay drug clearance in the kidney. A long-acting release formulation of exenatide was also developed by coupling the peptide to microspheres, which slows drug release; this formulation can be administered once a week instead of twice daily.

"GLP1RAs can also reduce the risk of atherosclerotic cardiovascular disease … and kidney outcomes"

Early clinical trials showed that both DPP4 inhibitors and GLP1RAs were well tolerated and that, compared with placebo, they could reduce HbA_{1c} in patients with T2D being treated with metformin. Subsequent studies have indicated that GLP1RAs are more effective than DPP4 inhibitors in lowering HbA_{1c}. In general, short-acting GLP1RAs strongly inhibit gastric emptying and delay glucose absorption, but this effect is not sustained as GLP1R activity levels decline after drug administration. By contrast, human GLP1-based drugs maintain GLP1R activation over longer periods of time, and have been shown to lower HbA_{1c} and fasting plasma glucose levels to a greater extent than exendin-4-based drugs, with the exception of once-weekly exenatide, which is also a long-acting drug.

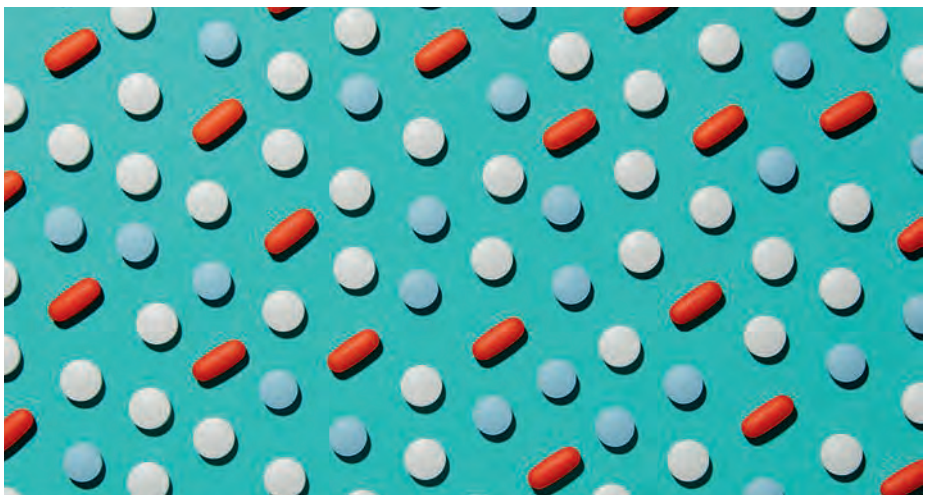
A systematic review and comparison analysis published in 2016 concluded that GLP1RAs are generally well tolerated and, although they can potentiate hypoglycaemia, the risk of this adverse event is reduced compared with that associated with other glucose-lowering agents such as short-acting insulin. Gastrointestinal symptoms are also common, although they are often transient; long-acting compounds seem to have the lowest risk of nausea, diarrhoea and vomiting.

Data collected in several clinical trials indicate that, compared with placebo, GLP1RAs can also reduce the risk of atherosclerotic cardiovascular disease (including cardiovascular and all-cause mortality) and kidney outcomes in patients with T2D. However, the benefits of incretin drugs extend beyond the treatment of T2D. Obesity, for example, can reduce the release of GLP1 and blunt its glycaemic control, although the underlying mechanisms remain unclear. In patients with obesity, a combination of GLP1RAs and lifestyle therapy improves weight loss compared with placebo.

GLP1RAs also have therapeutic benefits in other conditions associated with obesity and insulin resistance. In patients with nonalcoholic steatohepatitis, GLP1RAs improved weight loss and the liver phenotype, potentially through metabolic and anti-inflammatory effects.

The demonstrated ability of GLP1RAs to lower HbA_{1c} and fasting plasma levels of glucose confirms that they are promising anti-hyperglycaemic agents, and their additional metabolic effects suggest that their benefits might extend well beyond the treatment of patients with T2D.

Monica Wang Senior Editor,
Nature Reviews Nephrology



CREDIT: SHANA NOVAK

Milestone study

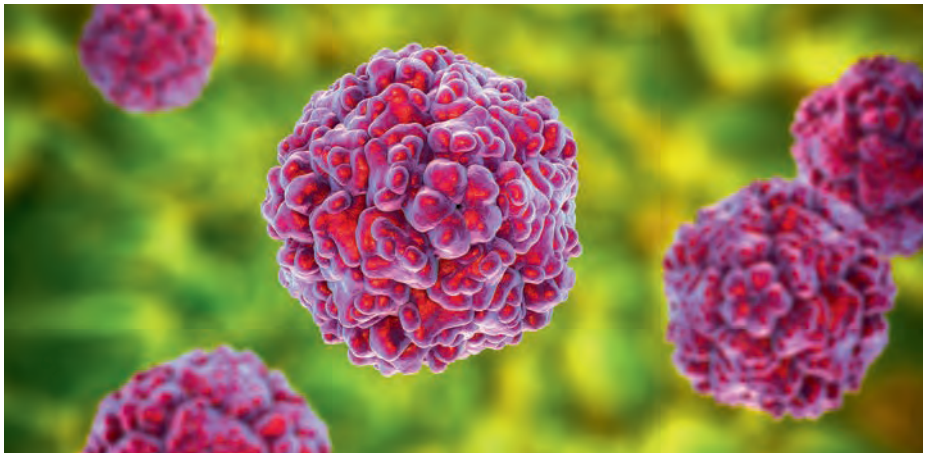
Htike, Z. Z. et al. Efficacy and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: a systematic review and mixed-treatment comparison analysis. *Diabetes Obes. Metab.* **19**, 524–536 (2017)

Further reading

Please visit the online article for a full list of further reading.

Milestone 23

An infectious cause for T1D?



CREDIT: KATERYNA KON / SCIENCE PHOTO LIBRARY

The development of type 1 diabetes (T1D) depends on a complex interplay between genetic factors (Milestone 3) and environmental factors that ultimately result in the autoimmune destruction of pancreatic β-cells (Milestones 7 and 8). Viruses have long been prime suspects as environmental triggers of T1D (particularly in the young), with the first investigators drawing on early epidemiological studies showing that disease incidence has a seasonal pattern and increases after enterovirus epidemics, and subsequent work showing that certain enteroviruses can infect β-cells and have a causative role in animal studies. It was the isolation of the enterovirus coxsackie virus B4 in 1979 from a 10-year-old boy who died as a result of T1D following a flu-like illness that drove the search for a viral aetiology of T1D (Yoon et al., 1979). Importantly, the isolated virus could cause hyperglycaemia following inoculation of mice. Forty years later, with the arrival of improved, more sensitive, sequencing technologies, Vehik et al. provided tantalizing evidence that prolonged enteroviral infection is a precursor to the development of autoimmune diabetes in young children.

Over the years, various mechanisms have been evoked to explain the link between viruses and autoimmune disease, including molecular mimicry and indirect immune activation. As evidence to support molecular mimicry, Atkinson et al. (1994) described a major T cell epitope of the β-cell enzyme glutamate decarboxylase recognized by individuals with T1D that has significant sequence similarity to a coxsackie viral peptide. By contrast, Horwitz et al. (1998) provided evidence suggesting instead that diabetes induced by coxsackie virus infection results from local inflammation, tissue damage and release of islet antigens that stimulate autoreactive T cells. A later study did not observe destructive islet inflammation following β-cell infection and instead linked infection to β-cell dysfunction and natural killer cell-mediated insulitis (Dotta et al., 2007). The exact mechanisms of potential viral causality or contribution to T1D are still a matter of debate.

"prolonged enteroviral infection is a precursor to the development of autoimmune diabetes in young children"

The report by Vehik et al. was one of a series of papers stemming from The Environmental Determinants of Diabetes in the Young (TEDDY) study — the largest multicentre prospective study of young children with a genetic susceptibility to T1D that aims to identify the environmental causes of T1D. Vehik et al. performed meta-genomic sequencing of monthly stool samples collected from newborn babies until detection of islet autoimmunity or T1D along with controls. They identified enterovirus B as the only virus in the human virome with a significant association with islet autoimmunity. Although the frequencies of enterovirus B infection did not differ between cases and controls, children with prolonged shedding of enterovirus B were more likely to develop islet autoimmunity. By contrast, human mastadenovirus C infection early in life was less frequent in children who developed islet autoimmunity than in those who did not, suggesting a protective effect. Finally, identification of an association between polymorphisms in the coxsackie virus and adenovirus receptor gene and susceptibility to T1D led the authors to propose that competition for receptor binding between adenovirus and coxsackie virus confers the protective effect of adenovirus. It is important to note that this study was limited to very young children, who account for only 10–20% of total new cases of T1D worldwide, and evidence that enterovirus B causes direct β-cell destruction is still lacking.

The TEDDY study also allowed the first extensive characterization of the gut microbiome in relation to T1D. A report by Vatanen et al. (2018) showed that the microbiomes of control children were enriched for genes related to fermentation and biosynthesis of short-chain fatty acids (SCFAs) compared with children with T1D, suggesting a protective effect of SCFAs. Moreover, the TEDDY cohort has provided insight into early colonization of the gut microbiome and the impact of breastfeeding, birth mode and other factors, as described in a companion paper (Stewart et al., 2018). Although only subtle compositional differences were observed between T1D cases and controls and cause or effect could not be discriminated, higher levels of *Streptococcus* sp. and *Lactococcus* sp. and lower levels of *Akkermansia* sp. in infants at risk of T1D provide further hints for the involvement of an altered microbiome in the development of T1D. Placing viral infection at the forefront of T1D aetiology has spurred current approaches that target viral infection to prevent islet autoimmunity.

Lucy Bird Senior Editor,
Nature Reviews Immunology

Milestone study
Vehik, K. et al. Prospective virome analyses in young children at increased genetic risk for type 1 diabetes. *Nat. Med.* **25**, 1865–1872 (2019)

Further reading
Please visit the online article for a full list of further reading.

Milestone 24

CREDIT: IT TIPOL NAMPOCHAI / EYEEM

Getting to the heart of the matter

In 2008, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) issued draft guidance to the pharmaceutical industry to address the need for more thorough evaluation of cardiovascular safety in diabetes drug development. They did so as several trials suggested intensive reduction of glucose may not necessarily lessen cardiovascular events and may even cause harm. This was worrying because diabetes is associated with increased risk of cardiovascular events. The FDA and EMA recommended that new trials for anti-diabetes drugs be designed to demonstrate, at minimum, no increased risk of cardiovascular disease in individuals with type 2 diabetes (T2D).

HbA_{1c} levels, the gold standard for assessing glycaemic control, remained the recommended primary outcome for new trials. However, sponsors were recommended to involve an independent cardiovascular endpoints committee in new trials, as well as designing phase II and phase III studies in such a way that cardiovascular endpoints could be readily compared in meta-analyses.

Clinical trials of dipeptidyl peptidase 4 inhibitors were among the first to measure cardiovascular outcomes. These studies were largely successful in demonstrating non-inferiority for risk of cardiovascular events compared with placebo, but there was no evidence of benefit. In 2015, the EMPA-REG study, assessing the effects of empagliflozin (a sodium–glucose co-transporter 2 inhibitor (SGLT2i) in people with T2D, over 60 years of age and with known cardiovascular disease, took the world by storm. The study demonstrated not only no increased risk of cardiovascular disease but that empagliflozin treatment resulted in a significant beneficial reduction in a composite cardiovascular outcome (fatal and non-fatal myocardial infarction and stroke), plus substantial reductions in heart failure and chronic kidney disease, over a median time of 3.1 years.

"The unexpected yet overwhelming clinical benefits conferred by new-generation glucose-lowering therapies have brought about a veritable revolution in T2D management"

In 2019, Zelniker et al. reported findings from a meta-analysis of three major trials of SGLT2is that included 34,322 patients, 60% of whom had established atherosclerotic disease. They found that treatment with SGLT2is across the trials reduced incidence of major adverse cardiovascular events (MACE) by 11%, suggesting a moderate class-effect cardiovascular benefit for SGLT2is. However, this benefit was only seen in patients with existing atherosclerotic disease. The analysis also showed substantial reductions in cardiovascular death and hospitalizations for heart failure, regardless of whether patients had a history of atherosclerotic disease or heart failure. Other secondary outcomes, including chronic kidney disease (CKD), were also found to be positively affected by treatment with SGLT2is. Subsequent trials of SGLT2is in patients with heart failure and CKD, with and without T2D, also showed clinical benefit.

Another meta-analysis from 2019 reported cardiovascular outcomes of seven trials testing glucagon-like peptide 1 receptor agonists, with data from 56,004 participants. Kristensen et al. reported a 12% overall reduction in MACE, as well as reductions in all-cause mortality, hospitalizations due to heart failure and a composite kidney disease outcome.

The question of whether the beneficial effects on cardiovascular and renal disease observed are attributable to improved glycaemic control or whether they are mediated by independent mechanisms are subject to debate and ongoing research. However, the mixed results for cardiovascular outcomes in individual trials, as well as heart failure and kidney benefits extending to patients without diabetes, suggest that these effects cannot be due to improved glycaemic control alone.

The unexpected yet overwhelming clinical benefits conferred by new-generation glucose-lowering therapies have brought about a veritable revolution in T2D management, providing patients and physicians with more options to reduce the burden of chronic complications associated with the disease.

Jennifer Sargent Senior Editor, *Nature Medicine*

Milestone studies

Zelniker, T. A. et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* **393**, 31–39 (2019) | Kristensen, S. L. et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol.* **10**, 776–785 (2019)

Further reading

Please visit the online article for a full list of further reading.

Diabetes: Following the science in the search for a cure

AstraZeneca

Diabetes is one of the priority cardiovascular, renal and metabolic diseases for which AstraZeneca is developing novel therapies. The global number of people living with diabetes is expected to rise to 700 million by 2045 (ref. 1). AstraZeneca's ambition is not just to reduce the raised blood glucose typical of the disease, it is to tackle the underlying pathophysiological drivers in order to instigate diabetes remission, prevent diabetic complications and deaths and, ultimately, to deliver a cure.

"AstraZeneca recognises that diabetes is more than a disease of the pancreas, and our research is focused on the underlying mechanisms that link diabetes to comorbidities, especially the main causes of death from the disease, including myocardial infarction and stroke, as well as heart and kidney failure," says David Baker, Head of Metabolism Bioscience, Biopharmaceuticals R&D.

INDUCE REMISSION

New approaches for the treatment of type 2 diabetes aim to induce remission as soon as possible after diagnosis by achieving durable responses to novel medicines designed to target the causes of type 2 diabetes and its related complications.

Obesity and insulin resistance are two key drivers in the development of type 2 diabetes, and are also connected to the development of diseases of the heart and circulation, liver and kidneys.

In people with obesity, bariatric surgery can cause remission of type 2 diabetes, and therapeutic alternatives are being sought to achieve levels of weight loss comparable to surgery[2]. Insulin resistance (reduced cell response to the glucose-lowering pancreatic hormone, insulin) has been a target for anti-diabetic medicines for many years. However, growing understanding of its central role in the development of blood vessel, kidney and liver diseases, has made it a research priority for addressing a key gap in the therapeutic armoury available to doctors and patients.

PRECISION MEDICINE

There is no single gene for type 2 diabetes but identification of the genomic drivers of the multiple biological mechanisms that lead to diabetes holds considerable promise. It is expected that new genomic understanding, linked to clinical phenotypes, will help to differentiate sub-groups of patients according to their risk of developing diabetes and progressing to diabetic complications. This segmentation of type 2 diabetes could also indicate opportunities for targeted therapies to intervene early in the disease.

NOVEL THERAPEUTICS

Advancing our understanding of disease biology enables us to uncover potential novel approaches for future diabetes treatments. At AstraZeneca, our toolbox of drug modalities enables us to address almost any drug target in diabetes using a range of therapeutics from classical small drug molecules to novel agents such as oligonucleotide and mRNA therapies. Approaches are being developed that can repair or modify a cell's blueprint for making key proteins that can be applied to type 2 diabetes and its complications.

Antisense oligonucleotides are short, synthetic, chemically modified pieces of RNA that can be used to 'silence' genes and prevent detrimental proteins from being made. Antisense oligonucleotides have been designed for delivery specifically into the insulin-producing beta-cells of the pancreas whose dysfunction can lead to diabetes[2]. This has opened the door to antisense oligonucleotide therapy aimed at restoring beta-cell function in diabetes and potentially 'knocking out' other genes in a way that could support long-term remission or even cure (**Fig. 1**).

Figure 1. Pancreactic beta cells at different stages of regeneration.

Messenger RNA (mRNA) has also been investigated for its potential in treating the small blood vessel damage, which commonly occurs with diabetes and can lead to organ failure. Encouraging results were obtained with injections of modified mRNA encoding for vascular endothelial growth factor A (VEGF-A), a protein involved in blood vessel formation. Local expression of VEGF-A was accompanied by improved blood flow in the skin of men with type 2 diabetes[3].

Making insulin-resistant cells more sensitive to insulin is another goal of novel therapeutics for diabetes. In preclinical research, insulin sensitivity has been improved with gene therapy, antisense oligonucleotides and novel biologic approaches to decrease ectopic fat, particularly in the liver, which in turn alleviates insulin resistance and rejuvenates beta-cell function, leading to diabetes remission.

FROM REMISSION TO CURE

If we want to change the trajectory of rising diabetes cases, we need to use novel approaches that target the drivers of diseases.

At AstraZeneca, we are using our genomics expertise and state-of-the-art drug discovery technologies to build on strong science to develop potential medicines aimed at treating – and ultimately curing – diabetes.

AUTHORS

Christopher Rhodes[1], David Baker[2] & Regina Danielson Fritsche[3].

ADDRESSES

[1] One MedImmune Way, Gaithersburg, Maryland 20878, US.
[2] Granta Park, Great Abington, Cambridge, CB21 6GH, UK.
[3] Pepparedsleden 1, Mölndal, SE-431 83, Sweden.

REFERENCES

1. International Diabetes Federation. Diabetes Atlas (2019).
2. Ämmälä, C., *et al. Sci. Adv.* 4, eaat3386 (2018).
3. Gan, L.-M., *et al. Nature Communications* 10, 871 (2019).

Artificial intelligence: The next frontier in diabetes therapy

Medtronic

For decades, Medtronic has focused on improving glycemic outcomes and reducing the management burden of people with diabetes through innovative cutting-edge technology. Medtronic pioneered many of the 'firsts' that have transformed diabetes care since the 1990s (**Fig. 1**). The company's interest in the field started with its first-to-market wearable pump, which was followed by real-time continuous glucose monitoring. By the mid-2000s, the technology evolved to continuous glucose monitor-directed predictive insulin suspension to prevent hypoglycaemia, which demonstrated an 83% reduction in severe hypoglycaemic events[1]. In 2016, the introduction of the hybrid closed-loop algorithm (MiniMed™ 670G) automated basal insulin delivery every five minutes. The 670G study reduced HbA1c, increased time-in-range (70-180 mg/dL,) and reduced time below range (70 and 50 mg/dL) in a clinical study[2]. An advanced hybrid closed-loop system, the MiniMed™ 780G*, demonstrated even greater glycaemic control by providing a lower glucose target and auto-correction boluses delivered every five minutes, which mitigated post-prandial hyperglycaemia resulting from missed, late or incorrectly calculated meal boluses[3,4].

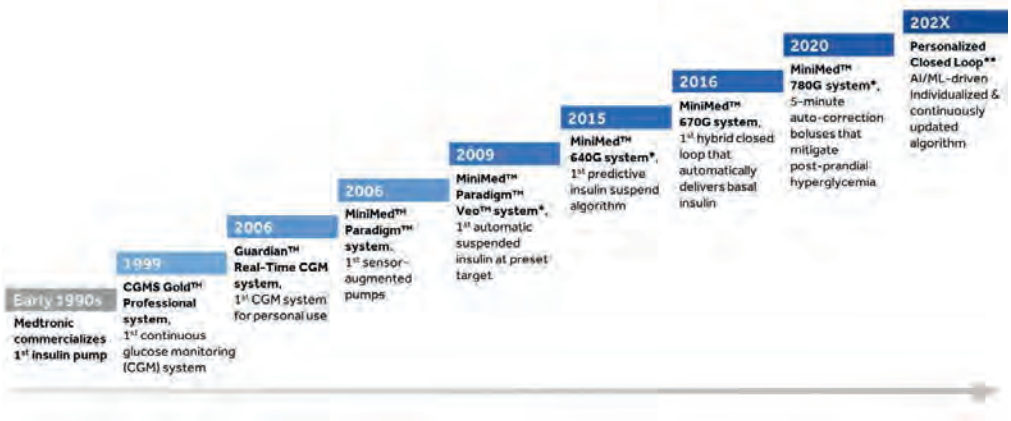


Figure 1. The evolution of glycemic control pioneered by Medtronic.

Figure 2. The "internet of things" applied to persons with diabetes includes using physiologic or activity sensors incorporated into smartphones or wearables that associate with a "digital twin" to provide important behavioral data that can be used to improve glycemia, personalize therapy, and minimize the burden of diabetes management.

Artificial intelligence (AI) will be critical to achieve the ultimate dual goals of near-normal glycaemia and reduced burden. Reducing mental burden in diabetes management cannot be over-emphasized. The multitude of decisions throughout the day can overwhelm patients, which can lead to abandonment of even the best technologies. Current systems continue to require multiple dosing decisions surrounding meals, exercise and other activities. Incorporating AI through a frequently updated 'digital twin' can circumvent daily challenges by promoting pharmaco-adherence and providing personalized management. **Fig. 2** describes how machine learning can use physiologic and/or activity sensors incorporated into smartphones or wearables to communicate with the digital twin and deliver personalized therapy that improves glycaemia while minimizing burden. For example, geolocation on a smartphone can predict the contents of an upcoming meal based on past behaviour and deliver the appropriate insulin dose before the meal. Also, hand gesture-sensing can determine when the meal starts or ends and the quantity of food consumed. Fitness monitors can identify the quantity, intensity and glycaemic response to exercise so insulin delivery adjusts accordingly.

Iteratively updating algorithms maximizes glycaemic control by personalizing therapy in a way that puts us on the cusp of developing a true artificial pancreas. Successfully incorporating AI into future products will fulfil Medtronic's mission of using biomedical engineering to restore health and reduce the management burden for people with diabetes.

*Approved for use only outside the United States
**In development. Not approved by the FDA for any use and not available for research or commercial use in the United States.

AUTHORS

Robert A. Vigersky, MD,
Toni L. Cordero, PhD,
Janice MacLeod, MS,
Andrew Rhinehart, MD,
Ohad Cohen, MD,
Yaron Hadad, PhD

ADDRESS

Medtronic Diabetes, 18000 Devonshire St. Northridge, CA 91325, US.

AUTHOR

Scott W. Lee, MD

ADDRESS

Loma Linda University, 11370 Anderson St # 3600, Loma Linda, CA 92354, US.

REFERENCES

1. Bosi, E., *et al. Lancet Diabetes Endo.* **7**, 462–472 (2019).
2. Bergenstal, R. M., *et al. J. Am. Med. Assoc.* **316**, 1407–1408 (2016).
3. Collyns, O. J., *et al. Diab. Care* **44**, 969-975 (2021).
4. Carlson, A. L., *et al. Diabetes* **202**, 69 (Supplement 1): 97-LB (2020).

Driving change to defeat diabetes and other serious chronic diseases

The history of Novo Nordisk dates back to the time when insulin was discovered. In 1922, the Danish physiologist August Krogh met with to-be fellow Nobel Prize laureates Frederick Grant Banting and John James Richard Macleod in Toronto, Canada, and secured the rights to supply insulin in Scandinavia.

Today, Novo Nordisk has become a leading healthcare company and we strive to use our capabilities to tackle unmet medical needs in diabetes, obesity and other serious chronic diseases (**Fig. 1**).

A SUSTAINABLE BUSINESS

We engage with partners globally to address the root causes of disease, for example with the United Nations International Children's Emergency Fund (UNICEF) to prevent childhood obesity, and we established and continuously invest in the World Diabetes Foundation and the Novo Nordisk Haemophilia Foundation.

We commit to providing access to affordable medicines for vulnerable patients and we support capacity building within healthcare systems for the future.

CHANGING DIABETES

The discovery of insulin was not only a miracle for people living with diabetes, but a foundation for modern medicine and biotechnology.

To mimic physiological insulin release, both long-acting and short-acting insulin preparations are needed. After decades of optimizations to develop longer-acting insulin preparations to reduce the number of daily injections, in 1946 we introduced Neutral Protamine Hagedorn insulin for twice-daily injection.

In 1985, Novo Nordisk pioneered production of recombinant human insulin in yeast. In 1999, we introduced rapid-acting and, in 2004, long-acting insulin analogues produced by genetic engineering, allowing users to mimic physiological insulin secretion to avoid hypoglycaemia around mealtimes as well as overnight.

Novo Nordisk as a current market leader offers multiple widely used insulin analogues, including long-acting insulin degludec, rapid-acting insulin aspart, and premix insulin preparations including the combination of insulin degludec and aspart.

To improve the convenience of our medicines, we introduced the world's first pen-shaped devices for subcutaneous injection in 1985.

GLP-1 ANALOGUES

Collaborating with academic researchers, Novo Nordisk pioneered translating glucagon-like peptide 1 (GLP-1) from incretin hormone to pharmacologically useful GLP-1 analogues for the treatment of diabetes and obesity. Our expertise in protein engineering, originating in insulin research, has enabled once-daily and once-weekly GLP-1 analogues that effectively control blood glucose in type 2 diabetes with low risk of hypoglycaemia. GLP-1 analogues can also offer significant weight loss for people with diabetes or obesity.

Today, Novo Nordisk is the world's leading supplier of injectable GLP-1-based therapies for type 2 diabetes with once-daily liraglutide and once-weekly semaglutide, as well as for weight management. With oral semaglutide for type 2 diabetes, we were the first to introduce a GLP-1 analogue (a large peptide) delivered in a tablet for systemic exposure.

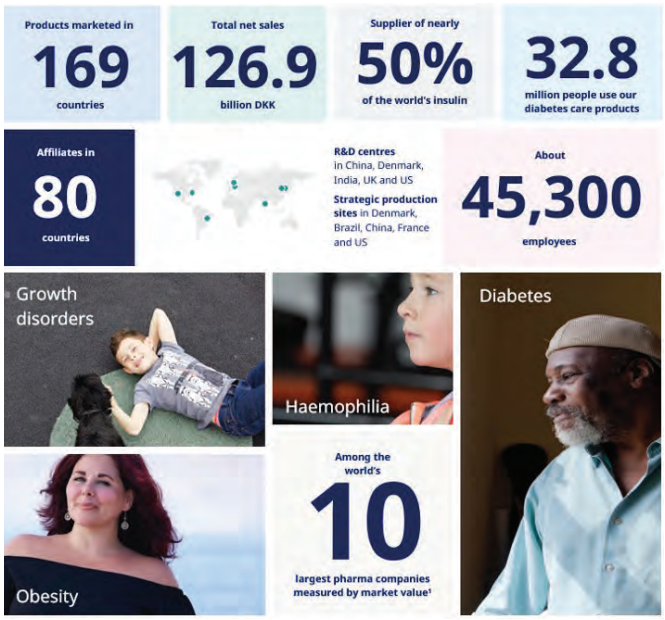


Figure 1. Novo Nordisk at a glance. Data from Novo Nordisk Annual Report 2020; S&P Global Market Intelligence on behalf of MedWatch.dk.

By developing next-generation GLP-1 analogues and other medicines in our pipeline, we envision a future where diabetes and other serious chronic diseases such as obesity and cardiovascular disease can be treated with safe medicines. Here, GLP-1 compounds are particularly promising: they effectively reduce blood glucose, weight and cardiovascular risk, and they are being explored in diseases with urgent unmet needs, including non-alcoholic steatohepatitis, chronic kidney disease and Alzheimer's disease.

RARE DISORDERS

For decades, we have produced advanced medicines to help individuals with haemophilia. In addition, we lead in growth hormone deficiency, which affects at least 1 in every 3,500 newborn children as well as many adults.

A BRIGHT FUTURE

Our commitment to finding preventive or protective interventions remains strong. We hope to leverage our 20-year experience researching stem-cell replacement therapies to introduce regenerative interventions.

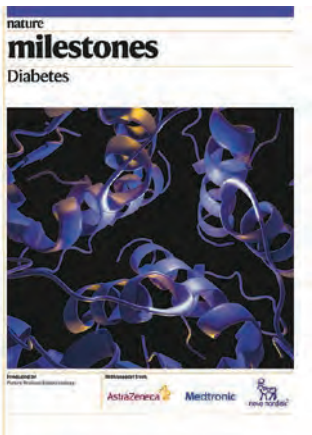
Novo Nordisk remains focused on raising the innovation bar and we continue to collaborate with academic and clinical scientists, and with biotech companies and pharmaceutical peers to develop innovative therapies for serious chronic diseases.

AUTHORS

Bernt Johan von Scholten, Frederik Flindt Kreiner, Stephen Charles Langford Gough and Peter Kurtzhals.

ADDRESS

Global Chief Medical Office, Novo Nordisk A/S, Vandtaarnsvej 110, DK-2860, Søborg, Denmark



nature.com/collections/diabetes-milestones

June 2021