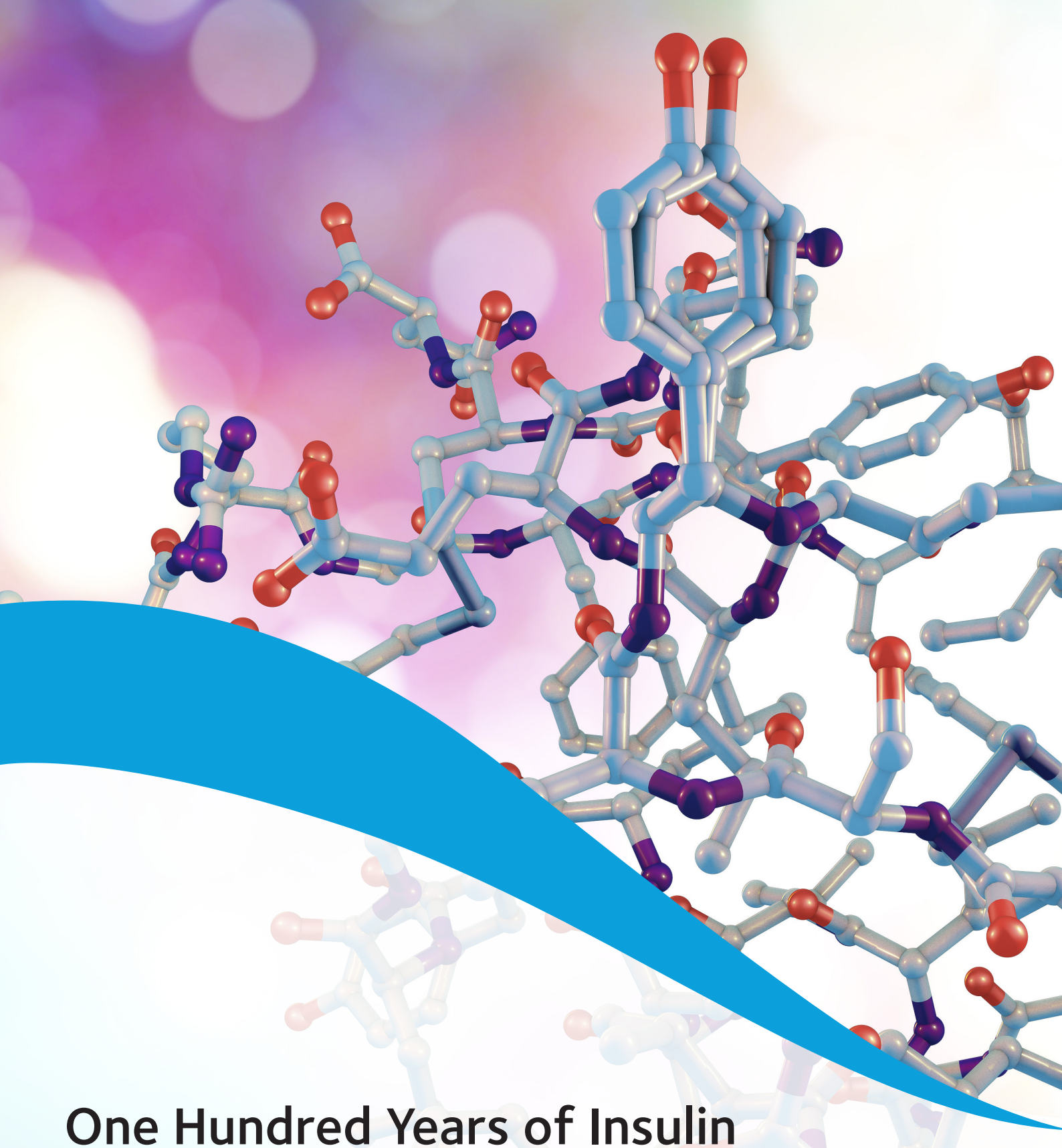

One Hundred Years of Insulin

Angus M Brown

The Physiological Society

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Angus M Brown *B.Sc., Ph.D., FPhysiol, SFHEA*

School of Life Sciences
University of Nottingham
Nottingham, NG7 2UH
UK

Department of Neurology
University of Washington
Seattle, WA 98195
USA

Email: ambrown@nottingham.ac.uk

In the aftermath of the First World War a young researcher working in a North American laboratory demonstrated dramatic reductions in the blood glucose concentration of dogs rendered diabetic by pancreatectomy when injected with solutions of freshly ground pancreas. He convincingly argued that the ability of the pancreatic extract to reduce diabetic hyperglycaemia was evidence of a role for the internal secretion of the pancreas in the origin of diabetes, and that this secretion could act as a potentially useful therapeutic tool in treating the disease. That this researcher was Israel Kleiner, an American now forgotten, and not the Canadian Frederick Banting, globally lauded as the pioneer of insulin, dispels the notion that the discovery of insulin emerged from a vacuum in the scientific backwater of Toronto, but rather was an inevitability founded on three decades of intensive study by numerous researchers, some of who came tantalisingly close to pre-empting Banting.

Diabetes

At the time of Banting's discovery of insulin in 1922 diabetes mellitus was considered a discrete condition; it was only in 1936 that the distinction between type 1 and type 2 diabetes was made (Himsworth, 1936), where the former results from lack of insulin production (insulin dependent diabetes), and the latter from insensitivity to insulin (insulin independent diabetes). The requirement for an effective treatment for diabetes was painfully obviously to the sufferers, their families and the doctors treating diabetic patients (Joslin, 1916). The disease occurred typically in pre-pubescent children, its victims indiscriminately struck down. The disease onset was rapid, the predominant symptoms being hunger, thirst, weight loss and excessive urination (polyuria) with a definitive diagnosis confirmed by the presence of glucose in the urine (glucosurea) and elevated blood glucose concentrations (hyperglycaemia; DiMeglio et al., 2018). The patients would die within a year emaciated and wretched. The only effective treatment, promoted by eminent endocrinologist Frederick Allen, was a reduced calorie diet that restricted the carbohydrate that fuelled hyperglycaemia, ketosis and coma that inevitably preceded death (Allen et al., 1919; Mazur, 2011). However, this seemingly inhumane treatment of starving emaciated patients only extended life by a few months.

Diabetes research up to 1920

In 1869 the German medical student Paul Langerhans proposed that the pancreas contained two distinct types of cell (Langerhans, 1869). The acinar cells were ordered in clusters, and produced the digestive enzymes secreted into the duodenum via the pancreatic ducts that expedite food digestion. The second cell type was expressed in islands or islets arranged as distinct clumps throughout the pancreas, but no function was ascribed to them. The French expert Laguesse subsequently named these cells the islets of Langerhans, and suggested that if the pancreas had a function other than its role in food digestion these islet cells were most likely involved (Laguesse, 1893). A major breakthrough

in diabetes research occurred in 1889 when Minkowski and von Mering, at the University of Strasburg, ligated the pancreatic ducts of dogs, thereby preventing secretion of the digestive enzymes and showed this did not cause diabetes. However upon complete removal of the pancreas (pancreatectomy) the dog became diabetic, exhibiting glucosurea (Mering & Minkowski, 1889). The absence of the pancreas caused diabetes. This was followed up in 1893 by, the French researcher Hédon, who removed the majority, but retained a small amount of pancreas in dogs, which reduced the supply of pancreatic juices: the dogs did not become diabetic. However when Hédon removed the remainder of the pancreas the dogs immediately developed diabetes and died within a week (Hédon, 1893). In 1901 Eugene Opie, at Johns Hopkins, established the correlation between damage to islets of Langerhans cells and the development of diabetes, which led to the belief that these cells produced an internal secretion that was the key to diabetes (Opie, 1910). The fundamental clinical conclusion drawn was that diabetes could be treated with extract of the pancreas containing the internal secretion.

These results were convincing evidence that the pancreas performed two distinct roles, producing the digestive enzymes (external secretion) and a blood borne internal secretion that controlled blood glucose. These early studies suggested a model for diabetes research that became the standard for the next thirty years: prepare an extract of pancreas, which was injected subcutaneously into a dog rendered diabetic by pancreatectomy to determine its ability to decrease glucosurea. The relative simplicity of the procedure and the urgent clinical need for an effective treatment for diabetes attracted hundreds of researchers, but the collective results were inconclusive and discouraging, a seemingly unavoidable consequence of the procedure were harmful side effects such a fever, convulsions and infections. It was concluded that degradation of the internal secretion by the digestive enzymes of the external secretion might explain the unpredictable potency of the extracts. To circumvent this issue Rennie and Fraser, at the University of Aberdeen, used pancreatic extracts from fish, where the islet cells are anatomically distinct from the acinar cells, but their results were inconclusive (Rennie & Fraser, 1907).

Georg Zuezler

In 1906 Zuelzer, a German researcher, prepared extracts from farm animal pancreas obtained for a local slaughterhouse and demonstrated reduced glucosurea in diabetic dogs. He also injected the extract into a diabetic patient, and although he could not measure the glucose in the urine, the patient's moribund state improved. He received financial support from the Schering drug company, and in 1907 showed the extract was effective in reducing glucosurea in diabetic humans, but side effects included fevers, vomiting and convulsions (Zuelzer, 1908). In 1911, funded by Hoffman-La Roche, he produced an extract that caused convulsions in the test animals, and the studies were discontinued due to the difficulty in isolating the internal secretion from the impurities assumed to underlie the side effects. In 1912 EL Scott, at the University of Chicago, considered the failures of the previous studies were due to the external secretion destroying the internal secretion, and reasoned that ligating the ducts, which caused the acinar cells to atrophy, would eliminate the external extract, but was unable to successfully carry out this surgical procedure. Instead he used alcohol as a solvent to isolate the internal secretion, which Zuelzer had also used. Scott optimised his extraction technique and found the dogs' glucosurea disappeared and that their temperament improved (Scott, 1912; Scott, 1913). However his advisor Carslon was sceptical of the results, thinking the experiments were not sufficiently controlled. Scott consulted JJR Macleod about this work, who informed him of studies by the British researchers Knowlton and Starling, whose work failed to show any effect of the extract on dogs. Discouraged, Scott moved on to research other areas. In the decade leading up to the 1920s technical improvements (see later) made measuring blood glucose concentrations

easier, faster, and more reliable, which was a major advance for researchers, since blood glucose concentration was a far more accurate indicator of glycaemic control than detecting the presence of glucosurea (Clarke & Foster, 2012).

Israel Kleiner

In 1914 Israel Kleiner, working with Meltzer in the Rockefeller Institute, demonstrated that injection of a glucose bolus into a pancreatectomised dog increased the blood glucose concentration three fold compared to a normal dog, and that co-injection of pancreatic extract with the glucose bolus prevented the increase. The First World War interrupted the research, but in 1919 Kleiner published a landmark paper in which he showed that the pancreatic extract reduced the blood glucose levels in dogs, the first such measurement. Kleiner stated that the ability of the pancreatic extract to reduce blood glucose was supportive of the role for the internal secretion in diabetes, and that human diabetic patients could be effectively treated with pancreatic extract, which if sufficiently pure would limit the toxic side effects (Kleiner, 1919).

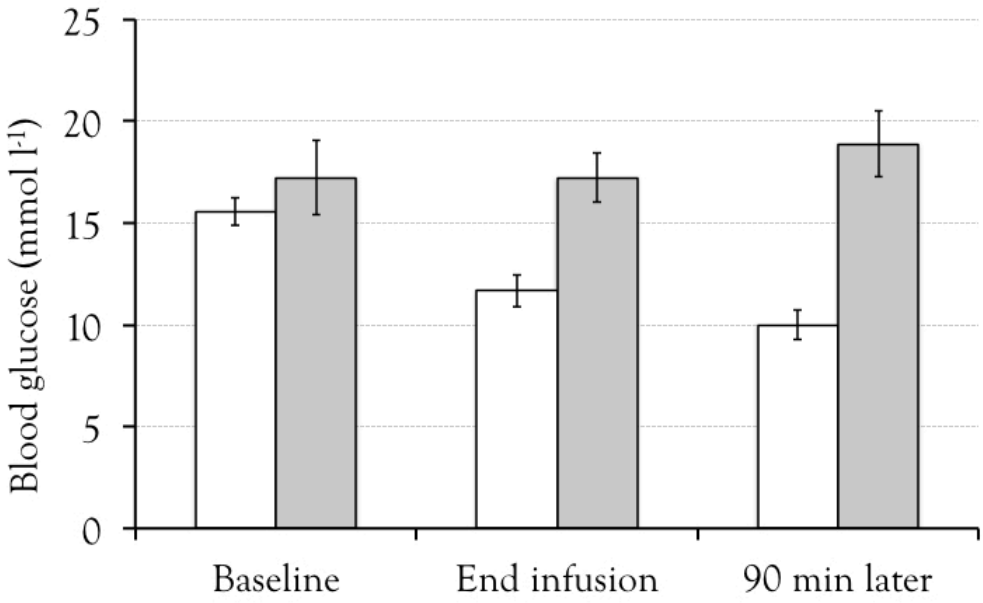

Figure 1 *- Infusion of pancreatic extract (open columns) reduced blood glucose concentration in dogs rendered diabetic by pancreatectomy, whereas extract of submandibular gland (grey columns) had no significant effect. Adapted from Friedman's reanalysis of Kleiner's original data with glucose expressed in mmol* l^{-1} *(Friedman, 2010; Kleiner, 1919).*

The Discovery of Insulin

Frederick Banting, as his biographers emphasise, was an extremely unlikely scientific hero (Bliss, 1992). He failed his 1st year at the University of Toronto, Canada, studying arts, and was only allowed to enrol in medicine in 1912 after petitioning the University Senate, having lied about his age on his application. Banting placed about average in his class. The start of the First World War interrupted Banting's medical education with his 5-year course compressed into 4 years, leaving Banting feeling deficient in his training. He specialised in

surgery and graduated in December 1916, reported for military duty the next day and sailed for England in March 1917. He was sent to France in June 1918 and served as a front line medical officer where he treated the wounded. However on the 28th Sept 1918, just six weeks before the Armistice, he was wounded by shrapnel and awarded the Military Cross, the citation noting, "his energy and pluck were of a very high order". He was invalided to the UK and treated in Manchester for 9 weeks when the wound became infected. He convalesced in Scotland and was recalled to Canada in February 1919, where he spent 6 months treating wounded soldiers at the Christie Street Hospital in Toronto. He was discharged in summer 1919 and completed his surgical training at the Toronto Hospital for Sick Children, where by February 1920 he had assisted in 232 surgeries. He left the Hospital for Sick Children in June 1920 and commenced private practice in London, Ontario. His practice was slow in becoming established and he was in debt. He carried out some additional work as a demonstrator in surgery and anatomy at Western University in London for FR Miller, Professor of Physiology, whom he also helped in the lab. The momentous events of 31st October 1920, the most propitious date in diabetes research, arose as a result of a lecture Banting was due to deliver on the role of the pancreas, about which only the basics of its exocrine function were known. Banting read a recent publication by Moses Barron in the journal *Surgery, Gynecology and Obstetrics* entitled "The relation of the islets of Langerhans to diabetes with special reference to cases of pancreatic lithiasis", which reported on an autopsy in which a pancreatic stone had occluded the pancreatic ducts, leading to loss of acinar cells, although the islet of Langerhans cells survived. These observations supported the previously reported ligation studies on dogs. The key information Banting absorbed was that ligation of the pancreatic ducts caused the pancreas to atrophy, the acinar cells died, but diabetes did not develop. As he tried to sleep in the early hours Banting scribbled the famous lines:

Diabetus
Ligate pancreatic ducts of dogs. Keep dogs alive till acini degenerate leaving Islets.
Try to isolate the internal secretion of these to relieve glycosurea.

Banting would later refer to this as 'the Idea'. However it was far from original, but Banting's knowledge of the diabetes literature was superficial, and he was unaware that EL Scott had proposed ligating ducts and that Zuezler and Kleiner had tested extracts of pancreas on diabetic dogs. Miller put Banting in touch with JJR Macleod, Professor of Physiology at the University of Toronto, a world-renowned expert on carbohydrate metabolism. Macleod had a voluminous knowledge of metabolism and in 1913 published a book entitled *Diabetes: Its Pathology and Physiology*, which was a summary of the search for the pancreatic internal secretion. The famous meeting between Banting and Macleod at the University of Toronto on Monday 7th November 1920 has been recounted many times (Bliss, 1983) and can be summarised as follows. Banting suggested experiments in which the pancreatic ducts of dogs were ligated, and after allowing for the pancreas to atrophy, it would be removed and grafted into another dog rendered diabetic by complete pancreatectomy. The dog's urine would be tested for the presence of glucose, its absence indicative of the glucose lowering effects of the graft. Macleod suggested at this meeting freezing the pancreas prior to treatment with alcohol to extract the internal secretion. Macleod provided Banting with the use of a laboratory, about a dozen dogs, and a laboratory assistant, undergraduate student Charles Best (Bliss, 1983).

The experiments commenced on 17th May 1921 and were completed nine months later. An important feature of the studies was that they evolved towards an identified end goal, to produce a purified pancreatic extract with glucose lowering properties. An under

appreciated aspect of the experiments, but one that ultimately led to success, was Banting and Best's willingness to immediately abandon dead ends and to rapidly adapt their protocol based on the success or failure of their most recent experiments. This led to rapid changes in the experimental design agreed with Macleod. Banting and Best realised that delays in production of the pancreatic extract from ligated dogs was the bottleneck in their progress. Banting was aware of Laguesse's description of (b)ovine pancreas, where the islet cells are fully developed in foetal and young calves, but the acinar cells are immature (Laguesse, 1896). Banting suggested that they obtain extract from foetal calf pancreas, an inspired idea that bypassed the limitations of working with ligated dogs. Banting then suggested using adult cow rather than foetal calf pancreas, which was successful, and at around this time they adopted Macleod's suggestion of using alcohol as a solvent. Several researchers had reached the stage of demonstrating extract from dogs had glucose lowering properties, but were unable to proceed any further due to the harmful side effects that accompanied injection of the extract. Whether by deliberate design, or most likely as a result of serendipitous gut feeling, Banting and Best incorporated these two critical modifications into their experimental design, which allowed them to maintain their momentum towards the success that had eluded so many other researchers. As their productivity increased in November 1921 Macleod acceded to Banting's request for additional manpower and JM Collip joined the group. James Collip was a similar age to Banting but was Professor of Biochemistry, an experienced researcher and knowledgeable in current biochemistry techniques. Collip started working in the first half of December 1921 on purifying extracts of beef pancreas. Macleod suggested using rabbits as the test animal as they were cheaper and easier to work with than dogs. Collip realised that working with normal rabbits, as opposed to diabetic rabbits, was viable as they responded with decreased blood glucose concentrations to injection of the extract. Two additional experiments completed their agenda by the end of 1921. The first showed that injection of the extract into a dog increased its hepatic glycogen concentration, normally negligible in a diabetic dog. The second experiment was their demonstration that regular injections of extract kept a diabetic dog alive for seventy days, whereas most pancreatectomised dogs died within a week (Bliss, 1983).

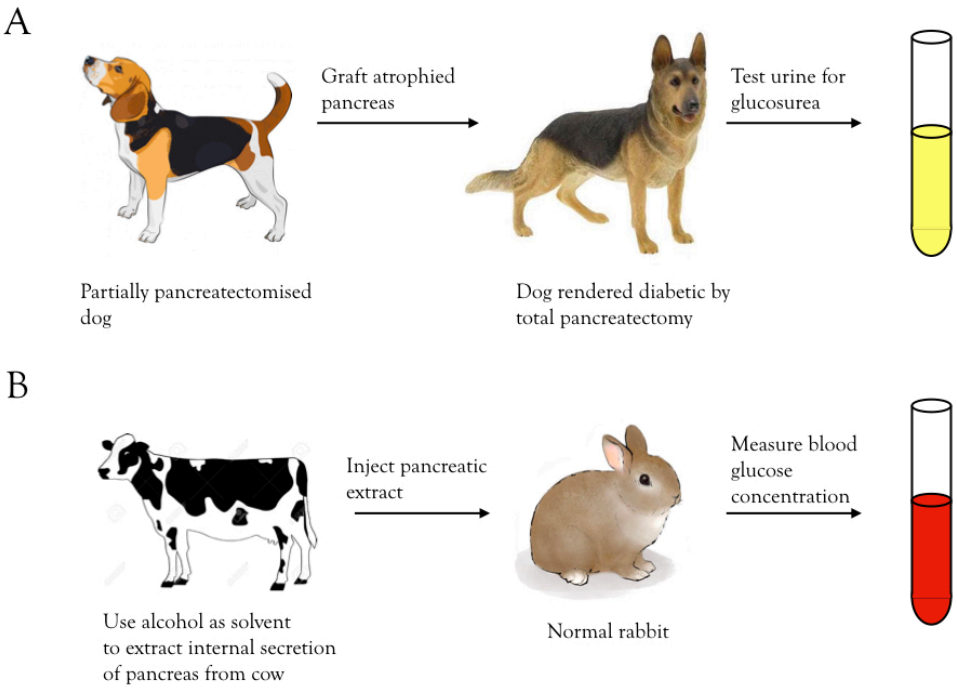

Figure 2 *- Schematic illustration of the Toronto group's experiments. A. The plan proposed by Banting and agreed by Macleod at their first meeting involved removing a substantial amount of pancreas from a dog, the remaining atrophied part of the pancreas to be grafted into a separate dog rendered diabetic by complete pancreatectomy, with measures of glucose in the urine to test the glucose lowering power of the procedure. B. The experimental protocol evolved rapidly, with bovine pancreas extract injected into rabbits, whose blood glucose was measured to determine the effectiveness of extract.*

At a conference in Yale on 30th December 1921 Banting, Best and Macleod presented their results to cautiously interested diabetologists. However Banting felt that Macleod was taking undue credit for his work and his simmering resentment of Macleod developed into irrational hatred. Banting also quickly became jealous of Collip who had parachuted into the study and immediately proved more adept at obtaining viable extract than himself and Best. In an attempt to re-establish his primacy Banting made a very poorly judged clinical decision when he persuaded Macleod to allow a human diabetic patient to be injected with extract he had prepared with Best. The date was the 11th January 1921 and the patient was 14-year-old Leonard Thompson. The extract produced a modest decrease in blood glucose from 24.4 mmol l^{-1} to 17.8 mmol l^{-1}, but the presence of ketones persisted. A sterile abscess appeared at the injection site and the procedure was judged a failure. The extract was described as 'thick brown muck' and the patient was injected with 15 ml in total, an enormous volume for a subcutaneous injection. This description clearly illustrates the limitations of Banting and Best's extract, which was too dilute and too impure for clinical use. Collip continued to work on purifying the extract and on January 23rd 1921 Thompson was administered with the Collip extract as a result of which his blood glucose concentration fell from 28.7 mmol l^{-1} to 6.7 mmol l^{-1}, his demeanour improved and he felt stronger with continued treatment over the next few days. It was the first convincing demonstration of

the anti-diabetic qualities of pancreatic extract. At this point however Banting's meaningful contribution to the work was complete. The Connaught Anti-Toxin Laboratories in Toronto purified the extract on an industrial scale under Collip's direction and patients were treated by qualified diabetologists. By February 1922 Dr Walter Campbell was treating six patients at the diabetes clinic he founded at the University of Toronto, and as word spread of the miraculous discovery in the press patients and doctors clamoured for insulin. Macleod presented the group's results on 3rd May 1922 at a meeting of the Association of American Physicians in Washington DC. A publication of the results presented at this meeting included the word insulin (page 4 of Banting et al., 1922), Macleod using the Latin translation for islet to describe the purified internal secretion.

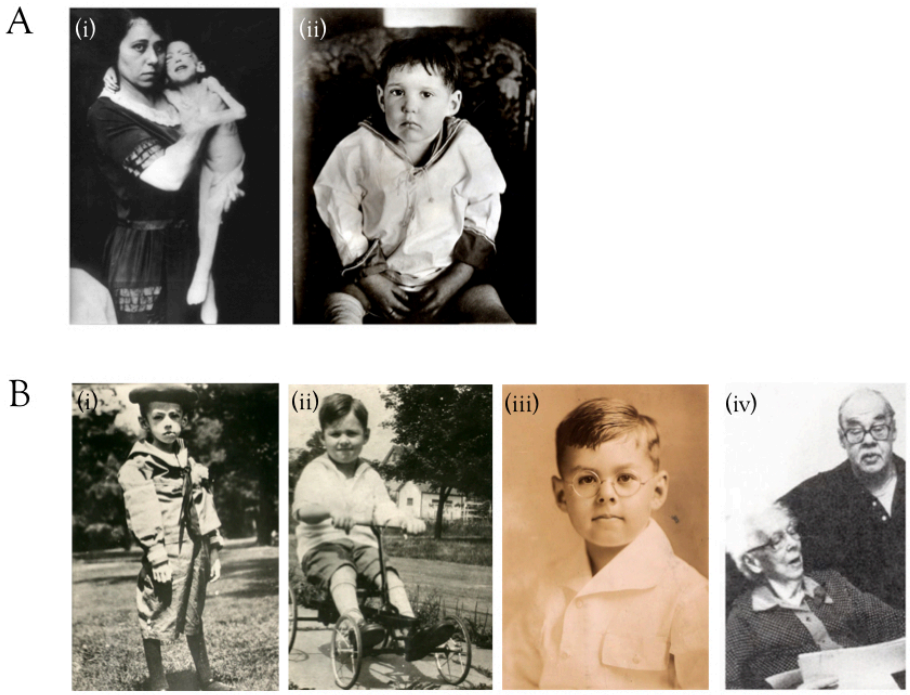

Figure 3 *- The miracle of insulin therapy. A. (i) Patient JL aged 3 years suffering from type 1 diabetes. Weighed 15 lbs on Dec 15th 1922, (ii) but after undertaking insulin treatment was transformed, weighing 29 lbs by Feb 15th 1923. B. (i) Theodore (Teddy) Ryder, diagnosed with diabetes at age 4, came to Toronto for treatment as a 5 year old weighing 26 lbs in July 1922. The life changing effect of insulin therapy is evident in photographs from (ii) 1923 and (iii) 1929. Ryder is pictured at age 66 (iv) and lived to 76 years of age, at the time of his death he was longest surviving patient treated with insulin in the world. However Ryder's longevity was the exception for those diabetics treated with insulin before the advent of modern glucose monitoring and accurate insulin administration.*

Readers interested in the work carried out in Toronto are directed to the critically acclaimed (Clarfield, 2009; Matz, 2000; Trehtewey, 1988) book *The Discovery of Insulin* (Bliss, 1983), which describes in forensic detail the background to the studies, the experiments, the extraction procedure and the aftermath. There is also a shorter accessible summary, which focuses on key experiments (Cardoso et al., 2017). Numerous review articles have been written on the discovery of insulin over the last one hundred years, of

which the following can be recommended (Barthold, 2004; Bliss, 1993; Bliss, 2013; de Leiva et al., 2011; Feasby, 1958; Fralick & Zinman, 2021; Karamitsos, 2011; Macleod, 1978; Pratt, 1954; Rosenfeld, 2002; Roth et al., 2012; Stansfield, 2012; Vecchio et al., 2018; Whitford et al., 2012). Banting, Best, Macleod and Collip, in various combinations, published over fifty papers between 1922 and 1924 (listed in the Bibliography section of *The Discovery of Insulin*), which if read in the appropriate chronological order provide a detailed account of their progress from the original studies on dogs to the purification of the extract and its use in treating diabetic patients. The city of Toronto is justifiably proud of the discovery of insulin and several websites link to historically fascinating content (CBC, 1990; U of Toronto 1; U of Toronto 2; U of Toronto 3).

Nobel Prize

In late 1923 the Nobel Prize Committee announced that Banting and Macleod were to be awarded the Prize for Physiology or Medicine for the discovery of insulin. This award required no justification as insulin was then in widespread use in North America and Europe. Banting was only 32 years old and a century later remains the youngest recipient of the Prize for Physiology/Medicine. However Banting was furious with Macleod: in his mind he had discovered insulin in spite of Macleod, not as a result of his support and encouragement. Banting seriously considered refusing the award but settled on sharing his prize with Best, a sentiment echoed by Macleod who shared his prize with Collip. The hatred, for this is not too strong a word, that Banting felt for Macleod lasted until his death twenty years later (Bliss, 1983; Bliss, 1992). Unlikely as it may sound Banting and Macleod were complementary, if not ideal, partners whose interpersonal chemistry fuelled their success. Banting, although untrained in research, had a driving ambition to undertake research, an unhappy engagement and financial problems prompting him to leave London for Toronto. Around this time Banting communicated with the great Sherrington in England on the topic of reflexes, evidence of his emerging interest in research. Banting's naiveté and superficial knowledge of the difficulties of his endeavour ensured he was not deterred by the failure of so many others. Macleod was widely read on the topic proposed by Banting and ideally informed to offer advice. Banting's skill as a surgeon, and his suggestion of grafting degenerated pancreas into dogs, likely appealed to Macleod, previous attempts at this procedure having ended in failure. Banting's proposal clearly intrigued Macleod sufficiently to induce him to provide costly facilities and resources. If Banting had been aware of the fate of Kleiner, who was forced to abandon his research while of the cusp of success for lack of funding (Friedman, 2010), he might have been more appreciative of Macleod's support. It is characteristic of naïve researchers to value the idea more than the ability to bring the idea to fruition via a well-funded and equipped laboratory. Self-awareness was not in Banting's make up. He probably did not appreciate that his great idea, duct ligation, was wrong, but it was the catalyst that secured him access to Macleod's lab, resources and the expertise of three co-workers, each contributing varied but vital skills. This was what led to the discovery of insulin. The initial progress of Banting and Best piqued Macleod's interest and he continued to fund and then expand support for the endeavour by enlisting the help of the experienced biochemist Collip. Banting failed to appreciate Macleod's input, interpreting the robust constructive criticism that is the bedrock of scientific intercourse as unjust criticism. Banting lacked Macleod's knowledge and the technical ability of Collip, and on three separate occasions came very close to fistfights with Best, Macleod and Collip, probably from the realisation of his limitations as a researcher in comparison with his co-workers. Indeed if any of the group were justified in feeling aggrieved for lack of recognition it was Collip, for it was his crucial purification process that produced an extract of internal

secretion of sufficient purity to use on diabetic patients, a feat no previous researchers had achieved. It is now widely accepted that complex teamwork led to the discovery of insulin with all four members of the Toronto group providing vital input. It is a profound tragedy that Banting felt unable to enjoy his success, any mention of insulin awakening memories of the unmerited apportioning of credit to Macleod, with whom his name would be forever linked. If only Banting had been able to console himself with the realisation that there were very few people in the history of medicine who have relieved the suffering and improved the quality of life for so many millions of people (Bliss, 1983; Bliss, 1992; Bliss, 1993; Bliss, 2013; Cardoso et al., 2017; Fralick & Zinman, 2021; Trehtewey, 1988; Whitford et al., 2012).

Standardisation

The discovery of insulin in Toronto led to an immediate and enormous global demand. The purification of insulin was optimised by Collip, and Best wrote a fascinating description of the process in which he offered generous praise to the researchers who came close to purifying insulin (Zuelzer, Scott, Rennie and Fraser, Knowlton and Starling, Murlin and Kramer, Kleiner, et al.) and provided detailed descriptions of the method used by himself and Banting in 1921 and Collip's method, commenced in December 1921. The painstaking optimisation of the purification is described in detail, clarifying Collip's vital contribution and justifying his inclusion as a core member of the Toronto group (Best & Scott, 1922). Several other researchers had progressed to the stage of demonstrating pancreatic extract lowered glucose, but failed to progress to the critical stage of testing the extract in human diabetic patients, as the impurities in their extract caused harmful side effects (Bliss, 1983). It was Collip's forensic attention to detail, borne out by his experience of internal secretions and making tissue extracts that accounted for his success where all others had failed. The key to Collip's success was the use of alcohol as a solvent at a variety of concentrations, and ether to extract insulin from lipoid material (Best & Scott, 1922). In Toronto Connaught AntiToxin Laboratories proceeded with large-scale production, whilst Eli Lilly was granted the exclusive right to produce and distribute insulin in the USA for one year. The Nobel Prize winning scientist Arthur Krogh, whose wife had recently been diagnosed with diabetes, visited Toronto in 1922 at Macleod's invitation, and secured exclusive permission to produce insulin in his native Denmark, establishing the Nordisk Insulin Laboratory, which had access to a plentiful supply of pork pancreas from the famous Danish bacon processing plants. The manufacture of insulin by different companies led to a call for standardisation, the process by which insulin is produced at equal potency irrespective of manufacturer, to ensure equivalent doses were given to patients. As the structure, and hence molecular weight of insulin, was unknown it could not be quantified in terms of moles, but instead was quantified by its glucose lowering ability, known as the biological system. In Toronto, in the spring of 1922, the effectiveness of insulin was measured by its ability to reduce blood glucose, where one physiological unit of insulin equalled the number of cubic centimetres (cc equivalent to 1 ml) of suspension that caused the blood glucose of a 2 kg rabbit, fasted for 24 hours, to fall to 2.5 mmol l^{-1} in 4 hours (Eadie & Macleod, 1922). Thus the researchers quantified the dose of insulin as a volume of solution. There were clearly problems associated with this means of standardisation, notably the biological variation among rabbits where some might be more sensitive to insulin than others, which required a complex method of normalising the response (Fieller, 1940). When insulin was injected into dogs, and corrected for weight differences, the Toronto workers found that insulin was far more potent, with only one third of the volume required to produce an equivalent effect to that in rabbits (Banting et al., 1922). In addition patients

required varying volumes of insulin indicative of differences in the purified insulin's potency, due to variations in manufacture.

This standardisation was subsequently modified by the Toronto workers, which reduced the physiological unit to one third of its original size as that value was considered too large a number, and was renamed the Clinical Unit, which was calculated as:

$$CU\ cc^{-1} = \frac{3}{2}\frac{Wt\ Rabbit}{No\ cc}\frac{IBS - FBS}{IBS - 0.045}$$

where *CU* cc^{-1} is the clinical unit per ml, *No cc* is the number of cubic centimetres injected, *Wt rabbit* is the rabbit weight in kg, *IBS* is the initial blood sugar percentage prior to injection and *FBS* is the final blood sugar percentage after insulin injection (Fieller, 1940). Plotting *CU* cc^{-1} versus *FBS* returns a linear relationship but plotting *CU* cc^{-1} versus *No cc* injected returns a non-linear relationship and reveals how small variations in the *No cc* injected can lead to large variations in the *CU* cc^{-1}.

The need for standardisation was based on the following considerations: (1) given the limited production it was important not to waste insulin in the early days of manufacture by administering imprecise dosages, (2) excess insulin dosage could lead to potentially fatal side effects associated with hypoglycaemia, (3) introduction of a standard unit would ensure that non-specialist doctors and nurses could safely administer insulin to patients, and (4) irrespective of its source all insulin would have the same potency (Fields, 2011). Since insulin was ineffective when taken orally (Rennie & Fraser, 1907) it was prepared as a water-soluble hydrochloride salt for subcutaneous injection. Henry Dale was consulted on behalf of the MRC in the UK and travelled to Toronto where he expressed reservations about the standardisation of insulin relative to its ability to induce hypoglycaemia (Feldberg, 1970). Dale consulted with the League of Nations Health Committee who sponsored a Conference in Edinburgh in 1923 to resolve the issue. Dale proposed abandoning the standardisation relative to biological systems i.e. the ability of insulin to produce hypoglycaemia, and instead proposed a standardisation relative to weight of purified dried insulin, with a correlation between weight of the powder and the experimental activity of 1 insulin unit (IU international unit) (Murnaghan & Talalay, 1992). Dried insulin from 5 sources was combined and ampoules sent to labs for comparison. Each mg of solid contained 8 IU, or 1 IU = 0.125 mg (Lacey, 1967). A detailed history of the early days of insulin standardisation in Toronto is available (Sinding, 2002). This was modified in 1935 to 1 IU of insulin = 1/22 mg of new standard, with the fourth international standard in 1959 defining 24 IU of insulin per mg of standard where the factor 7.147 was used to convert between IU and moles (Bangham & Mussett, 1959). In 1986 it was further revised to 26 IU per mg (Bristow et al., 1988). However the 1986 standard contained water and salts and required correction to 6 nmol per 1 IU, which is equivalent to 28.8 IU per mg. Thus in 2010 the WHO International Standards defined 1 IU as 0.0347 mg equivalent to 28.8 IU per mg. However many online calculators and references use 6.944 to convert between IU and mg, based on the rounding up of the MW of human insulin from 5808 to 6000 (Knopp et al., 2019).

Insulin - the molecule

Banting and Best initially used dogs as their principle laboratory model, since they were the standard animals used in previous diabetes studies and were readily available in Macleod's laboratory at the University of Toronto. The premise of Banting and Best's work was to isolate the internal secretion from a dog, then test its efficacy on a separate dog. A distant goal was to isolate an extract of internal secretion from dog of sufficient purity to be used on human diabetic patients, a procedure that had been attempted by Zueler almost 15

years previously (Zuelzer, 1908). Nowhere in the published literature from the Toronto group are the implications of cross-species effectiveness of pancreatic extract discussed, i.e. the ability of an extract from dog to produce an effect in humans. We now know this is due to the evolutionary conservation of the insulin molecule. In the Toronto studies humans, rabbit, dogs, cats, pigs, cow and ox all featured, the first three to test the effect of the extract and the last five as sources of the extract. Indeed anatomical knowledge of inter-species variations in the anatomy of the pancreas led Macleod to seriously consider fish (Bliss, 1983), where the acinar and islet cells are anatomically distant, as a suitable source for the internal secretion. Collip hypothesised that a substance equivalent to the internal secretion of the pancreas was present in all plants and even showed the glucose lowering ability of an extract from lawn grass cuttings in rabbits (Collip, 1923). Collip realised that 'where glycogen occurs in nature a related compound to insulin will be there too'. These studies in Toronto revealed that insulin is widespread in the animal and plant kingdoms, where it plays a fundamental role in carbohydrate metabolism.

Insulin is manufactured in the β cells in the islets of Langerhans in the pancreas in humans. The insulin gene is encoded by a 14 kilobase DNA fragment sequence on the short arm of chromosome 11 (Owerbach et al., 1980) at position 15.5 (Mutskov & Felsenfeld, 2009). Insulin is synthesised initially as part of preproinsulin in the β cells, a polypeptide that contains a 24 amino acid (AA) residue signal peptide, which directs the molecule to the rough endoplasmic reticulum (RER) where it is cleaved leaving proinsulin. In the RER the molecule folds to the correct conformational 3D shape and is then transported to the transGlogi network. Enzymes cleave the molecule to separate insulin from the C peptide (Champe & Harvey, 2008). Insulin is made up of an A and B chain connected by 2 disulphide bonds and consists of 51 amino acids (Fig 6A), with 10 AA residues fully conserved during vertebrate evolution. Human insulin has a molecular mass of 5808 Da and a molecular formula of $C_{257}H_{383}N_{65}O_{77}S_6$. Insulin is stored within granules awaiting release by signals such as glucose and sympathetic nervous system β2 receptor activation (Champe & Harvey, 2008). Insulin is released via elevated subcutaneous glucose, which is taken up by the β cells ultimately producing ATP, which closes ATP sensitive K^+ channels leading to depolarisation of the cell membrane, activation of voltage gated Ca^{2+} channels and Ca^{2+} influx (Ashcroft et al., 1984). This influx causes docking of the secretory vesicle containing the insulin with the β cell membrane and release of insulin into the blood stream.

Insulin and glucagon exist as antagonistic hormones, each opposing the effect of the other, where the former is anabolic in nature and released from pancreatic β cells, and the latter is catabolic in nature and released from pancreatic α cells. In response to increased blood glucose concentrations insulin is secreted from β cells, which also acts as a signal to inhibit α cell glucagon secretion (Cooperberg & Cryer, 2010). The insulin causes cellular uptake and storage of glucose from the blood into liver and adipose cells, thereby reducing the blood glucose concentration and diminishing insulin's release. In response to low blood glucose the β cells are inhibited from releasing insulin and this signals the α cells to release glucagon, which causes liver glycogenolysis and gluconeogenesis, both of which increase systemic glucose concentrations (Cryer, 2012; Figure 4A). In non-diabetic individuals the blood glucose never falls below about 3.9 mmol l^{-1} (Cryer, 2012) and since the threshold for insulin secretion is 3.3 mmol l^{-1} (Henquin et al., 2006) there is a constant tonic release of insulin. However in type 1 diabetes the β cell response is diminished or absent thus attenuating the α cell activation, which results in a limited glucagon release in response to hypoglycaemia (Cryer, 2012), which extends the depth and duration of hypoglycaemia.

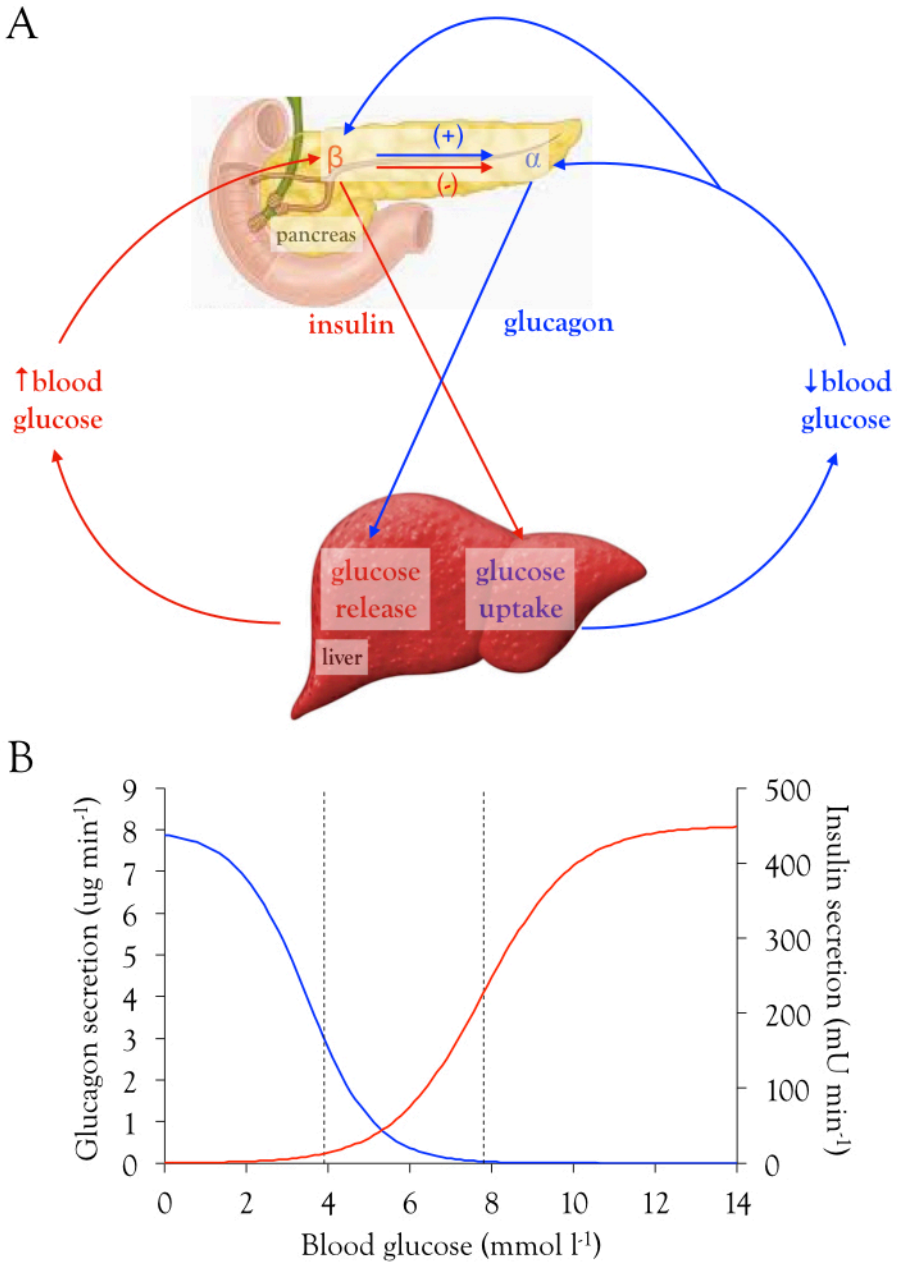

Figure 4 *- The reciprocal relationship between insulin and glucagon release. A. The circuit whereby glucagon and insulin release responds to blood glucose concentration. Elevated blood glucose activates the pancreatic β cells to release insulin into the portal vein (red). This activation of the β cells also acts to inhibit the α cell release of glucagon. The insulin causes cellular storage of blood borne glucose, thereby lowering glucose concentrations (blue). The low blood glucose directly stimulates the α cells, but also inhibits activation of the β cells, which releases the tonic inhibition on the α cells, releasing glucagon into the portal vein causing hepatic glycogenolysis and gluconeogenesis, elevating blood glucose concentrations. B. As the blood glucose concentration increases the insulin secretion increases, the steepest part of the slope coinciding with the upper limit of the normoglycaemic range (7.8 mmol l^{-1}). No insulin is secreted below about 3.3 mmol l^{-1} glucose. Glucagon is released when blood glucose concentrations are low, with little insulin released at the lower limit of the normoglycaemic range (3.9 mmol l^{-1}) (Guyton & Hall, 2006).*

The insulin molecule may have originated over two billion years ago, with a biologically similar molecule present in *E. coli* (de Souza & López, 2004). The release of insulin from vesicles via Ca^{2+} sensitive protein (Carafoli & Krebs, 2016) also points to an ancient heritage. Insulin is stored in the β cells as hexamers stabilised by zinc molecules, but once released into the blood stream the zinc is diluted and the bonds holding the insulin molecules together weaken, disassembling into insulin monomers, which can diffuse into the blood vessels (Hirsch et al., 2020). Since insulin is released into the portal vein the insulin molecules are cleared by the liver (about 80%) and kidney (20%) thereby limiting insulin availability in the periphery, where it acts on muscle and adipose cells (Hirsch et al., 2020).

Insulin analogues

The insulin used to treat human patients from 1922 to the early 1980s was animal in origin, predominantly pig and cow, which have 1 and 3 AA residues different from humans, respectively (Pickup, 1986) (Figure 5A). The problems associated with these animal insulins were supply and allergic reaction. Introduction of animal insulin into humans led to the formation of anti-insulin antibodies, causing insulin resistance in patients (Schernthaner, 1993), mandating the use of human insulin to treat human diabetic patients. The AA sequence of human insulin was published by Sanger in the early 1950s for which he was awarded the Nobel Prize for Chemistry in 1958 (Sanger & Thompson, 1953; Sanger & Thompson, 1953; Sanger & Tuppy, 1951; Sanger & Tuppy, 1951). The 3D crystal structure of insulin was revealed by Dorothy Hodgkin (Nobel Prize for Chemistry 1964) using X-ray crystallography in 1969 (Adams et al., 1969), which facilitated its mass production and the potential to alter its structure. Insulin was subsequently purified to exclude pro-insulin and other peptides in the 1970s. The discovery of the insulin gene (Owerbach et al., 1980) and recombinant DNA technology (Baeshen et al., 2014) enabled large-scale production of human insulin, where the insulin gene was incorporated into *E. coli* by Eli Lilly or yeast by Novo Nordisk and produced en masse. The first of these mass-produced recombinant human insulins was Humulin manufactured by Eli Lilly in 1982, followed by Novolin manufactured by Novo Nordisk in 1991 and Insuman manufactured by Hoecht in 1997 (Hirsch et al., 2020). This major advance in manufacturing allowed patients to be treated with human insulin for the first time. However, it is important to appreciate the differences in the kinetics of natural insulin versus subcutaneous injection of human insulin in the blood. The natural release of insulin can be viewed simplistically as a negative feedback mechanism, where elevated blood glucose concentrations promotes insulin release, which facilitates cellular storage of glucose thereby reducing systemic blood glucose concentrations, which restricts insulin release. A decrease in blood glucose concentration promotes glucagon release, which causes blood glucose concentrations to rise (Fig 4). Thus blood glucose concentrations determine the balance between insulin and glucagon release (Cryer, 2012). At normoglycaemic concentrations of glucose there is a continuous tonic release of insulin, but ingestion of food stimulates a bolus of insulin release, which can lead to elevated blood insulin levels for several hours (Fig 5B). The kinetics of insulin in the blood are defined by two separate but related properties, pharmacokinetics, which is the time course of the circulating insulin, and pharmacodynamics, which refers to the blood glucose concentration. A comparison of the pharmacokinetic profile of subcutaneous injection versus endogenous insulin shows a slower rise, later peak and prolonged presence (Fig 5C), which may result a mismatch between prevailing glucose concentrations and insulin dosage leading to hypoglycaemia (see later; Hirsch et al., 2020). In order to combat these mismatches genetically engineered insulin analogues were developed, based on human

insulin, but incorporating structural alterations to increase the rate of absorption, called rapid acting insulin. It should be noted that all human insulin analogues were developed to alter their absorption into the blood stream once injected subcutaneously and do not affect insulin's binding to its target receptors or the resulting physiological effect (Hirsch et al., 2020).

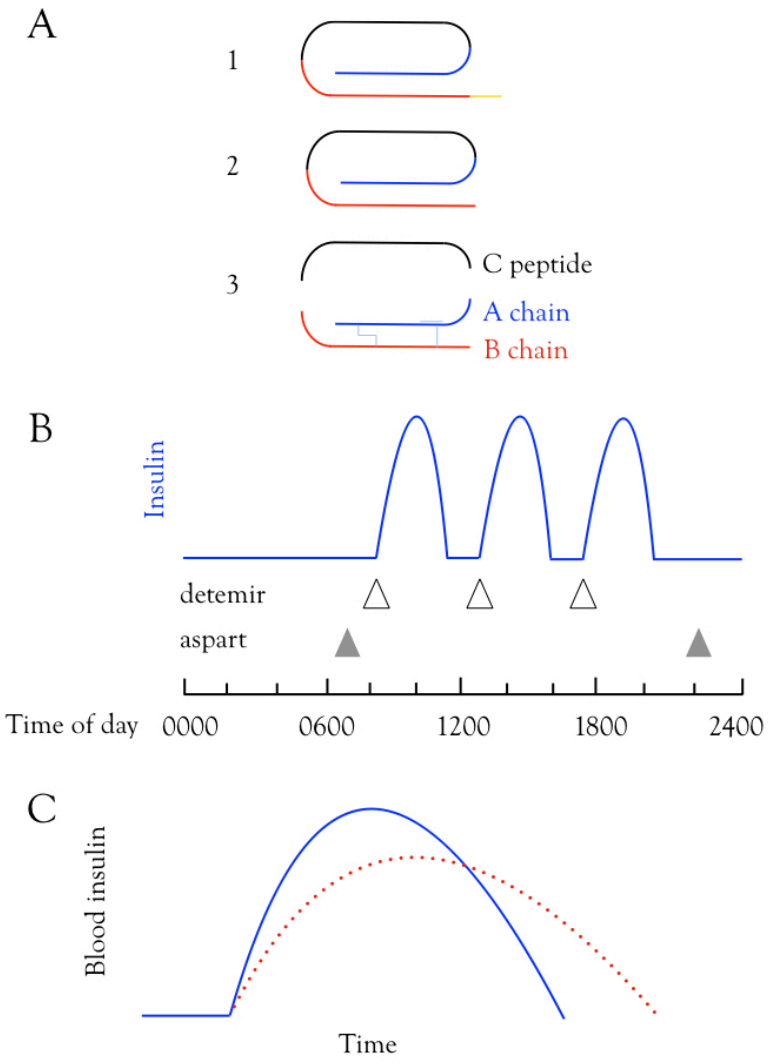

Figure 5 *- Kinetics of blood insulin. A. (1) Insulin is synthesised as a polypeptide (2) prior to cleavage of the 24 AA signal peptide (orange) then (3) excision of the C peptide (black), which is released with insulin and can be used as a measure of insulin release. B. Insulin levels throughout the day derive from two separate components, a tonic basal release throughout the day evident as a tonic release at night time, coupled with bolus release of insulin in response to meal ingestion. The insulin injection regime (triangles) via insulin pens shows two injections of long acting aspart at 0630 and 2230 and 3 injections of detemir prior to meals at 1 IU per 10 g carbohydrate. C. Profile of endogenous blood insulin levels in response to meal ingestion (blue) compared to subcutaneous ingestion of insulin prior to meal ingestion (red).*

The three main insulin analogues developed for rapid absorption were Insulin lispro marketed as Humalog by Eli Lilly in 1996, Insulin aspart marketed as NovoLog and NovoRapid by Novo Nordisk in 2000, and Insulin glulisine marketed as Apidra by Sanofi in 2004 (Hirsch et al., 2020). The AA substitutions weaken the bonds that hold the insulin hexamers together leading to rapid dissociation into monomers in the subcutaneous space and absorption, where the rate of insulin increase and decrease in the systemic circulation post injection more accurately match that of endogenous insulin. These developments, along with careful coordination between meal ingestion and insulin dosing, resulted in a pharmacokinetic profile that more accurately matched natural insulin release in response to meal ingestion. These rapid acting insulins are not suited to matching the sustained basal level of insulin release at the lower end of the normoglycaemic range, thus slower acting insulin analogues were developed. Conjugating protamine with insulin results in crystallisation of the hexamers, which must dissolve before the insulin can be absorbed. Insulin glargine, marketed a Lantus, was introduced by Sanofi in 2000, Insulin detemir, marketed as Levemir, was introduced by Novo Nordisk in 2005, and Insulin degludec, marketed as Tresiba, was introduced by NovoNordik in 2015. They were all engineered for extended absorption, the alterations in structure prolonging the latency for the hexamers to dissociate into monomers. Degludec acts for more than 24 hours requiring only one injection per day and has the considerable advantage of reducing the risk of nocturnal hypoglycaemia. However these developments still require multiple injections of fast acting insulin per day (Hirsch et al., 2020) (Fig 5B).

Insulin is expressed as mol l^{-1} or Units ml^{-1} (Frier et al., 2014), where a fasting level of inulin in non-diabetic adult humans ranges from about 70 to 150 pmol l^{-1} (Owens et al., 2001), equivalent to about 10 to 20 mUnits ml^{-1}. A typical type 1 diabetic patient requires 0.5 to 1 IU kg^{-1} day^{-1}, but in the early stages of the disease, when there is some remaining β cell function, may require only 0.2 to 0.6 IU kg^{-1} day^{-1} (Janez et al., 2020). Type 1 diabetic patients are typically supplied with insulin formulations of 100 IU ml^{-1} (Lane et al., 2017), which limits the volume of solution injected to a couple of mls at most per day. However some patients with type 2 diabetes may require more than 200 IU day^{-1} due to insulin insensitivity and increased body weight (Fig 8A & B), and may be supplied with formulations of up to 500 IU ml^{-1} (de la Pena et al., 2011). Insulin mixtures have been developed to include basal insulin and rapidly acting insulin to reduce the number of daily injections and to more accurately match the kinetic profile of endogenous insulin release (Hirsch et al., 2012). However these mixes of insulin cannot be altered and suit patients with regular, routine and equivalent meals.

A

	A8	A9	A10	A15	A18	A19		B1	B2	B21	B25	B27	B28	B29	B30		Diff
Human	T	S	I	Q	N	C		F	V	E	F	T	P	K	T		
Dog															A		1
Pig															A		1
Rabbit															S		1
Cow	A		V												A		3
Cat	A		V		H										A		4
Sheep	A	G	V												A		4
Sturgeon	H		P	D				A	A				N		V		7
Chicken	H	N	T			Y		A	A			S			A		8
Python	E	N	T	E				A	P	D	Y	S		R	S		11

B

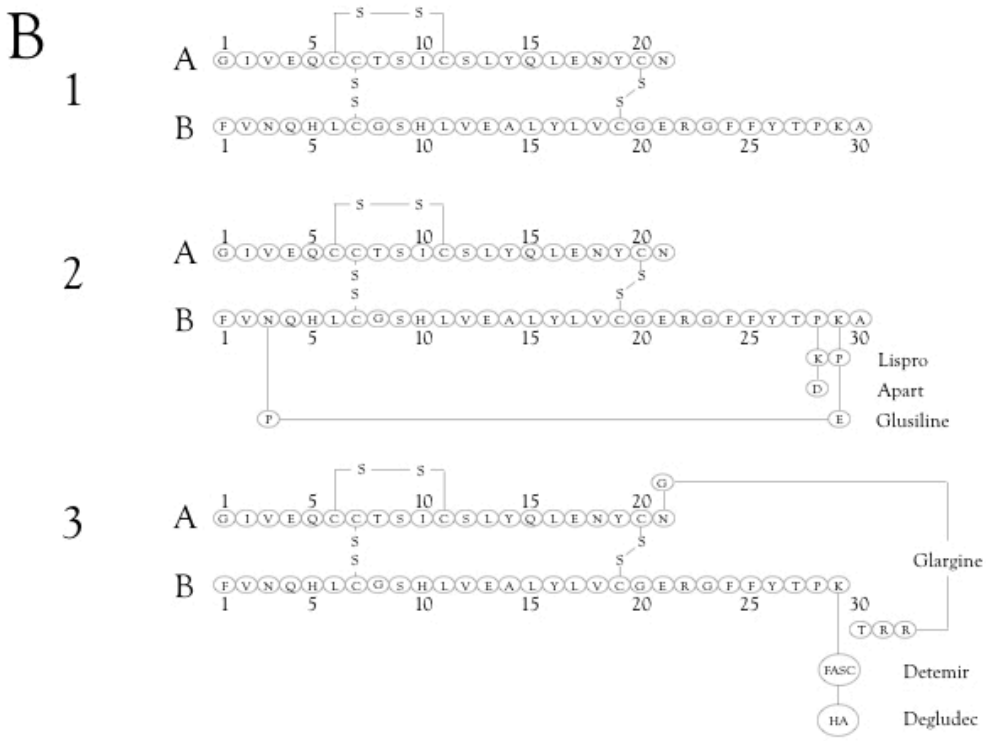

Figure 6 *- Structure of insulin. A. Comparison of human insulin AA residues with a variety of vertebrates. B. (1) Human insulin comprises an A chain (21 AA residues) and B chain (30 AA residues), interlinked with two disulphide bonds with an intra disulphide bond on the A chain. (2). Fast acting human insulin analogues showing the alterations in AA residues for Lispro, Aspart and Glulsine. (3). Slow acting analogues showing the AA substitutions for Glargine and the replacement of the AA residues 30 and 31 on the B chain with large fatty acid groups for Detemir and Degludec.*

Glucose monitoring

Disruption to the insulin signalling pathway that regulates blood glucose underlies diabetes, leading to its cardinal symptom, hyperglycaemia. Measurement of blood glucose, prior to the introduction of measuring HbA1c, was used as a clinical test to initially diagnose diabetes, and as a means of assessing glycaemic control in the diabetic patient. Thus the therapy for diabetes comprises two separate, but related, components; to administer the appropriate dose of insulin according to the blood glucose concentration, and is dependent upon a rapid and accurate means of measuring blood glucose. In sufferers of type 1 diabetes, if the degree of hyperglycaemia exceeds 10 mmol l^{-1}, the renal threshold for glucose absorption (Triplitt, 2012), glucose appears in the urine, roughly in proportion to the blood glucose concentration (Griffin et al., 1979). Prior to the introduction of accurate blood glucose measurements (around 1920) detecting the presence of glucosurea was the standard method of diagnosing diabetes. The early diabetes researchers used the reduction or absence of glucosurea after pancreatic extract injection as evidence of its glucose lowering ability. However the absence of glucosurea could mean that the blood glucose was mildly hyperglycaemic, normoglycaemia or hypoglycaemia (Clarke & Foster, 2012), clearly indicating the requirement for accurate measures of blood glucose. It was with the development of the Benedict solution, known as the copper reduction reaction, in the early 20th century, that an accurate means of measuring glucose in the urine became widely available (Benedict, 1908). This complex test required boiling of the reagent and used the presence of alkaline sodium carbonate to convert glucose to a strong reducing agent, which was oxidised by Cu^{2+} to produce Cu^{+}, visible as an insoluble red copper oxide, the intensity of the colour proportional to the glucose concentration. The colour of the sample was compared to a standard colour chart to estimate the glucose concentration. Variations of this reaction were introduced over the next few decades, but they all utilised the 'copper reaction', which required laboratory analysis and their accuracy was questioned (Shaffer & Hartmann, 1921; Somogyi & Kramer, 1928; Van Slyke & Hawkins, 1928). A significant advance occurred in 1941 with the first test that could be carried out at home by the patient. The introduction of the Clinitest tablet, which, when added to the urine sample, induced a boiling reaction, promoting the formation of copper oxide (Free & Free, 1964). In the 1950s the Clinistix was introduced, a new technology in which the enzyme glucose oxidase was embedded on a stick that was inserted into the urine sample (Kohn, 1957). The glucose oxidase catalysed the oxidation of glucose to form hydrogen peroxidase, which reacted with potassium iodide catalysed by hydrogen peroxidase to produce iodine, whose brownish colour indicated the presence of glucose, the strength of the colour proportional to the glucose concentration. The use of urine glucose concentrations as a proxy for blood glucose concentrations was fraught with limitations (Miller et al., 1983), which included, but were not limited to the following. Urine volume and concentration could affect the accuracy of the strips, the presence of glucose in urine was only detected when glucose exceeded the threshold for renal clearance, and comparison between the colour on the strip and the standard colour chart introduced the element of subjective judgement.

The need for blood glucose testing became overwhelming with the realisation that measures of urine glucose as a proxy for blood glucose were hugely inaccurate, leading to poor glycaemic control and high incidence of morbidity and mortality. In 1965 the Dextrostix was introduced, which was based on the same principle as the Clinistix, but was capable of measuring blood glucose (Free & Free, 1964). The strip used the glucose oxidase/peroxidase reaction, but came with a semi-permeable outer layer, which occluded the red blood cells from the strip, while allowing access of plasma. At about the same time Boehringer

introduced the Chemstrip bG, which used the same oxidase reaction but offered the user a clearer view of the strip. The urine and glucose tests described so far are single point estimates of glucose concentration at the time of sampling. As such they are useful for determining if the patient is hypo- or hyperglycaemic, but give no indication of the long-term maintenance of blood glucose concentrations. Additional considerations include how accurate a representation of blood glucose interstitial glucose is, limitations in accuracy of measures of glucose at either end of the range, and the use of finger stick in the calibration process (Frier et al., 2014). In the 1970s the introduction of the measurement of glycated haemoglobin (HbA1c) (Rahbar et al., 1969) was an enormous step forward in estimating long-term glycaemic control in patients (Koenig et al., 1976; Koenig et al., 1976). The average life span of red blood cells is about 120 days and the reaction between haemoglobin and glucose to form glycated haemoglobin has been used as an estimate of the degree to which the red blood cells have been exposed to glucose during their lifetime. Values exceeding the threshold of 6.5% or 48 mmol mol^{-1} signify diabetes (Garber et al., 2016).

The inaccuracies of comparing the strip colour with a standard colour chart were bypassed in 1970 by the introduction of the Dextrostix reflectance meter by Ames (Hedner et al., 1974). The device was used in doctors' offices, and required the insertion of a Dextrostix strip dipped in blood sample into the meter. A spectrophotometer measured the reflectance from the strip and the blood glucose concentration was indicated by a needle superimposed on one of three scales, which required no interpretation of the part of the user (Schersten et al., 1974). Inaccuracies in the Dextrostic meter at the extremes of the blood glucose range routinely encountered in diabetic patients prompted development in 1973 of the Eyetone meter, which displayed the glucose concentration on one easily readable scale (Schersten et al., 1974). In 1980 a Dextrometer, which used the Dextrostix as the glucose sensor, was developed for home use complete with a digital readout for accuracy. The introduction of small battery operated glucose meters with digital readouts commenced with the GlucoMeter M in 1980, the AccuCheck in the mid 1980s and the OneTouch Ultra in 2000 (Clarke & Foster, 2012). This allowed the patient to test their blood *ad libitum* without a visit to the doctor. The use of glucose meters at home introduced the concept of self-monitoring of blood glucose (SMBG), the most important development in diabetes therapy since the introduction of insulin six decades previously.

In 1993 an enormously influential study, whose conclusions remain the basis for current diabetes therapy, convincingly demonstrated the link between poor glycaemic control and increased probability of developing microvascular complications. The study demonstrated that intensive insulin therapy, which attempts to maintain the blood glucose concentrations as close to the normal range as possible for as long as possible, significantly reduced the principal complications associated with diabetes: retinopathy, nephropathy and neuropathy, offset however, by an increased incidence of hypoglycaemia. Increases in HbA1c values were correlated with increasing retinopathy, whereas lower values, below the diabetic threshold of 6.5%, were associated with increased incidence of hypoglycaemia. The conclusion from this study was that although no specific target value of HbA1c could be recommended, maintaining the glycaemic status as close to the normal range as safely possible delayed progression of microvascular complications, but also increased the incidence of hypoglycaemic episodes (Nathan et al., 1993). The target blood glucose range was between 70 - 180 mg dl^{-1} (3.9 - 10 mmol $l^{-1)}$ (Nathan et al., 1993) with patients' adherence to this range quantified as the Time in Range (TiR), and expressed as a percentage (Fig 7A).

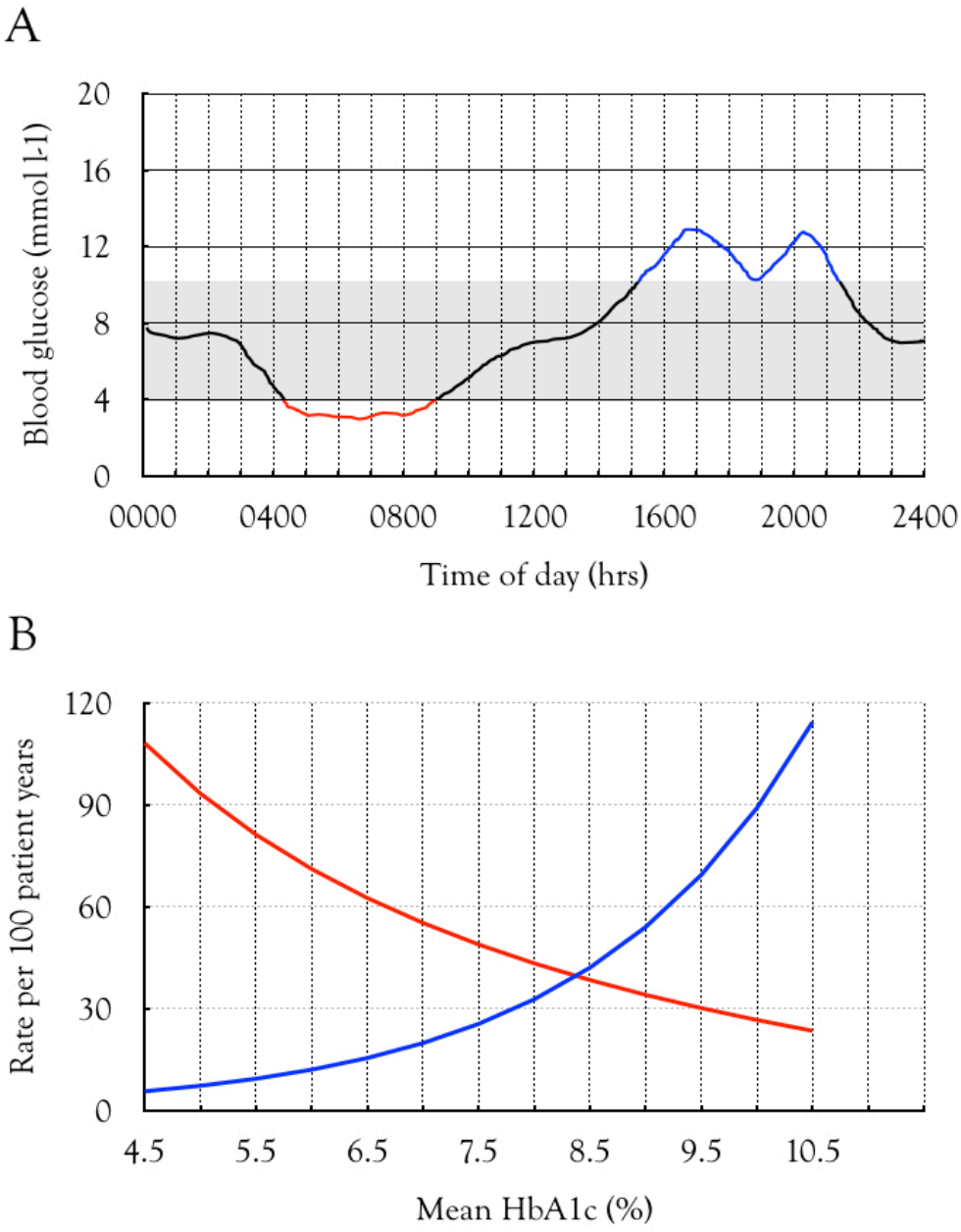

Figure 7 *- Glycaemic control and risk of microvascular complications. A. The glucose profile throughout a 24 hour period where the grey region indicates the desirable range of glucose concentrations from 3.9 to 10 mmol* l^{-1}*, showing an episode of hypoglycaemia during the night (red) and hyperglycaemia during the day (blue). B. The relationship between HbA1c, an index of glycaemic control, where 6.5% is the diabetic threshold, and risk of microvascular complications and hypoglycaemia. Increasing values of HbA1c (blue) increase the probability of microvascular complications, whereas decreases in HbA1c (red) lead to increased probability of hypoglycaemia episodes.*

In order to achieve a close relationship between prevailing glucose concentration and target glucose concentrations continual glucose monitoring (CGM) was introduced in the early 2000s. The first devices measured glucose by passing very low amplitude currents across the skin to attract glucose molecules. The first such commercially available device was the Glucosewatch, introduced in 2002 and featured in the contemporaneous film *Panic Room*, which displayed digitally the prevailing blood glucose concentration on a watch-type device (Chan & Hurel, 2002). Continual advancements have been made in the development of CGM with the prescription-only DextComms STS, which had a sensor, transmitter and receiver that output the data to a computer. In 2008 the FreeStyle Navigator CGM was introduced, but it required a complex and lengthy calibration. In 2015 the DexCom G4 Platinum was a major advance as it was approved for used by under 18s. In 2016 the Medtronic MiniMed 'iPro2'G5 mobile was introduced and in 2017 the FreeStyle Libre Pro Flash was introduced which required no calibration (Clarke & Foster, 2012). These current systems use a sensor coated with glucose oxidase that penetrates the skin and sends regular updates of the current glucose concentration to an app on the user's mobile phone. These devices allow storage and display in graphical form of long-term data, and quantify the TiR for defined periods of time. Longitudinal studies have shown CGM is more effective than SMBG as measured by an increased percentage of TiR and lower incidence of hypoglycaemia (Danne et al., 2017).

The units by which glucose is expressed vary. Early experimenters expressed glucose concentration as a %, where 1% equals 1 g of glucose in 100 ml of urine/blood, although clinicians favour mg dl^{-1} and lab scientists mmol l^{-1} (mM). The inter-conversion between these units is straightforward (Sawbridge et al., 2014). A baseline fasting glucose concentration in a non-diabetic adult of 0.09% is equivalent to 90 mg dl^{-1} or 5 mmol l^{-1}. The blood glucose concentration range of a non-diabetic individual is between 3.9 and 7.8 mmol l^{-1} (70 - 140 mg dl^{-1}; Frier et al., 2014). It is enlightening to put into perspective the glucose concentration of blood relative to the glucose concentration in common drinks and food. A can of coke (355 ml) contains about 21.3 g of glucose (350 mmol l^{-1}), and an equivalent amount of fructose (Varsamis et al., 2017). Instantaneous absorption of this amount of glucose into the blood of an 80 kg man (blood volume of 5.6 litres) would increase the blood glucose concentration by 21 mM, far in excess of the hyperglycaemic threshold of 7.8 mmol l^{-1}. A diabetic patient undergoing a hypoglycaemic event (blood glucose of 3 mmol l^{-1}) would only need to drink 50 ml of coke (although 150 ml is recommended (Frier et al., 2014)), or eat 4.2 g of pasta (72 g of carbohydrate per 100 g) to increase blood glucose concentration by 3 mmol l^{-1}.

Life Expectancy

A longitudinal examination reveals the effects of various developments in diabetes therapy on life expectancy. About half of those diagnosed with type 1 diabetes are under the age of 14 (Rawshani et al., 2014), hence the description of childhood onset diabetes. Prior to insulin therapy the life span of these children was between 1 and 2 years, although older patients lived longer, between 4 and 9 years (Brostoff et al., 2007). By 1945 the life expectancy for type 1 diabetics had dramatically increased by 45 years for children diagnosed under the age of 10, by 30 years for those diagnosed at age 30 and 16 years for those diagnosed at age 50 (Brostoff et al., 2007). It must be borne in mind that this time period pre-dates the advent of SMBG, thus the patients in these studies were most likely poorly controlled. The trend to increased longevity continued with those born between 1956 and 1980 living 15 years longer than those born between 1950 and 1964 (68.8 versus 53.4 years) (Miller et al., 2012). The improvements in blood glucose monitoring underlie

these increases in life expectancy and the introduction of SMBG and CGM (see later) should extend these even further.

A recent Swedish study revealed that the age of diagnosis was strongly correlated with life expectancy, those diagnosed under 10 years of age lived approximately 6 years less than those diagnosed between 26 and 30 years of age (Rawshani et al., 2018). The likelihood of death and the increased risk of complications is directly related to the degree of glycaemic control, the higher the per cent of time spent in TiR, the lower the risk of complications such as retinopathy, neuropathy and nephropathy. The greatest risk in these patients was of cardiovascular disease and endocrine disorders, which accounted for 70% of deaths in those diagnosed between 0 and 10 years old, which fell to 61% for those diagnosed between 26 and 30 years old. However younger onset patients had up to a 30-fold increased risk of cardiovascular disease or acute myocardial infarction (Rawshani et al., 2018). Age of onset is considered a critical factor in determining life expectancy, the rapid death of β cells in young children not only exposing them to high glycaemic load at the onset of the disease, but leading to extended exposure to high glycaemic loads. The vasculature, and in particular the coronary arteries, are vulnerable to increasing glycaemic load, again underlining the importance of maintaining strict glycaemic control (Aronson & Rayfield, 2002).

Prevalence of diabetes

The world wide number of individuals diagnosed with diabetes quadrupled from 108 million in 1980 to 422 million in 2014, a global prevalence increase from 4.9% to 8.5% (NCD-RisC, 2016), with a projection of 592 million by 2035 (Guariguata et al., 2014) and 642 million by 2040. In the UK the number of diagnosed diabetics has risen from 1.4 million in 1996 to 4.7 million in 2019, a prevalence of 7% (Whicher et al., 2020), and is estimated to reach 5.5 million in 2030. About 90% of these diabetics have type 2 diabetes and 10% have type 1 diabetes, although type 1 diabetes incidence is increasing in Europe, possibly due to life style, increasing weight and height, C-section deliveries and reduced frequency of early infections (Patterson et al., 2009). One third of people at the time of their diagnosis have irreversible micro vascular complications, but fewer that half of these patients receive the recommended eight annual health checks putting them at increased risk of complications (Diabetes UK, 2017). In the USA there are currently 34.2 million people with diabetes, 10.5% of the population, with 1.7 million having type 1 diabetes. However the prevalence increases with age; 26.8% of people over 65 are diagnosed as diabetic (CDC, 2020) (Fig 8).

Insulin Pumps

The ideal delivery of insulin to the diabetic patient should mimic the natural release of insulin from the pancreas as closely as possible. In non-diabetic individuals there is a continual tonic low level release of insulin throughout the day and night, referred to a basal release. Insulin is released as a bolus in response to meals with blood concentrations reaching ten times higher than basal levels (CDC, 2020). In the early days of therapy insulin was administered subcutaneously via injection by syringe, whereas endogenous insulin is realised into the hepatic vein and is cleared by the liver, with residual insulin travelling to the periphery in the systemic circulation where it acts on skeletal muscle and adipose cells. There are numerous problems associated with subcutaneous injections of insulin, which include variations in absorption dependent upon injection site, patient compliance with a repetitive painful process, a large bolus of insulin present in periphery can lead to excessive

uptake of glucose into adipose cells leading to weight gain, inaccuracies in dosage, sufficiently concentrated insulin solutions required to limit volume of injections, and sensitivity/irritation at injection site (Kesavadev et al., 2020). However subcutaneous injection was the only means of administering insulin in the sixty years after its discovery. This, combined with the limited ability to accurately measure blood glucose concentrations with any regularity, led to poor glycaemic control resulting in high levels of morbidity and mortality. The longevity of Theodor Ryder (Fig 3), one of the first patients to use insulin, was the exception rather than the rule (Jones, 1983). In the 1980s the insulin pen was developed which operated in a similar manner to the syringe, but came equipped with an adjustable nozzle for accurately varying dosages. The insulin pen offers more flexibility than the syringe, is more discreet, more accurate, more cost effective in the long term and leads to improved treatment compliance. In controlled studies patients treated with insulin pens achieved greater glycaemic control and fewer complications than patients treated with conventional syringes (Kesavadev et al., 2020).

In order to address the issues related to insulin administration with syringes or pens the insulin pump was developed in the 1960s, but only became a viable option for patients at the turn of the century (Alsaleh et al., 2010). The design of the insulin pump, or continuous subcutaneous insulin infusion (CSII), incorporates a reservoir of insulin solution connected to a subcutaneous catheter via tubing, with the site of subcutaneous injection changed every 3 days (McAdams & Rizvi, 2016). The pump is programmable and can deliver insulin at variable rates (0.01 to 50 IU min^{-1}). The insulin pump is suitable mainly for type 1 diabetics but about 10% of pump users are type 2 diabetics and only rapid insulin analogues are used (Janez et al., 2020). The insulin pump continuously delivers insulin (basal delivery), is tailored to the patient's daily glucose cycle and can also deliver a bolus injection of insulin with meals, although the user has to calculate the appropriate dose based on the carbohydrate content of the meal and manually input this information (usually 1 IU per 10 g carbohydrate). The insulin pump offers the benefit of no injections and the insulin dose more closely mimics the physiological response, meaning TiR is greater, the variations in absorption are reduced, and glycaemic control is improved with a decreased risk of complications (Group, 2017).

The artificial pancreas

The continual improvements in CGM and the advent of the insulin pump led to these two technologies being combined as the artificial pancreas or closed loop insulin delivery, where readings from the glucose sensor are transmitted wirelessly to a receiver that contains a computer algorithm for calculation of the insulin dosage delivered to the patient via the insulin pump (Elleri et al., 2011). There are types two of algorithm used to optimise insulin delivery relative to prevailing glucose levels (Bequette, 2013). The proportional integral derivative (PID) algorithm can be considered reactive as it responds to observed glucose concentrations, whereas the model predictive control (MPC) algorithm is proactive at forecasting glucose levels in anticipation of the glucose concentrations and the effects of administered insulin (Pinsker et al., 2016). MPC algorithms calculate the delivery of insulin via the pump by minimising the difference between the desired glucose level and the measured glucose level over a future time horizon. The PID algorithm alters the insulin delivery by measuring the glucose level deviation from three perspectives (1) difference between glucose level and target glucose level (proportional component), (2) area under curve between target and measured glucose level (integral component), and (3) rate of change of measured glucose (derivative component) (Steil, 2017; Steil et al., 2011). An important early development in closed loop systems was a suspend function where

decreases in sensor readings of glucose temporarily stopped infusion of insulin. The primary goal of the closed loop system is to achieve a high level of glycaemic control, whilst minimising the risk of hypoglycaemia. Several recent trials have demonstrated the superiority of the closed loop system over the SMBG when comparing TiR and episodes of hyper- and hypoglycaemia (Benhamou et al., 2019; Brown et al., 2019; Weisman et al., 2017). A significant factor in the improvements of TiR with the closed system is related to the variations in insulin delivery that occur between day and night. The two main factors that affect blood glucose concentration, and hence insulin release, are intake of food and exercise, which are absent at night. As such there is less variability in blood glucose concentration and hence less variation in insulin release and glycaemic control is more easily achieved at night. During the day the variations in composition of meals, time of consumption and exercise can lead to blood glucose concentrations straying from the target levels due to the rapid increase in blood glucose concentrations resulting from food ingestion, which requires significant release of insulin. Postprandial time covers about 65 - 70% of the daytime and consumption of meals require user input to administer the appropriate insulin dosage. In a randomised study the majority of the improvement in TiR for the closed loop system came from overnight and evening improvements and highlights the extremely important point that the majority of the time not in range occurs as a result of meals and physical activity (Kovatchev et al., 2020). In future the use of a closed loop with fully automatic insulin delivery in combination with ultrafast acting insulin will improve TiR. These closed loop systems are not entirely independent as they require the user to input when meals occur to account for the bolus delivery of insulin (Boughton et al., 2020). However true independent closed loop systems have been developed in which the algorithm for delivering the insulin is embedded within the pump as opposed to be present on a smartphone device (Wang et al., 2021). A bionic pancreas, which will deliver both insulin and glucagon was invented in 2015 and approved by FDA in 2019, and is in the development stage (Kesavadev et al., 2020).

Cost

Diabetes is a very costly disease to treat. There are several reasons for this, including patients with type 1 diabetes can suffer with the disease for six decades or more based on a childhood diagnosis, and type 2 diabetic patients can suffer for four decades or more, given a middle age diagnosis. There is a direct correlation between duration since diagnosis and probability of developing complications (Rawshani et al., 2018), which include retinopathy leading to blindness, nephropathy leading to kidney failure and neuropathy which can lead to amputations, all of which are time consuming and costly to treat. In the UK it is estimated that diabetes costs the NHS £10 billion per year, 10% of its entire budget, with 80% of these costs going to treat complications (Diabetes.UK, 2017). In the United States a similar picture emerges, where diabetes consumed $237 billion in direct medical costs in 2017, accounting for about 25% of health care spending (American Diabetes Association, 2018; CDC, 2020). Since about 10% of adult USA citizens have a diabetes diagnosis it is clearly a disproportionally costly condition to treat. The increasing prevalence of diabetes can only lead to increases in these costs.

Diabetes can also be expensive for the patient depending upon where they live. In Europe the cost of insulin to the patients is limited by the national health care systems and in the UK patients receive insulin and related supplies such as CGM equipment free if they have diabetes mellitus and qualify for a medical exemption certificate. In Scotland, Wales and Northern Ireland prescriptions for insulin are free. In the United States the system is radically different and can be a huge financial burden to the patient, which can cost up to

$900 per month, and it is estimated that 1 in 4 patients in the USA ration their insulin (Herkert et al., 2019), leading to complications and even death (Rajkumar, 2020). Each diabetic patient on average incurs costs of $9,600 per year, about 2.3 times more than non-diabetic individuals. The scandal of patients unable to afford life saving insulin is a regular media story in the USA, but untangling the details of why this occurs is complicated. There are only three manufacturers of insulin supplied to the USA, Novo Nordisk, Eli Lilly and Santori. There are 1.7 million type 1 diabetes sufferers in the USA, and about 7.5 million type 2 diabetics in the USA use insulin. Although only 7% of the worlds diabetics live in the USA 50% of worldwide insulin cost occurs in the USA, whereas China accounts for 25% of worlds' diabetics but only 4% of insulin sales (Bliss, 1983). The price of insulin products in the USA tripled between 2002 and 2013 and the price paid for insulin doubled between 2012 and 2016. One explanation for the increased costs is that insulin analogues are seven times more expensive than human insulin, and the vast majority of the insulin currently in use are analogues. Although the cost to manufacture insulin has remained stationary for the past two decades, the price of a vial of Humalog increased from $35 to $234 between 2001 and 2015, despite only costing $4 to produce (Hirsch, 2016). Globally insulin was a $7.3 billion industry in 2005, $21 billion in 2013 and will reach $28 billion by 2025 (Tsai, 2016). It is a bitter irony that the only thing the Toronto group agreed upon by mid 1922 was that they should not benefit financially from their discovery and sold the patent to the University of Toronto for a nominal sum, to ensure patients would have access to low cost insulin (Bliss, 1983).

The complex pathway between insulin manufacturer and the patient is opaque with lack of clarity of the costs involved in each stage. A large amount of the cash goes to pharmacy benefit managers (PBM), whose role is to agree contracts between manufacturers and pharmacies, for which they receive large rebates from the manufacturers (Cefalu et al., 2018). It is estimated that half of the list price of insulin goes to PBMs, a $200 billion a year industry in the USA. Even if cheap biosimilars (Gotham et al., 2018), essentially generic versions of insulin, are introduced it is likely that the PBMs will intervene in the supply chain significantly raising costs. There is no easy solution to the problem, with the USA Congress refusing to limit drug costs, as is the case in Europe, mandating via Medicare that free market theories should determine the cost of insulin.

It is clear that the pharmaceutical companies are guilty of rampant and blatant profiteering. They routinely issue lawsuits involving patent extensions to zealously protect their monopoly on supply of insulin in the USA, and spend millions of dollars lobbying politicians to bar cheaper biosimilar compounds from entry into the market place (Know, 2020). This issue is the focus of political attention at the highest level, with insulin caravans routinely crossing the border to Canada, where patients can buy insulin freely over the counter for a fraction of the cost in the USA. Such trips, although highly visible via media coverage, are available to only a limited number of the USA diabetic patients (Gambino, 2019). These patients are the beneficiaries of the discovery of insulin, but are cynically and mercilessly exploited by Big Pharma, who view them as cash cows, willing to pay exorbitant prices for the insulin that keeps them alive, that they will use daily for decades, and for which there is no substitute. Banting, a war hero who risked his life treating his fellow soldiers in the First World War, who refused to personally profit from his discovery despite a precarious personal financial situation, and who died on a secret mission serving his country during the Second World War, would have been absolutely disgusted.

Islet transplantation

Since diabetes results from the autoimmune destruction of pancreatic β cells, thus depriving the body of the insulin required to control blood glucose, an obvious therapeutic strategy is to introduce the missing component, thus injection of exogenous insulin has been the sole therapy for type 1 diabetes for the last 100 years. However there are difficulties in matching delivery of insulin to prevailing glucose concentrations as previously described. Within the last 20 years transplanting a donor pancreas into diabetic patients has become a reality and to date about 48,000 have been carried out worldwide (Bellin & Dunn, 2020). The recipients of these transplants are almost exclusively type 1 diabetic patients, with lifelong immunosuppression therapy required to prevent tissue rejection. In the USA a controlled follow up study ten years after transplantation found that 40% of the recipients remained insulin independent, an impressive statistic, although the limited number of donor pancreas makes it an unrealistic therapeutic intervention for most type 1 diabetics (Bellin & Dunn, 2020). An alternative therapy is to implant pancreatic islets, which contain both insulin-secreting β cells and glucagon-secreting α cells, introducing the potential to counteract hypoglycaemia (Rheinheimer et al., 2015). Islets are harvested from human donor pancreas and more than one pancreas donor may be required per patient depending upon yield. The islets are purified to rid the tissue of the exocrine secretion, an attraction of this preparation is that it allows more scope for innovation, particularly regarding immunosuppression (Rheinheimer et al., 2015). A study of this procedure demonstrated that 87% of patients reported no severe hypoglycaemia and 52% were insulin independent one year after implantation (Hering et al., 2016). Once the islets are cleaned they are introduced into the portal system where they became implanted in the liver developing their own blood supply (Bellin & Dunn, 2020). However this can lead to a rapid inflammatory reaction (IBMIR), which can kill a sizeable proportion of the implanted cells and lifelong immunosuppressive drug therapy is required (Bellin & Dunn, 2020). There is also the problem of implanting an appropriate number of cells. It is estimated in the adult human there are about 1,000,000 β cells. Implanting an excess of β cells in the expectation of considerable cell death post-implant may lead to hypoglycaemia, whereas implanting too few would fail to achieve insulin independence (Matsumoto, 2010). In order to deal with the immune response islet cells can be encapsulated, which isolates them from the circulation and alleviates the need for immunosuppressive therapy, although this is still in development. Another method is to use pig β cells. It is known that pig insulin is effective in humans, and there is a plentiful supply of pig pancreas. This would obviously lead to an immunogenic response but emerging technologies are being used to reduce this effect (Matsumoto, 2010).

The demand for donor pancreases far outstrips supply and introduces the potential use for insulin producing cells (IPCs) derived from stem cells as the most advanced approach for a sustainable therapy (Chen et al., 2020). Many studies have been carried out in this area, the IPCs derived from either embryonic stem cells (Thomson et al., 1998) or from human pluripotent stem cells (Pagliuca et al., 2014). The former comes with ethical issues, whereas the latter involves adult cells, which are programmed back into an embryonic pluripotent state (Takahashi et al., 2007). To circumvent rejection issues the induced pluripotent stem cells may be taken from the patient (Millman et al., 2016), which can also be encapsulated within a matrix that allows interstitial fluid access to the grafted cells but prevents immune cell reaction (Kepsutlu et al., 2014). Such cells resemble the adult primary cells regarding their ability to produce insulin. Trials involving newly diagnosed type 1 diabetic patients demonstrate that introduction of IPCs derived from liver mesenchymal striomal cells achieved an increase in both insulin and C peptide secretion, which fell in control diabetic patients (Cai et al., 2016). However, despite enormous progress in the field, it must be considered a technique in development with numerous obstacles to be overcome

before it becomes a viable therapy. The technique must improve upon the islet and pancreas transplantation where up to 60% of treated patients remain insulin independent 5 years after treatment (Rickels & Robertson, 2019).

Hypoglycaemia

In non-diabetic individuals the normal blood glucose concentration lies between 3.9 and 7.8 mmol l^{-1} and never deviates from this range (Frier et al., 2014). The direct activation of α cells by blood glucose is of secondary importance to the release of the tonic inhibition that β cells exert over α cells in the presence of normoglycaemic glucose (Cryer, 2012). When glucose falls to the lower end of the normal range the decreased release of insulin by β cells acts to release the tonic inhibition of glucagon release from α cells, thus insulin and glucagon act in an antagonistic manner in determining the glucose concentration in the blood. In type 1 diabetic patients the loss of β cells removes the stimulus for α cell release of glucagon, thus falls in blood glucose concentration are not counteracted by glucagon release (Cryer, 2012). Hypoglycaemia is extremely uncommon in non-diabetic individuals where the principle cause is insulinoma, a rare condition (Frier et al., 2014). Thus 100 years ago hypoglycaemia was a condition of which most doctors were unaware.

In the early diabetes studies on dogs a commonly reported consequence of injection of pancreatic extract was convulsions, but these were considered comparable to fevers and infection and were classified as harmful side effects resulting from impurities in the extract (Bliss, 1983). Indeed Zuezler reported severe convulsions in dogs injected with his extract prior the First World War (Zuelzer, 1908). That convulsions were a symptom of hypoglycaemia was recognised by Collip while purifying extract in the winter of 1921/22. Measurement of the blood glucose of rabbits rendered convulsive by extract injection showed very low readings: the injection of glucose precipitated the animal's recovery (Bliss, 1983). This was the first appreciation that pancreatic extract could not only render hyperglycaemic animals normoglycaemic, but could render normoglycaemic animals hypoglycaemic. In early January 1922 Banting persuaded Macleod to allow his extract to be injected into a type 1 diabetic patient for the first time. It is not clear if Banting was aware of Collip's discovery given the breakdown in communications within the group, but in retrospect it must be considered a very poor clinical decision (Bliss, 1983). As more patients were treated with insulin in Toronto the clinicians became extremely vigilant for signs of hypoglycaemia (Banting et al., 1923). The Toronto group had not only created a new therapeutic strategy to effectively treat diabetes via exogenous application of insulin, they had also created a new pathological condition, iatrogenic hypoglycaemia, and those treating type 1 diabetic patients with insulin became familiar with the causes, symptoms and treatments (Fletcher & Campbell, 1922).

Hypoglycaemia is a condition in which the brain receives insufficient glucose i.e. the demand is not matched by the supply, and the absence of β cells limits the extent to which glucagon can increase systemic glucose concentrations (Cryer, 2012). The principle symptoms of the response to hypoglycaemic attack betray the systems sensitive to decreased glucose and are categorised as being either autonomic (Lin et al., 2021) or neuroglucopenic (Garcia et al., 2021), with symptoms of sweating, trembling, weakness, hunger, pounding heart, belonging to the former category and visual disturbances, difficulty concentrating and confusion belonging to the latter (Frier et al., 2014). The glucose threshold for the autonomic warning signs is higher than that for the neuroglucopenic signs allowing the patients time to recognise the symptoms and take appropriate action by ingesting glucose. However a complication of hypoglycaemia is that repeated exposure

decreases the gap between the threshold for autonomic and neuroglucopenic symptoms such that the patients may be rendered incapable of reacting to the autonomic symptoms, a process of unknown mechanism named hypoglycaemia unawareness (Cryer, 2013).

Hypoglycaemia is the main reason that patients fail to comply with an insulin regime designed to maintain strict glycaemic control (Cryer, 2002). The Diabetic Control Study demonstrated the effectiveness of a strict insulin regime in reducing the microvascular complications that results from hyperglycaemia, but it came at a price, increased episodes of hypoglycaemia (Fig 7B)(Cryer, 2002). The practical measures that have been installed in modern insulin therapy to prevent hypoglycaemia include equipping closed loop systems with a stop function that prevents insulin injection when blood glucose readings are too low and alarm activation in CGM systems when blood glucose levels fall towards hypoglycaemic concentrations (Boughton & Hovorka, 2021).

Insulin is potentially fatal and has been used occasionally in murder and suicide; the lack of a counter-regulatory glucagon response makes injected insulin particularly dangerous. The first suicide by insulin was recorded in 1927 by a diabetic patient and there have been sporadic cases reported since (Russell et al., 2009). The first murder attributed to insulin occurred in 1957 when Kenneth Barlow, a hospital worker who presumably had access to insulin, was convicted of murdering his pregnant non-diabetic wife Elizabeth by injection of insulin for which he was imprisoned for 26 years (Marks & Richmond, 2008). Similar cases are routinely reported in the medical literature (Marks & Richmond, 2008). The Claus von Bulow case, in which the wealthy socialite was tried twice for the attempted murder of his wife, presumably by insulin injection, attracted headlines worldwide and was made into the film *Reversal of Fortune*. Bulow was acquitted at the second trial, the case resting on his wife's low blood glucose and high insulin levels, although whether von Bulow had access to insulin was a key aspect of the defence case (Marks, 2007). Given the limited access to insulin it tends to be used by patients or those in the health care professions and there have been several cases of healthcare workers who use insulin to kill patients (Pidd & Grierson, 2015). However insulin is considered a poor means of killing, as it can have variable effects on victims, and exogenously applied insulin can be detected if the ratio of C peptide to insulin is lower than six to one in living people and twenty to one in corpses (Marks, 1999).

A

B

C

Figure 8 *- The obesity epidemic underlies the increase in type 2 diabetes. A & B. Unrestrained consumerism, manifest as morbid obesity, subsidizes the fast food industry, the dieting industry and ultimately corporate medicine. C. Portion size plays a significant role in obesity.*

Future Directions

The core principle of current diabetes therapy is to match as closely as possible exogenously administered insulin to the physiological release of insulin. The incremental steps towards this goal have been documented in this review and comprise two independent but related processes, accurate blood glucose monitoring coupled to administration of an appropriate dose of insulin. It is only within the last 20 years that advances in these technologies have progressed sufficiently that CGM coupled with insulin pumps have resulted in desirable TiR being achievable by the majority of patients. The development of glucose–responsive insulin bypasses the requirement for continuous insulin infusion, where a pool of latent insulin is only activated with increasing concentrations of glucose (Wang et al., 2021). Unfortunately hypoglycaemia remains an ever-present risk, as this strategy does not address the impaired glucagon response in the absence of β cell input. Curing diabetes, as opposed to effectively treating it, will require the introduction of an autonomous endogenous glucose sensitive insulin release mechanism that accurately mimics physiological insulin (and glucagon) release across the broad range of blood glucose concentrations experienced by diabetic patients. To date whole pancreas and β cell transplantation are effective in achieving insulin independence in type 1 diabetic patients, but limits in donor pancreas availability have prompted development of stem cell therapies to create patient-derived glucose responsive IPCs that will both secrete insulin and modulate glucagon release.

Conclusion

The current obesity epidemic (Fig 8) underlies the explosion in type 2 diabetes, where, allowing for population increase, global incidence rates have doubled in the last 40 years. In the USA in the last 20 years the incidence of type 2 diabetes has increased from 6% to over 10% of the general population, 13% if only adults are considered. This relatively recent development is extremely concerning for a variety of reasons. Firstly, the developed world (north America, Europe and Australia) acts as aspirational capitalist model for developing counties (south America, Africa and Asia). There is a positive, direct relationship between incidence of obesity and Gross Domestic Product, which is an indicator of a county's economic development (Kinge et al., 2015). A sense of entitlement also prevails, where economic prosperity liberates individuals from a subsistence diet to one where freedom of choice dominates, and medical guidance to limit junk food consumption is considered interfering, unwelcome and generally unheeded. Such economic freedoms invariably lead a large proportion of the population towards obesity and diabetes. A prime target in reducing diabetes is prevention and delaying its onset, but strategies to reduce obesity on a global scale have been unsuccessful (NCD-RisC, 2016). Individuals who are pre-diabetic or with short duration diabetes, can reverse the condition with a regimen of caloric restriction and exercise with long-term sustained weight reduction maintaining the post-diabetic state (Lim et al., 2011; Taylor, 2019). However the increasing incidence of diabetes suggests that few people manage to accomplish this and highlights a worrying underlying trend, where patients refuse to adopt behaviour that will favour their long-term health, probably as a result of the difficulties in reversing a lifetime of bad habits. Secondly, there are enormous cost implications of increasing incidence of diabetes, since it is a disproportionately expensive disease to treat. In the UK one in six hospital inpatients has diabetes, and in the USA, although 10% of the population have diabetes, it consumes over 25% of the healthcare budget. In the USA about 25% of patients ration their insulin, which will only lead to increases in the rates of complications. How health care systems are planning to cope when diabetes incidence approaches 20% is unknown.

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