Hypoglycaemia

Merchant, Heather J.; McNeilly, Alison D.

Published in:
Diabetic Medicine

DOI:
10.1111/dme.14721

Publication date:
2021

Document Version
Peer reviewed version

Title: Hypoglycaemia: Still the main drawback of insulin 100 years on: “From man to mouse”

Short running title: Hypoglycaemia: Still the main drawback of insulin 100 years on: “From man to mouse.”

Heather J. Merchant and Dr Alison D. McNeilly

Systems Medicine, Medical School, University of Dundee, Dundee, DD1 9SY

Corresponding Author: Alison McNeilly, a.d.mcneilly@dundee.ac.uk

Manuscript: 3,937 **Abstract**: 142

No conflict of interest

Keywords:

Type 1 diabetes, Hypoglycaemia, Translational Research, Diabetic Complications, Experimental Animal Models

Abstract

One hundred years on from the initial discovery of insulin, we take this opportunity to reflect on the scientific discoveries that have improved so many lives. From its original crude form, insulin therapy has improved significantly over the past century. Despite this, hypoglycaemia remains an ever-present fear for people with Type 1 diabetes. As such, it is essential that research now looks to minimise the frequency and severity of insulin-induced hypoglycaemia and its complications, some of which can be life-threatening. Over the last century, one thing that has become apparent is the success and need for translational diabetes research. From its origin in dogs, insulin treatment has revolutionised the lives of those with Type 1 diabetes through the coordinated effort of scientists and clinicians. In this review, we recount the more recent research that uses a mouse-to-man approach, specifically in hypoglycaemia research.

A diagnosis of Type 1 diabetes once came attached with radical, unsuccessful treatments and a dire prognosis. One hundred years from the first administration of exogenous insulin to treat symptoms of diabetes, we take this opportunity to reflect on the resources which made this scientific discovery possible and address the obstacles that insulin treatment still presents. In addition, this article will focus on how treatment has been developed and could be furthered by translational research.

The Discovery of Insulin

The initial discovery of insulin occurred approximately a decade before its first therapeutic use [1]. Frederick Banting and Charles Best are recognised for discovering insulin in 1920 while working under the directorship of James McLeod at the University of Toronto. Using pancreatic extracts containing insulin, Banting and Best were able to treat symptoms of diabetes in dogs who had undergone a total pancreatectomy. James B. Collip, a talented biochemist, working alongside Banting and Best, purified the insulin protein used to treat individuals with diabetes at Toronto General Hospital [2]. This treatment was successful in reducing hyperglycaemia and glucosuria. Subsequent work by Collip and the involvement of Eli Lilly led to the first mass production of insulin and the first case of Type 1 diabetes which did not have a prognosis of death [2]. Banting and Macleod received the Nobel Prize in Physiology or Medicine in 1923 for the discovery of the insulin protein; this accolade was split with their colleagues Best and Collip in recognition of their contribution [3]. This pioneering work highlights the importance and success of translational research. *Figure 1* depicts the progression of insulin therapy from its discovery to the current day.

Insulin - A double-edged sword

Exogenous insulin administration overcomes the initial symptoms of Type 1 diabetes, namely hyperglycaemia and glucosuria, thereby protecting the individual from potentially fatal diabetic ketoacidosis. However, due to bolus injections, miscalculated doses, and reduction of other components of the counterregulatory response, hypoglycaemia remains a real threat.

The Counterregulatory Response to Hypoglycaemia

The onset of hypoglycaemia is typically accompanied by an array of symptoms classified into three categories: autonomic, neuroglycopenic and malaise. These symptoms are outlined in *Table 1.* Typically, a series of events occur in response to reducing blood glucose, known as the counterregulatory response (CRR). Initially, insulin secretion is suppressed, followed by increased glucagon, epinephrine, cortisol, and growth hormone if blood glucose continues to fall. At around 3 mmol/L plasma glucose, behavioural responses promote exogenous glucose intake, such as the feeling of hunger to stimulate carbohydrate intake [4]. If blood glucose drops below 2.8mmol/L, a decline in cognitive ability will be apparent. Hypoglycaemia is rarely seen in individuals not taking insulin replacement therapy [4]. As a result of exogenous insulin administration, individuals with diabetes cannot “switch off” insulin secretion in response to falling blood glucose. Immediately, this puts these individuals at greater risk of hypoglycaemia. In addition, it is well appreciated that glucagon secretion in response to hypoglycaemia is severely reduced approximately five years post-diagnosis [5]. Type 1 diabetes alone suppresses the hormonal components of the CRR with prior exposure to repeated episodes of hypoglycaemia, only potentiating these defects [6].

Defective counterregulatory response to hypoglycaemia in Type 1 diabetes

Although features of the CRR can be maintained in Type 1 diabetes, repeated exposure to hypoglycaemia reduces the magnitude of the epinephrine response to hypoglycaemia and the glucose concentration at which it is initiated [7], leading to further potentiation of hypoglycaemia. The

reduced secretion of epinephrine in response to hypoglycaemia due to previous hypoglycaemia also extends to non-diabetic humans [8]. Collectively, these factors result in longer recovery times to glucose homeostasis and an increased risk of hypoglycaemia. For these reasons, despite improvements in the diagnosis and treatment of diabetes, exposure to hypoglycaemia remains the most feared consequence of insulin treatment. The recent GOLD-4 clinical trial reported that individuals with Type 1 diabetes and average blood glucose of 8mmol/L spend 12% of their time below 3.9mmol/L and 4.9% below 3mmol/L [9]. Plasma blood glucose was measured using self-monitoring blood glucose meters or continuous glucose monitors (CGM). Time spent in the hypoglycaemic range was reduced in individuals with higher average blood glucose and those using CGM [9]. Non-severe hypoglycaemia is estimated to occur twice weekly in people with Type 1 diabetes, whereas episodes of severe hypoglycaemia are predicted to occur between 1-3 annually in a large proportion of those with Type 1 diabetes [4, 10]. Severe hypoglycaemia is defined as an event that requires outside intervention in order to recover. The incidence of hypoglycaemia is greatly influenced by the duration of diabetes, with risk increasing by 3-fold between patients with < 5-year duration and >15 years of duration [10]. This increased risk can be attributed to further β-cell loss, decreased glucagon secretion in response to hypoglycaemia after five years of disease, and the development of impaired awareness of hypoglycaemia. Although the exact mechanisms underlying the loss of glucagon secretion in response to hypoglycaemia remain unclear, it has been hypothesised that the loss of regulatory beta-cell signals such as zinc, insulin or γ-aminobutyric acid (GABA) or basal hypersecretion of somatostatin may all contribute to diminished glucagon secretion[11]. Likewise, reduced alpha cell mass in long-duration Type 1 diabetes and the increase in bi-hormonal expressing cells suggest that alpha to beta cell transition may play a role in this phenomenon.

Complications of Insulin-Induced Hypoglycaemia

Hypoglycaemia and the brain

The incidence of recurrent hypoglycaemia has been shown to profoundly affect the brain, which is unsurprising due to the brain's reliance on glucose as a primary fuel source. In rats, repeated insulin-induced hypoglycaemia has been shown to impair the sympathoadrenal response, which in humans is part of the counter-regulatory response to hypoglycaemia [12]. In addition, this study showed a significant reduction in glucose sensing ability in the ventromedial hypothalamus (VMH) [13]. More recently, repeated insulin-induced hypoglycaemia has been shown to induce cognitive deficits in a streptozotocin (STZ) induced rodent model of Type 1 diabetes [14]. In both primary cortical neuronal cultures and various regions of rat brain, severe glucose deprivation has been shown to induce neuronal death [15]. This may account for cognitive deficits observed in response to severe hypoglycaemia.

Hypoglycaemia and cardiovascular disease

Acute hypoglycaemia has been reported to increase heart rate, systolic blood pressure and left ventricle ejection fraction whilst reducing diastolic blood pressure [16]. There is scant evidence to suggest that a single episode of hypoglycaemia can directly induce macrovascular complications; however, recurrent hypoglycaemia is likely to worsen pre-existing microvascular complications [16]. In some cases, severe episodes of nocturnal hypoglycaemia can result in cardiac arrhythmias, which lead to cardiac arrest [17]. This phenomenon is referred to as *dead in bed syndrome*. Prolonged QT intervals have been hypothesised to cause this syndrome, with hypoglycaemia possibly attributing to this prolongation [16].

Hypoglycaemia and renal disease

In individuals with existing nephropathy, acute hypoglycaemia may further potentiate the complication. However, acute hypoglycaemia has been shown to have no deleterious effects in individuals without diabetes and in those with diabetes who are free of nephropathy [16].

Impaired Awareness of Hypoglycaemia

Originally termed *hypoglycaemia unawareness* [5], impaired awareness of hypoglycaemia (IAH) refers to the clinical phenomena whereby reductions in blood glucose go undetected until much lower concentrations. The onset of IAH relates to the duration of diabetes and prior exposure to recurrent hypoglycaemia. Depth, duration, and the number of prior hypoglycaemic episodes are also contributory factors. IAH affects approximately 25% of people with Type 1 diabetes [18]. Although the mechanisms resulting in the development of IAH are poorly understood, it is likely to be a combination of multiple factors, including i) an adaptation to low blood glucose levels, ii) changes in glucose or alternative fuel transport and storage, iii) changes in intracellular glucose metabolism resulting in altered neuronal firing and iv) changes in intracellular communication and neurotransmitter release [19]. *Figure 2* depicts the shift in symptomatic response to hypoglycaemia in those without diabetes and people with Type 1 diabetes with differing awareness of hypoglycaemia. Hormonal responses to hypoglycaemia in those with IAH occur at lower glucose concentrations when compared to those with normal awareness [5]. IAH was initially diagnosed using symptom questionnaires; however, this has obvious flaws. Therefore, Mokan et al. [5] implemented a more robust determination of IAH by inducing hypoglycaemia and defining individuals who exerted autonomic symptoms two standard deviations below people with normal awareness as having IAH.

The barrier of hypoglycaemia

Hypoglycaemia remains the main drawback of insulin therapy. Despite vast improvements in insulin formulation, education, treatment plans, technology, the incidence of hypoglycaemia has remained constant. These categories are addressed in depth in a comprehensive review by Farrell and McCrimmon [20]. Notable advancements have been accomplished in insulin preparations and technology. Since the original animal-derived insulins there has much research into the formulation of long and short acting insulin and finally to analogue insulins. These are scientifically engineered preparations that improve the overall action of therapeutic insulin [20]. Technological advancements include the generation of insulin pumps and closed-loop systems, acting as an artificial pancreas, in addition to widely available continuous-glucose-monitoring (CGM) equipment [20], removing the requirement for finger-pricking.

Interestingly, recent research has reported that CGM significantly underestimates the degree of hypoglycaemia [21]. CGMs were reported to measure 8% higher during euglycaemia and 12% higher during hypoglycaemia compared to arterialised-venous blood measured using a bedside glucose analyser [21]. This disparity is likely due to CGMs measuring interstitial fluid rather than plasma directly. As a result, the degree of hypoglycaemia is severely underestimated, and consequently, the number of hypoglycaemic events reported will be inaccurate. Ultimately, this may put individuals with IAH at higher risk of severe hypoglycaemic events.

Currently, there is no cure for IAH other than strict avoidance of hypoglycaemia, a challenging feat when an individual is unaware of hypoglycaemia. Further, the mechanisms by which IAH develops remain unclear. Translational research using cellular and rodent models has provided insight into potential factors contributing to IAH and other complications associated with Type 1 diabetes.

Current rodent models of diabetes

Animal models allow researchers to investigate the behavioural, physiological and biochemical responses to hypoglycaemia. Animal models enable the researcher to control the depth, duration, and frequency of each hypoglycaemic episode and present researchers the opportunity to delve into mechanistic features of the disease, which are not possible in clinical research.

The main characteristic of Type 1 diabetes is pancreatic β-cell destruction which ultimately leads to the requirement of exogenous insulin administration. The mode of insulin delivery to a diabetic model is an essential consideration as left untreated, the animals will show severe weight loss, polyuria and polydipsia. Slow-release insulin implants (Linbit) or osmotic mini-pumps placed subcutaneously beneath the skin are favoured over daily injections. Several models (rat and mouse) have been used to address specific clinical features of Type 1 diabetes and *Table 2* highlights some of the most well-characterised rodent models used in Type 1 diabetes research (for detailed reviews, see [22, 23]). Although these models can never completely recapitulate the human condition, when combined with pharmacological and biochemical measures, they can provide vital insight into mechanisms contributing to the diabetic phenotype. The following sections will address some examples in which rodent models have played a fundamental role in furthering clinical advancements.

Example 1: Glucose sensing – The ATP-sensitive Potassium Channel

With glucose as its primary fuel source and lack of storage capacity, glucose-responsive machinery must exist in the brain. The hypothalamus has been identified as a centre for glucose-sensing and producing restorative outputs in response to hypoglycaemia [24-26]. In particular, the ventromedial hypothalamus (VMH), comprised of the ventromedial nucleus (VMN) and arcuate nucleus (ARC), has been highlighted as an invaluable brain region in the detection of and response to hypoglycaemia [25, 26]. Distinct populations of glucose-responsive neurons were first identified in the VMH in the late 1960s by Oomura et al. [27] and have since been described numerous times [28]. These neurons are commonly referred to as glucose-excitatory (GE) and glucose-inhibitory (GI) neurons. GE neurons depolarise in response to high glucose concentrations, whereas GI neurons depolarise in low glucose concentrations. It is well appreciated that GE neurons are mechanistically similar to pancreatic β-cell, with both cell types containing the enzyme glucokinase and the Kir6.2/SUR1 ATP sensitive potassium channel. However, these cells are active over different glucose thresholds, with the periphery being exposed to ~ five-fold higher glucose concentrations than those of the brain. Despite this, both GE neurons and pancreatic β-cells depolarise in response to high glucose levels. This mechanism is depicted in *Figure 3*.

The K_{ATP} channel

The presence of Kir6.2/SUR1 ATP-sensitive potassium channels in the VMH and the effectiveness of potassium channel opening drugs were confirmed by McCrimmon et al. [29]. The authors demonstrated that recurrent exposure to hypoglycaemia modifies K_{ATP} channel function contributing to the counter-regulatory hormonal defect. Hypoglycaemia is known to increase the ADP: ATP ratio, suggestive of decreased ATP [30] and therefore decreased opening of the K_{ATP} channel. These findings identified a critical role for these channels in maintaining the cells glucose-sensing ability *in vivo* and led to investigations into the therapeutic potential of potassium channel openers in hypoglycaemia.

Potassium channel openers – from rodent

NN414 is an analogue of diazoxide with 100-fold increased potency and a receptor subtype specificity for Kir6.2/SUR1 channels [31]. Due to its increased subtype specificity, NN414 is expected to have fewer off-target effects than diazoxide [31]. NN414 has been shown to reduce blood glucose and improve glucose tolerance in Vancouver diabetic fatty (VDF) Zucker rats [32] as well as improve

glucose-related parameters in healthy male subjects [33]. These beneficial glucose handling effects are likely due to the NN414's action on hypothalamic K_{ATP} channels expressing the Kir6.2/SUR-1, rather than those present in β-cells. To explore the therapeutic potential of NN414, healthy non-diabetic Sprague-Dawley rats were subjected to insulin-induced hypoglycaemia or saline control for three consecutive days to induce a defective counterregulatory response to subsequent hypoglycaemia. On day four, NN414 (0.6mg/Kg, i.v.) was administered 30 minutes before a hyperinsulinaemic-hypoglycaemic clamp was performed. Animals receiving NN414 displayed an increased epinephrine response to hypoglycaemia and decreased glucose infusion rate [34]. Administration of the K_{ATP} channel blocker glibenclamide directly into the VMH post-NN414 injection decreased epinephrine response and increased glucose infusion rate, demonstrating that the improvements were due to effects on the K_{ATP} channel [34]. To explore whether similar improvements in the counter-regulatory response to hypoglycaemia could be achieved in Type 1 diabetes, the authors replicated the study in BB diabetic rats. As observed in healthy Sprague-Dawley rats, three days of antecedent hypoglycaemia significantly reduced the secretion of epinephrine and increased the glucose infusion rate to a hypoglycaemic challenge. Diabetic rats treated with NN414 showed a significant increase in epinephrine secretion and a decrease in glucose infusion rate in response to hypoglycaemia during the clamp. Although epinephrine secretion in response to hypoglycaemia was blunted in the BB diabetic rats compared to control rats, treatment with NN414 improved responses compared to vehicle-treated counterparts [34]. Overall, this study demonstrated a pivotal role for K_{ATP} channels in glucose sensing.

Potassium channel openers – to man

A clinical trial was devised to determine whether K_{ATP} channel opening drugs could improve the detection and responsiveness to hypoglycaemia in those with established Type 1 diabetes [35]. Participants were administered a single dose of diazoxide, a K_{ATP} channel opener, 2 hours before undergoing a hyperinsulinaemic-hypoglycaemic clamp. Ingestion of the K_{ATP} channel opener diazoxide amplified epinephrine and norepinephrine secretion and decreased glucose infusion rate during a hypoglycaemic clamp [35] in keeping with the findings of Fan et al., [34]. These results are indicative of improved glucose sensing and responsiveness. Several participants had a reduced response to diazoxide treatment compared to other participants. Genetic screening identified that participants with an E23K polymorphism of the K_{ATP} channel had reduced response to the drug [35]. This study highlights the importance of K_{ATP} channels in hypoglycaemia detection and strongly suggests that K_{ATP} channel opening is integral for a functional counterregulatory response in people with Type 1 diabetes. Notably, the E23K was present in 58% study population, suggesting the need for a more stratified dosing response in the future. Larger , more extensive studies are needed however to assess the therapeutic potential of K_{ATP} channel openers in clinical practice.

These studies collectively pose an alternative therapeutic use for KATP channel openers in treating defective CRR in Type 1 diabetes. *In vivo* studies allow initial drug efficacy testing, while *in vitro* studies allow further investigation into mechanisms. Clinical trials remain the gold standard for investigating novel therapies in Type 1 diabetes (Figure 4). Nevertheless, the impact of *in vivo* and *in vitro* studies on advancements in clinical practice should not be overlooked.

Example 2: Habituation, Exercise, and Dishabituation

First described in the early 1930s and later defined by Thompson and Spencer in the 1960s [36], habituation refers to the most basic form of memory. Simply put, habituation is defined as "a reduction in a behavioural response that is resultant from repeated exposure to a stimulus" [36, 37]. The principle also extends to reduced psychological and physiological responses [38]. An established

biological example of habituation is the gill-withdrawal reflex displayed in *Aplysia* (sea slugs). Repeated application of a tactile stimulus to either the siphon or mantle shelf of the *Aplysia* results in habituation to the stimulus, and the gill is no longer withdrawn [39]. Thompson and Spencer initially proposed nine well-described characteristics of habituation, with a tenth characteristic recently introduced [37].

Does IAH develop through habituation?

Many features associated with Type 1 diabetes and IAH can be viewed as a form of habituation. Impaired awareness of hypoglycaemia develops due to repeated exposure to a stimulus (hypoglycaemia). IAH leads to reduced hormonal and autonomic responses to hypoglycaemia which could be viewed as "habituation to hypoglycaemia" [40]. Furthermore, awareness of hypoglycaemia can be reinstated by avoidance of hypoglycaemia [41], in keeping with the second characteristic of habituation, termed "spontaneous recovery" [37]. Therefore, our group hypothesised that introducing an acute novel stimulus could potentially restore hypoglycaemia awareness in keeping with characteristic 8: dishabituation. Dishabituation is the interruption of the habituated response, usually by the introduction of a strong, novel stimulus.

Dishabituation – from rodent

In the following studies, a single episode of high-intensity exercise was used as a dishabituating stimulus. This hypothesis was initially tested in male Sprague-Dawley rats exposed to 4-weeks of recurrent insulin-induced hypoglycaemia or saline control three times weekly to induce defective counter-regulation [38]. Experimental groups were subdivided into i) no exercise, ii) low-intensity exercise or iii) high-intensity exercise. Animals underwent exercise 24 hours before being subjected to a hyperinsulinaemic-hypoglycaemic clamp. As anticipated, there was a significant reduction in the secretion of epinephrine and glucagon in response to hypoglycaemia in animals exposed to antecedent hypoglycaemia with no or low-intensity exercise.

In contrast, animals exposed to a single episode of high-intensity exercise following 4-weeks of recurrent hypoglycaemia had increased epinephrine and glucagon secretion to the hypoglycaemic challenge [38]. These increases were comparable to control animals [38]. This study supports the hypothesis that IAH may develop through habituation and, importantly, that restoration of hypoglycaemia awareness might be possible through dishabituation. Later work in this area successfully employed cold as an alternate dishabituating stimulus. Applying the same protocol, rats underwent 4-weeks of recurrent hypoglycaemia before cold-exposure intervention and a hyperinsulinaemic-hypoglycaemic clamp [42]. In line with the previous study, epinephrine secretion to experimental hypoglycaemia was significantly increased in animals exposed to antecedent hypoglycaemia and cold (4^{o}C for 4.5 hrs) compared to recurrent hypoglycaemia alone [42]. This study further supports the hypothesis that IAH is a form of habituation. However, neither of these studies included a model of Type 1 diabetes or diagnosed true IAH by way of impaired symptom response to clinical hypoglycaemia.

Dishabituation -to man

In a randomised cross-over clinical study, participants with Type 1 diabetes and IAH were subjected to a single intervention of high-intensity training (HIT) or rest before crossing over into the alternate arm of the study [40]. Participants were subjected to an episode of experimental hypoglycaemia induced by a hyperinsulinaemic-hypoglycaemic clamp preceding each intervention. Counterregulatory hormones along with symptom awareness and cognitive ability were tested pre-and post-intervention. As a result of HIT intervention, participants exhibited a significant increase in

epinephrine and glucagon secretion to experimental hypoglycaemia [40]. Additionally, both symptom awareness and cognitive ability were improved following the HIT intervention [40]. As previously shown *in vivo*, this study indicates that awareness of hypoglycaemia can be restored, at least partially, by introducing a dishabituating stimulus [40]. Cumulatively, this strongly implies that IAH may be a form of habituation arising from exposure to recurrent hypoglycaemia.

In this case, *in vivo* experimentation enabled the testing of novel therapies before trialling the hypothesis in a cohort of individuals with Type 1 diabetes and IAH. The latter clinical study allowed drawbacks experienced *in vivo* to be overcome, such as the inclusion of the Type 1 diabetes phenotype and collection of unfeasible data in rodents, i.e., symptomatic awareness. These examples highlight the benefits of translational research. If employed on a larger scale, this therapy could be revolutionary in treating IAH and improving our understanding of the mechanism responsible for the adaptation in the first instance.

Example 3: Neonatal diabetes – from man

So far, this review has discussed examples whereby data from *in-vivo* research has led to changes in clinical practice. Translational research, however, is a bi-directional process. In recent years, improvements in whole-genome sequencing and genome-wide analysis sequencing (GWAS) have revealed novel disease-associated mutations that can be introduced to rodent models to study mechanisms. One such example is the treatment regimen for individuals diagnosed with permanent neonatal diabetes (ND). Genetic sequencing of the KCNJ11 gene (coding for the Kir6.2 subunit of the K_{ATP} channel) identified several heterozygous missense mutations in babies with neonatal diabetes [43]. This mutation results in a reduced ability of the K_{ATP} channel to close in response to increased ATP, therefore inhibiting insulin release from pancreatic β-cells. Historically, neonatal diabetes was treated with exogenous insulin that can lead to hypoglycaemia. Sulfonylureas, such as glibenclamide, induce closure of the KATP channels, restoring glucose-stimulated insulin response lost in neonatal diabetes [44]. Treatment with sulfonylureas is a superior treatment option to insulin in this instance as they improve clinical presentation and quality of life [45]. As sulfonylureas promote endogenous insulin secretion, there is an increased risk of hypoglycaemia in individuals with impaired renal or hepatic function. The risk of hypoglycaemia is most apparent with long-acting drugs such as glibenclamide.

Neonatal diabetes – to mouse

Identification of the mutation underlying neonatal diabetes in humans led to a mouse model with the β- cell-specific human Kir6.2 mutation responsible for ND, dubbed the β-V59M model, to be generated [46]. These mice develop severe diabetes within 5-weeks of birth and present with hyperglycaemia and hypoinsulinemia. In addition, isolated islets from these animals have decreased β-cell and insulin content along with abnormal morphology [46]. This mouse model has a disease phenotype similar to that of human ND, allowing further research into the treatment of ND and investigations into molecular mechanisms that are unfeasible in humans (Figure 4).

Summary

One hundred years on, hypoglycaemia remains a major drawback of insulin therapy despite significant advancements in formulation, delivery, and education surrounding insulin. With impaired awareness of hypoglycaemia affecting around 25% of individuals with Type 1 diabetes and hypoglycaemia contributing to other complications, expanding our understanding of the mechanisms that underlie IAH is critical. In this review, we have drawn attention to some examples that put the “bench to

bedside" practice into use. *In vivo* models allow us to trial novel therapeutics such as NN414 and enable researchers to delve deeper into the mechanisms that underpin disease.

References (max. 50)

1. Vecchio I, Tornali C, Bragazzi NL, Martini M. The Discovery of Insulin: An Important Milestone in the History of Medicine. *Frontiers in Endocrinology* 2018; **9**:613.
2. Best CH, Scott DA. THE PREPARATION OF INSULIN. *Journal of Biological Chemistry* 1923; **57**:709-723.
3. Bliss M. Rewriting Medical History: Charles Best and the Banting and Best Myth. *Journal of the History of Medicine and Allied Sciences* 1993; **48**:253-274.
4. Cryer PE. The barrier of hypoglycemia in diabetes. *Diabetes* 2008; **57**:3169-3176.
5. Mokan M, Mitrakou A, Veneman T, Ryan C, Korytkowski M, Cryer P*, et al.* Hypoglycemia unawareness in IDDM. *Diabetes Care* 1994; **17**:1397-1403.
6. Powell AM, Sherwin RS, Shulman GI. Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. Reversibility and stimulus specificity of the deficits. *J Clin Invest* 1993; **92**:2667-2674.
7. Galassetti P, Tate D, Neill RA, Morrey S, Wasserman DH, Davis SN. Effect of antecedent hypoglycemia on counterregulatory responses to subsequent euglycemic exercise in type 1 diabetes. *Diabetes* 2003; **52**:1761-1769.
8. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans. *Diabetes* 1991; **40**:223-226.
9. Seyed Ahmadi S, Westman K, Pivodic A, Ólafsdóttir AF, Dahlqvist S, Hirsch IB*, et al.* The Association Between HbA1c and Time in Hypoglycemia During CGM and Self-Monitoring of Blood Glucose in People With Type 1 Diabetes and Multiple Daily Insulin Injections: A Randomized Clinical Trial (GOLD-4). *Diabetes Care* 2020; **43**:2017-2024.
10. Minimizing Hypoglycemia in Diabetes. *Diabetes Care* 2015; **38**:1583-1591.
11. Quesada I, Tuduri E, Ripoll C, Nadal A. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. *J Endocrinol* 2008; **199**:5-19.
12. Flanagan DE, Keshavarz T, Evans ML, Flanagan S, Fan X, Jacob RJ*, et al.* Role of Corticotrophin-Releasing Hormone in the Impairment of Counterregulatory Responses to Hypoglycemia. *Diabetes* 2003; **52**:605-613.
13. Song Z, Routh VH. Recurrent hypoglycemia reduces the glucose sensitivity of glucose-inhibited neurons in the ventromedial hypothalamus nucleus. *Am J Physiol Regul Integr Comp Physiol* 2006; **291**:R1283-1287.
14. McNeilly AD, Gallagher JR, Dinkova-Kostova AT, Hayes JD, Sharkey J, Ashford MLJ*, et al.* Nrf2-Mediated Neuroprotection Against Recurrent Hypoglycemia Is Insufficient to Prevent Cognitive Impairment in a Rodent Model of Type 1 Diabetes. *Diabetes* 2016; **65**:3151-3160.
15. Suh SW, Aoyama K, Chen Y, Garnier P, Matsumori Y, Gum E*, et al.* Hypoglycemic neuronal death and cognitive impairment are prevented by poly(ADP-ribose) polymerase inhibitors administered after hypoglycemia. *J Neurosci* 2003; **23**:10681-10690.
16. Fisher BM, Heller SR. Mortality, cardiovascular morbidity and possible effects of hypoglycaemia on diabetic complications. In *Hypoglycaemia in Clinical Diabetes (2nd edn)*. Chichester: John Wiley and Sons; 2007; 265-283.
17. Wright RJ, Frier BM. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes Metab Res Rev* 2008; **24**:353-363.
18. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. *Diabet Med* 2008; **25**:501-504.
19. McNeilly AD, McCrimmon RJ. Impaired hypoglycaemia awareness in type 1 diabetes: lessons from the lab. *Diabetologia* 2018; **61**:743-750.
20. Farrell CM, McCrimmon RJ. Clinical approaches to treat impaired awareness of hypoglycaemia. *Ther Adv Endocrinol Metab* 2021; **12**:20420188211000248.
21. Farrell CM, McNeilly AD, Hapca SM, McCrimmon RJ. Real-time Continuous Glucose Monitoring During a Hyperinsulinemic-Hypoglycemic Clamp Significantly Underestimates the Degree of Hypoglycemia. *Diabetes Care* 2020; **43**:e142-e143.

22. Pandey S, Dvorakova MC. Future Perspective of Diabetic Animal Models. *Endocr Metab Immune Disord Drug Targets* 2020; **20**:25-38.
23. Lenzen S. Animal models of human type 1 diabetes for evaluating combination therapies and successful translation to the patient with type 1 diabetes. *Diabetes Metab Res Rev* 2017; **33**.
24. Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI. Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest* 1994; **93**:1677-1682.
25. McCrimmon RJ, Shaw M, Fan X, Cheng H, Ding Y, Vella MC*, et al.* Key role for AMP-activated protein kinase in the ventromedial hypothalamus in regulating counterregulatory hormone responses to acute hypoglycemia. *Diabetes* 2008; **57**:444-450.
26. Fan X, Ding Y, Brown S, Zhou L, Shaw M, Vella MC*, et al.* Hypothalamic AMP-activated protein kinase activation with AICAR amplifies counterregulatory responses to hypoglycemia in a rodent model of type 1 diabetes. *Am J Physiol Regul Integr Comp Physiol* 2009; **296**:R1702-1708.
27. Oomura Y, Ono T, Ooyama H, Wayner MJ. Glucose and osmosensitive neurones of the rat hypothalamus. *Nature* 1969; **222**:282-284.
28. Routh VH, Hao L, Santiago AM, Sheng Z, Zhou C. Hypothalamic glucose sensing: making ends meet. *Front Syst Neurosci* 2014; **8**:236.
29. McCrimmon RJ, Evans ML, Fan X, McNay EC, Chan O, Ding Y*, et al.* Activation of ATP-Sensitive K+ Channels in the Ventromedial Hypothalamus Amplifies Counterregulatory Hormone Responses to Hypoglycemia in Normal and Recurrently Hypoglycemic Rats. *Diabetes* 2005; **54**:3169-3174.
30. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol* 2007; **8**:774-785.
31. Dabrowski M, Larsen T, Ashcroft FM, Bondo Hansen J, Wahl P. Potent and selective activation of the pancreatic beta-cell type K(ATP) channel by two novel diazoxide analogues. *Diabetologia* 2003; **46**:1375-1382.
32. Carr RD, Brand CL, Bodvarsdottir TB, Hansen JB, Sturis J. NN414, a SUR1/Kir6.2-Selective Potassium Channel Opener, Reduces Blood Glucose and Improves Glucose Tolerance in the VDF Zucker Rat. *Diabetes* 2003; **52**:2513-2518.
33. Zdravkovic M, Kruse M, Rost KL, Møss J, Kecskes A, Dyrberg T. The effects of NN414, a SUR1/Kir6.2 selective potassium channel opener, in healthy male subjects. *J Clin Pharmacol* 2005; **45**:763-772.
34. Fan X, Ding Y, Cheng H, Gram DX, Sherwin RS, McCrimmon RJ. Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K(+) channel opener? *Diabetes* 2008; **57**:3327-3334.
35. George PS, Tavendale R, Palmer CNA, McCrimmon RJ. Diazoxide Improves Hormonal Counterregulatory Responses to Acute Hypoglycemia in Long-standing Type 1 Diabetes. *Diabetes* 2015; **64**:2234-2241.
36. Thompson RF, Spencer WA. Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol Rev* 1966; **73**:16-43.
37. Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J*, et al.* Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiol Learn Mem* 2009; **92**:135-138.
38. McNeilly AD, Gallagher JR, Huang JT-J, Ashford MLJ, McCrimmon RJ. High-Intensity Exercise as a Dishabituating Stimulus Restores Counterregulatory Responses in Recurrently Hypoglycemic Rodents. *Diabetes* 2017; **66**:1696-1702.
39. Pinsker H, Kupfermann I, Castellucci V, Kandel E. Habituation and dishabituation of the gill-withdrawal reflex in Aplysia. *Science* 1970; **167**:1740-1742.
40. Farrell CM, McNeilly AD, Fournier P, Jones T, Hapca SM, West D*, et al.* A randomised controlled study of high intensity exercise as a dishabituating stimulus to improve hypoglycaemia awareness in people with type 1 diabetes: a proof-of-concept study. *Diabetologia* 2020; **63**:853-863.

41. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA. Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. *Lancet* 1994; **344**:283-287.
42. Vickneson K, Blackburn J, Gallagher JR, Evans ML, de Galan BE, Pedersen-Bjergaard U*, et al.* Cold-induced dishabituation in rodents exposed to recurrent hypoglycaemia. *Diabetologia* 2021; **64**:1436-1441.
43. Gloyn AL, Pearson ER, Antcliff JF, Proks P, Bruining GJ, Slingerland AS*, et al.* Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 2004; **350**:1838-1849.
44. Pearson ER, Flechtner I, Njølstad PR, Malecki MT, Flanagan SE, Larkin B*, et al.* Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 2006; **355**:467-477.
45. Ashcroft FM, Puljung MC, Vedovato N. Neonatal Diabetes and the K(ATP) Channel: From Mutation to Therapy. *Trends Endocrinol Metab* 2017; **28**:377-387.
46. Girard CA, Wunderlich FT, Shimomura K, Collins S, Kaizik S, Proks P*, et al.* Expression of an activating mutation in the gene encoding the KATP channel subunit Kir6.2 in mouse pancreatic beta cells recapitulates neonatal diabetes. *J Clin Invest* 2009; **119**:80-90.
47. Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. *Diabetologia* 1993; **36**:771-777.
48. King AJ. The use of animal models in diabetes research. *Br J Pharmacol* 2012; **166**:877-894.
49. Chatzigeorgiou A, Halapas A, Kalafatakis K, Kamper E. The Use of Animal Models in the Study of Diabetes Mellitus. *In Vivo* 2009; **23**:245-258.

446 **Table 1:** Overview of categories of symptomatic responses to hypoglycaemia [47].

Category	Symptoms
Autonomic	Sweating Palpations Shaking Hunger
Neuroglycopenic	Confusion Drowsiness Odd behaviour Speech difficulties Incoordination
Malaise	Nausea Headache

447

448 **Table 2:** Overview of frequently used rodent models of Type 1 diabetes along with their pros and cons
449 and method of disease induction [23, 48, 49].

Rodent Model	Pros	Cons	Method of induction
Chemical Induction			
Streptozotocin (STZ) or Alloxan	+ Simple + Inexpensive	- Does not mirror human disease well - Off-target effects on other essential organs - No strong autoimmune features	- STZ leads to the destruction of pancreatic β-cells mediated via GLUT2 transporters (high dose) or immune and inflammatory destruction of the β-cell (low dose). - Alloxan is a cytotoxic glucose analogue that accumulates within the pancreatic β-cell inducing ROS and superoxide radicals generation. - Selective inhibition of glucose-induced insulin secretion
Autoimmune Models			
NOD (non-obese diabetic) mice	+ More representative of human disease onset and progression	- Expensive - Gender bias (female > male) - Requires sterile conditions - Onset is unpredictable	- Leukocytic infiltration of pancreatic islets leading to insulitis
BB (bio-breeding) rats	+ Useful when investigating the genetics	- Lymphocytopenia	- Carry two T1D susceptibility genes MNC class II RT1u and GIMAP5. Gimap5

	+ Exhibit many clinical features typical of diabetes in humans		mutation leads to severe T cell lymphopenia and impaired development and function of regulatory T cells
LEW.1AR1-*iddm* (IDDM) rats	+ Long pre-diabetic state for immune profiles + Useful for intervention studies + Longer life expectancy compared to other models	- Expensive due to longevity	- Apoptotic β-cell death induced by pro-inflammatory cytokine release from infiltrating immune cells - Characterised by MHC Lewis.1AR1 haplotype
Genetic Induction			
AKITA	+ Very similar phenotype to human diabetes	- Complete loss of insulin, animals can become very unwell	- Spontaneous mutation of Ins2 gene leads to incorrect folding of insulin and toxicity in pancreatic β cells.

450

451

i. Figure legends

Figure 1:

Timeline highlighting the discovery of insulin and steps that led to the first successful treatment of Type 1 diabetes and subsequent discoveries that have continued to innovate and improve the treatment of Type 1 diabetes. Particular attention is drawn to the discoveries in which translational research played a crucial role. Image created with BioRender.com.

Figure 2:

A schematic highlighting the differences in symptomatic response to hypoglycaemia in people without diabetes, those with diabetes and intact awareness, and those with diabetes who have impaired awareness of hypoglycaemia (IAH). **a**: suppression of the insulin release, **b**: release of counterregulatory hormones, **c**: onset of neurogenic symptoms, and **d**: decline in cognition. As per the diagram, the first line of defence against hypoglycaemia is suppression of insulin secretion; however, this is lost in Type 1 diabetes. Next, the release of counterregulatory hormones, e.g. glucagon and epinephrine, occurs, followed by the onset of autonomic and neuroglycopenic symptoms in response to hypoglycaemia. In people with IAH, the glucagon response to hypoglycaemia is lost. In addition, the plasma glucose concentration at which the symptomatic response occurs is suppressed until lower glucose concentrations. In summary, those with IAH take longer to experience symptoms meaning they face more severe hypoglycaemic events and have reduced hormonal capacity to overrule hypoglycaemia. Figure created using Microsoft PowerPoint.

Figure 3:

Diagram showing mechanism of action in a glucose-excitatory neuron in response to a high glucose concentration. In short, glucose enters the cell via GLUT1/3 glucose transporter, is phosphorylated by glucokinase (GK) and ultimately converted to pyruvate before entering the mitochondria to fuel the tricarboxylic acid cycle (TCA). This process results in a high yield of ATP, which causes inhibition of the ATP sensitive potassium channel (K_{ATP}), resulting in cell depolarisation and opening the voltage-dependent calcium channel (VDCC). Finally, an increase in intracellular calcium induces neurotransmitter release. Image created with BioRender.com.

Figure 4:

A graphic summarising the strength of translational research and two examples showing that this relationship is not unilateral. The example marked by burgundy numbers refers to work discussed under *"other examples of translational research,"* The example marked by blue numbers refers to the work discussed under *"Example 1"*. Image created using BioRender.com.