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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.”

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1 **Abstract**

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

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

17 ***The Discovery of Insulin***

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

31 ***Insulin - A double-edged sword***

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

36 ***The Counterregulatory Response to Hypoglycaemia***

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

51 ***Defective counterregulatory response to hypoglycaemia in Type 1 diabetes***

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

55 reduced secretion of epinephrine in response to hypoglycaemia due to previous hypoglycaemia also
56 extends to non-diabetic humans [8]. Collectively, these factors result in longer recovery times to
57 glucose homeostasis and an increased risk of hypoglycaemia. For these reasons, despite
58 improvements in the diagnosis and treatment of diabetes, exposure to hypoglycaemia remains the
59 most feared consequence of insulin treatment. The recent GOLD-4 clinical trial reported that
60 individuals with Type 1 diabetes and average blood glucose of 8mmol/L spend 12% of their time below
61 3.9mmol/L and 4.9% below 3mmol/L [9]. Plasma blood glucose was measured using self-monitoring
62 blood glucose meters or continuous glucose monitors (CGM). Time spent in the hypoglycaemic range
63 was reduced in individuals with higher average blood glucose and those using CGM [9]. Non-severe
64 hypoglycaemia is estimated to occur twice weekly in people with Type 1 diabetes, whereas episodes
65 of severe hypoglycaemia are predicted to occur between 1-3 annually in a large proportion of those
66 with Type 1 diabetes [4, 10]. Severe hypoglycaemia is defined as an event that requires outside
67 intervention in order to recover. The incidence of hypoglycaemia is greatly influenced by the duration
68 of diabetes, with risk increasing by 3-fold between patients with < 5-year duration and >15 years of
69 duration [10]. This increased risk can be attributed to further β -cell loss, decreased glucagon secretion
70 in response to hypoglycaemia after five years of disease, and the development of impaired awareness
71 of hypoglycaemia. Although the exact mechanisms underlying the loss of glucagon secretion in
72 response to hypoglycaemia remain unclear, it has been hypothesised that the loss of regulatory beta-
73 cell signals such as zinc, insulin or γ -aminobutyric acid (GABA) or basal hypersecretion of somatostatin
74 may all contribute to diminished glucagon secretion[11]. Likewise, reduced alpha cell mass in long-
75 duration Type 1 diabetes and the increase in bi-hormonal expressing cells suggest that alpha to beta
76 cell transition may play a role in this phenomenon.

77 ***Complications of Insulin-Induced Hypoglycaemia***

78 *Hypoglycaemia and the brain*

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

89 *Hypoglycaemia and cardiovascular disease*

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

98 *Hypoglycaemia and renal disease*

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

102 ***Impaired Awareness of Hypoglycaemia***

103 Originally termed *hypoglycaemia unawareness* [5], impaired awareness of hypoglycaemia (IAH) refers
104 to the clinical phenomena whereby reductions in blood glucose go undetected until much lower
105 concentrations. The onset of IAH relates to the duration of diabetes and prior exposure to recurrent
106 hypoglycaemia. Depth, duration, and the number of prior hypoglycaemic episodes are also
107 contributory factors. IAH affects approximately 25% of people with Type 1 diabetes [18]. Although the
108 mechanisms resulting in the development of IAH are poorly understood, it is likely to be a combination
109 of multiple factors, including i) an adaptation to low blood glucose levels, ii) changes in glucose or
110 alternative fuel transport and storage, iii) changes in intracellular glucose metabolism resulting in
111 altered neuronal firing and iv) changes in intracellular communication and neurotransmitter release
112 [19]. *Figure 2* depicts the shift in symptomatic response to hypoglycaemia in those without diabetes
113 and people with Type 1 diabetes with differing awareness of hypoglycaemia. Hormonal responses to
114 hypoglycaemia in those with IAH occur at lower glucose concentrations when compared to those with
115 normal awareness [5]. IAH was initially diagnosed using symptom questionnaires; however, this has
116 obvious flaws. Therefore, Mookan et al. [5] implemented a more robust determination of IAH by
117 inducing hypoglycaemia and defining individuals who exerted autonomic symptoms two standard
118 deviations below people with normal awareness as having IAH.

119 ***The barrier of hypoglycaemia***

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

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

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

141 ***Current rodent models of diabetes***

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

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

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

158 With glucose as its primary fuel source and lack of storage capacity, glucose-responsive machinery
159 must exist in the brain. The hypothalamus has been identified as a centre for glucose-sensing and
160 producing restorative outputs in response to hypoglycaemia [24-26]. In particular, the ventromedial
161 hypothalamus (VMH), comprised of the ventromedial nucleus (VMN) and arcuate nucleus (ARC), has
162 been highlighted as an invaluable brain region in the detection of and response to hypoglycaemia [25,
163 26]. Distinct populations of glucose-responsive neurons were first identified in the VMH in the late
164 1960s by Oomura et al. [27] and have since been described numerous times [28]. These neurons are
165 commonly referred to as glucose-excitatory (GE) and glucose-inhibitory (GI) neurons. GE neurons
166 depolarise in response to high glucose concentrations, whereas GI neurons depolarise in low glucose
167 concentrations. It is well appreciated that GE neurons are mechanistically similar to pancreatic β -cell,
168 with both cell types containing the enzyme glucokinase and the Kir6.2/SUR1 ATP sensitive potassium
169 channel. However, these cells are active over different glucose thresholds, with the periphery being
170 exposed to \sim five-fold higher glucose concentrations than those of the brain. Despite this, both GE
171 neurons and pancreatic β -cells depolarise in response to high glucose levels. This mechanism is
172 depicted in *Figure 3*.

173 ***The K_{ATP} channel***

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

181 ***Potassium channel openers – from rodent***

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

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

206 ***Potassium channel openers – to man***

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

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

226 ***Example 2: Habituation, Exercise, and Dishabituation***

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

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

236 ***Does IAH develop through habituation?***

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

246 ***Dishabituation – from rodent***

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

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

268 ***Dishabituation -to man***

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

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

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

287 **Example 3: Neonatal diabetes – from man**

288 So far, this review has discussed examples whereby data from *in-vivo* research has led to changes in
289 clinical practice. Translational research, however, is a bi-directional process. In recent years,
290 improvements in whole-genome sequencing and genome-wide analysis sequencing (GWAS) have
291 revealed novel disease-associated mutations that can be introduced to rodent models to study
292 mechanisms. One such example is the treatment regimen for individuals diagnosed with permanent
293 neonatal diabetes (ND). Genetic sequencing of the KCNJ11 gene (coding for the Kir6.2 subunit of the
294 K_{ATP} channel) identified several heterozygous missense mutations in babies with neonatal diabetes
295 [43]. This mutation results in a reduced ability of the K_{ATP} channel to close in response to increased
296 ATP, therefore inhibiting insulin release from pancreatic β -cells. Historically, neonatal diabetes was
297 treated with exogenous insulin that can lead to hypoglycaemia. Sulfonylureas, such as glibenclamide,
298 induce closure of the K_{ATP} channels, restoring glucose-stimulated insulin response lost in neonatal
299 diabetes [44]. Treatment with sulfonylureas is a superior treatment option to insulin in this instance
300 as they improve clinical presentation and quality of life [45]. As sulfonylureas promote endogenous
301 insulin secretion, there is an increased risk of hypoglycaemia in individuals with impaired renal or
302 hepatic function. The risk of hypoglycaemia is most apparent with long-acting drugs such as
303 glibenclamide.

304 **Neonatal diabetes – to mouse**

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

312 **Summary**

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

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

320

321

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- 445

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 insulinitis
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.

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451

452 i. Figure legends

453 **Figure 1:**

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

458 **Figure 2:**

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

471 **Figure 3:**

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

479 **Figure 4:**

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

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