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# RD Lawrence Lecture 2015. Old habits are hard to break

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# RD Lawrence Lecture 2015
# Old habits are hard to break: Lessons from the study of hypoglycaemia



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# Figure 1.

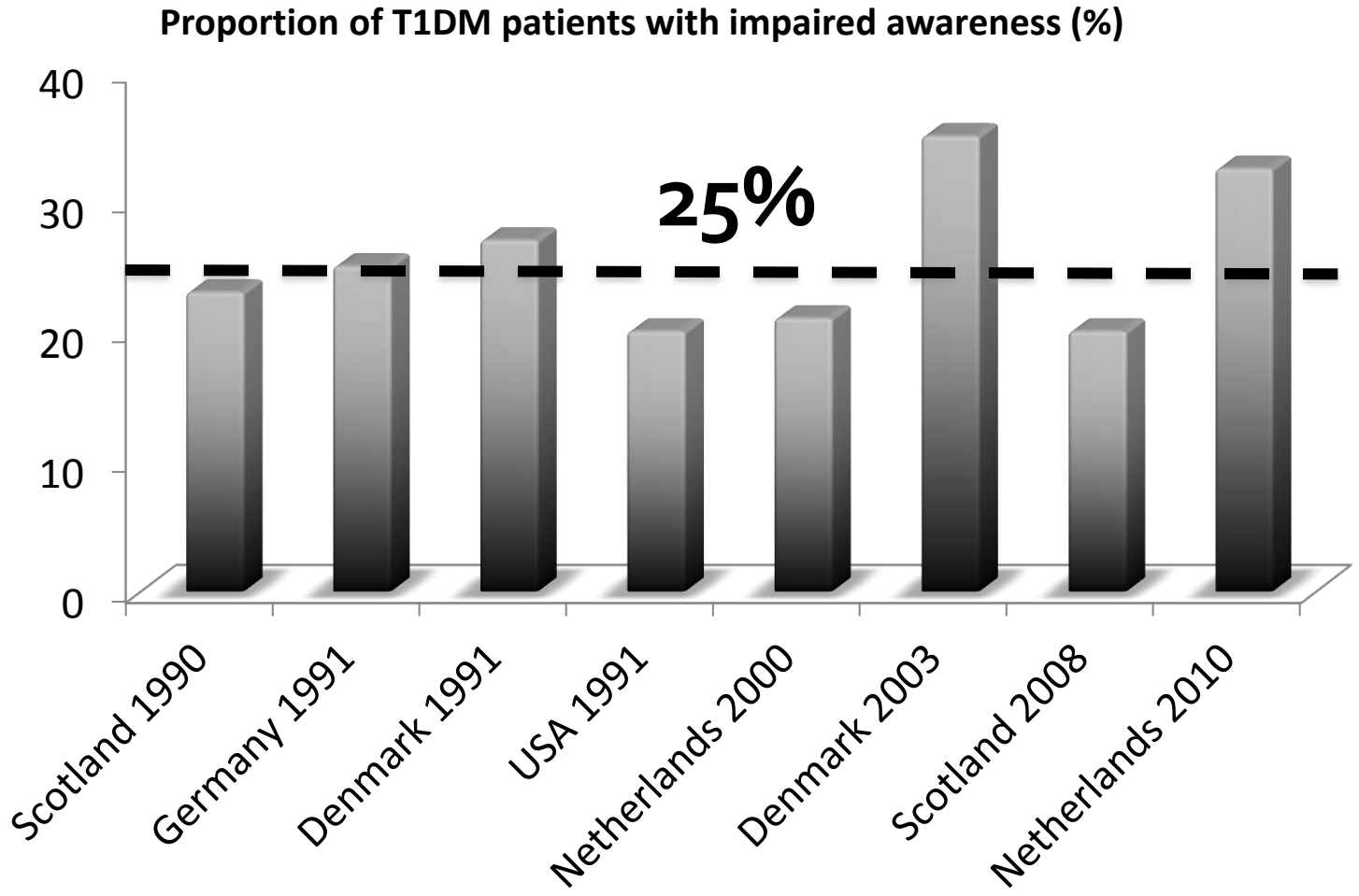

Proportion of T1DM patients with impaired awareness (%)
40
30
20
10
0
25%
Scotland 1990
Germany 1991
Denmark 1991
USA 1991
Netherlands 2000
Denmark 2003
Scotland 2008
Netherlands 2010

Figure 2.

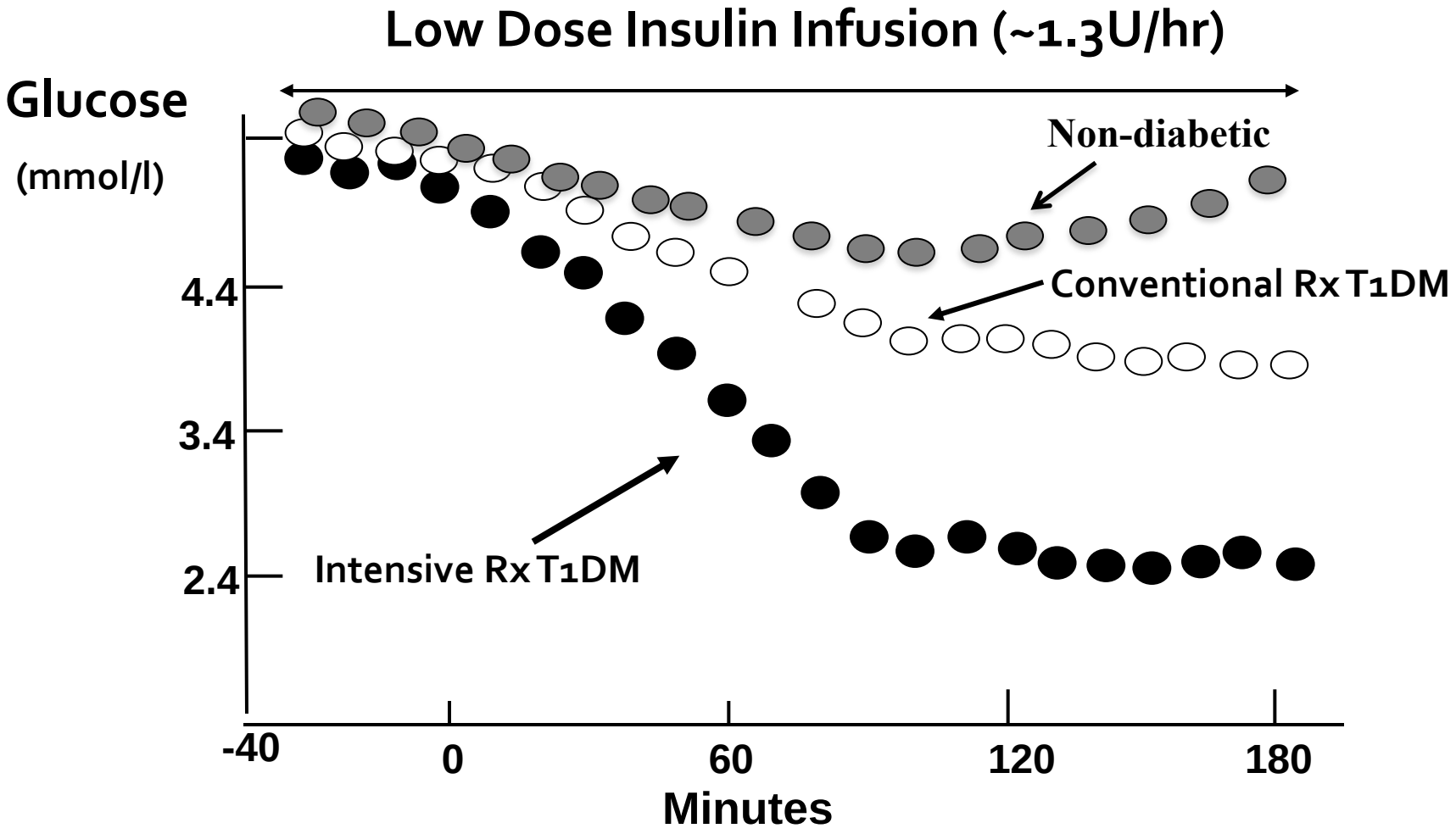
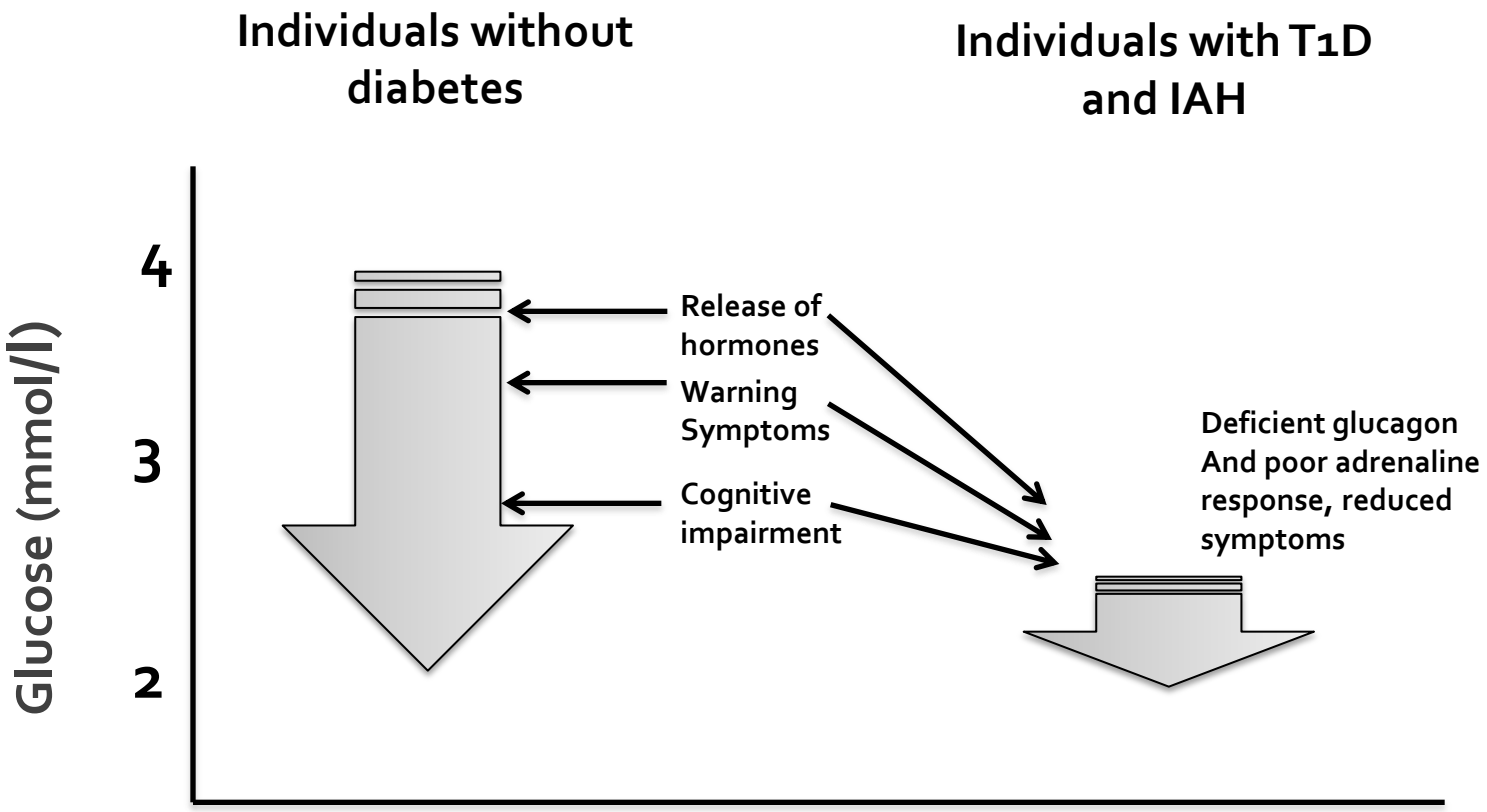
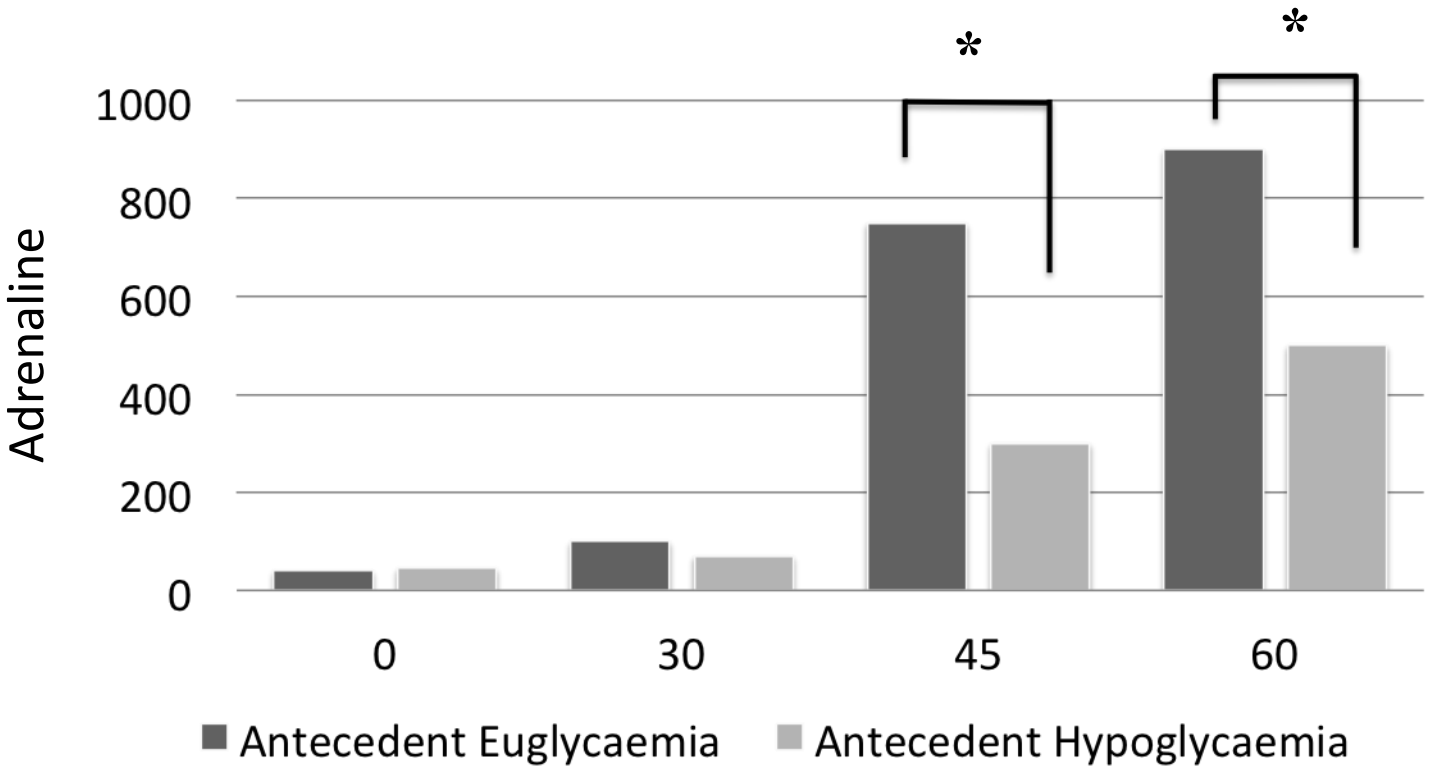

Low Dose Insulin Infusion (~1.3U/hr)
Glucose
(mmol/l)
Non-diabetic
Conventional Rx T1DM
Intensive Rx T1DM
4.4
3.4
2.4
-40
0
60
120
180
Minutes

**Figure 3.**

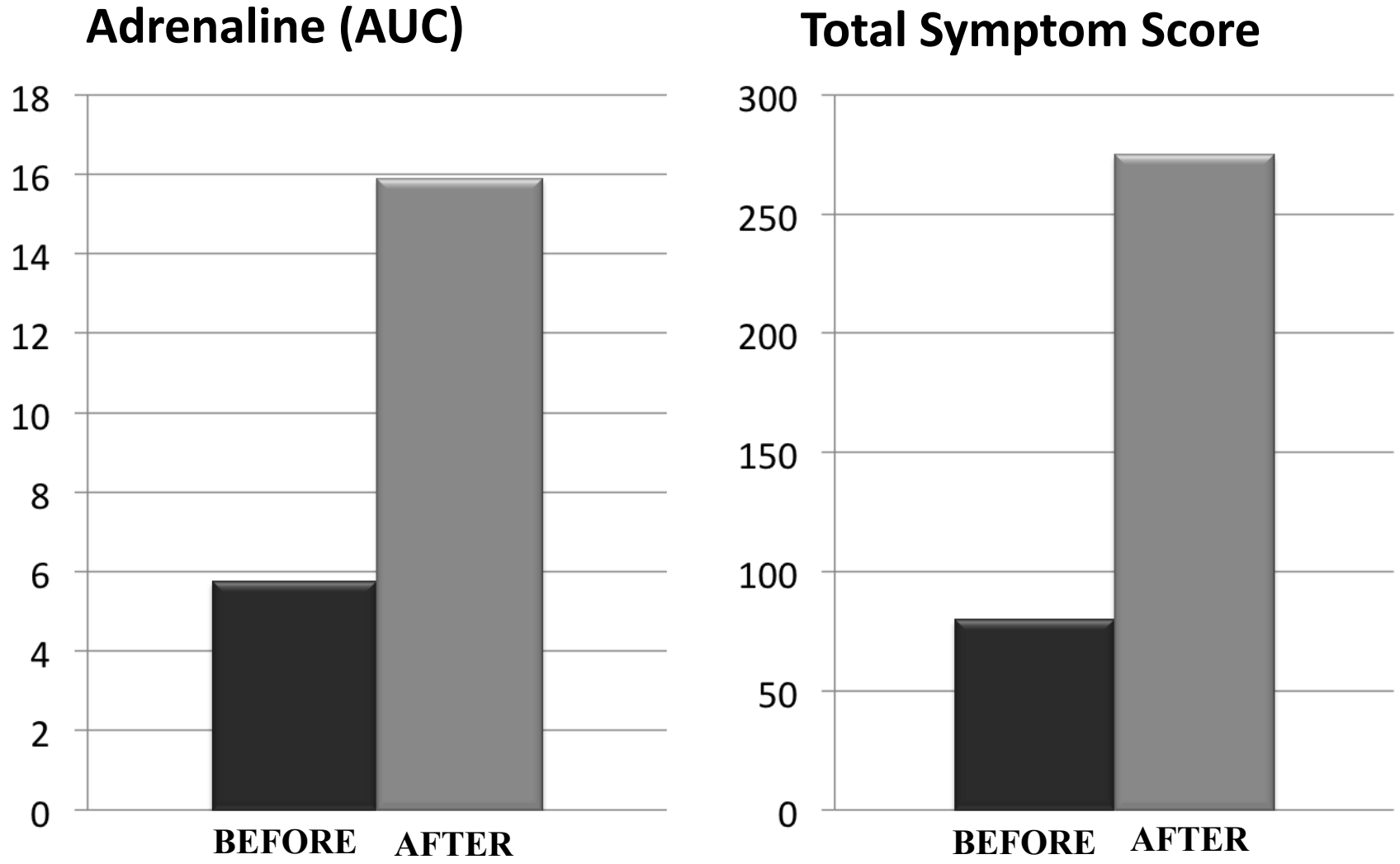

Individuals without diabetes
Individuals with T1D and IAH
Glucose (mmol/l)
4
3
2
Release of hormones
Warning Symptoms
Cognitive impairment
Deficient glucagon And poor adrenaline response, reduced symptoms

# Figure 4.




*Adapted from Heller and Cryer Diabetes 1991*

# Figure 5


Adrenaline (AUC)
18
16
14
12
10
8
6
4
2
0
BEFORE
AFTER
Total Symptom Score
300
250
200
150
100
50
0
BEFORE
AFTER

**RD Lawrence Lecture 2015**

# Old habits are hard to break: Lessons from the study of hypoglycaemia

**Rory J. McCrimmon**

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**Abstract:** Despite the introduction of newer technologies and improved insulin formulations, recurrent hypoglycaemia continues to affect the lives of many people with types 1 and 2 diabetes. Developing strategies or therapies designed to prevent or minimise hypoglycaemia risk is of outmost importance to help individuals safely achieve glycaemic targets. Novel, educational or behavioural approaches need to be based on a clear understanding of the mechanisms underpinning both the detection of hypoglycaemia and why repeated exposure to hypoglycaemia leads to the development of a clinical syndrome referred to as impaired awareness of hypoglycaemia (IAH). In this lecture, I propose that IAH may represent a form of learning called habituation; a response that at a cellular level represents a biological adaptation designed to protect the organism from future exposure to that stressor. In diabetes, this survival response to low glucose is, however, overwhelmed by high systemic insulin levels resulting from exogenous insulin therapy, leading to progressively more severe hypoglycaemia. Recognition of the underlying mechanism means that the development of IAH can perhaps be better understood and explained to individuals with diabetes, and novel therapeutic approaches such as dishabituation or cognitive behavioural therapies can be considered.

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## Introduction

Soon after the successful use of insulin in humans, physicians began to recognise that excessive insulin replacement lead to more marked falls in blood glucose and when the glucose fell below approximately 3.9 mmol/l the patient would become aware of hypoglycaemia with 'a characteristic train of symptoms' that became known as the 'hypoglycaemic reaction'[1]. RD Lawrence, in honour of whom this lecture is given, subsequently made the important observation in 1941 that the symptoms of hypoglycaemia changed over time and in fact 'reactions differ so much from the original ones that patients become dangerously unaware of their onset' [2]. This clinical phenomenon, came initially to be known as 'hypoglycaemia unawareness', but is now more appropriately referred to as 'impaired awareness of hypoglycaemia' (IAH). IAH is defined as 'a diminished ability to perceive the onset of acute hypoglycaemia' [3]. It is not a condition that is either present or absent in an individual, but reflects a continuum in which differing degrees of IAH can occur and can vary over time in any one individual. Why this happens in people with diabetes, and the mechanisms(s) that underpin this, remains unknown and will be the subject of this article.

The thesis of this lecture is that IAH results from co-ordinated intra- and extra-cellular physiological adaptations to recurrent hypoglycaemic stress that are in essence survival responses designed to protect the cell from subsequent exposure to glucose deprivation. I will also make the argument that these adaptations at the cellular level can be considered a form of learning that ultimately lead to a change in behaviour as well as physiological responses in humans. This change appears to bear the hallmarks of a behavioural response that is commonly referred to as 'habituation'. Therefore, this lecture will propose that habituation to hypoglycaemia may provide a paradigm in which we can consider how humans respond to recurrent hypoglycaemia and subsequently the approaches that may help in reversing this condition.

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## Hypoglycaemia in Clinical Practice

Hypoglycaemia is rare in people without diabetes, but due to fundamental defects in the mechanisms that regulate glucose homeostasis it is an all too common event in those with Type 1 (T1D) [4]. Mild hypoglycaemia is thought to occur on average about once or twice weekly [5], while severe hypoglycaemia (defined as the need for external assistance to recover) is experienced on average 1-3 times per person-year depending on duration of T1D. (e.g.[6]). Many people with T1D will not experience severe hypoglycaemia, and the average rate reflects a higher frequency of events in a small cohort of people.

Severe hypoglycaemia has a well-recognised morbidity and mortality in T1D [7], while, more recently, a concern has also been raised about the possible consequences of hypoglycaemia in people with T2D and established cardiovascular disease [8, 9]. However, it is important to also recognise that hypoglycaemia has more widespread effects on the brain, inducing a negative mood state (tense-tiredness) as well as negative appraisals of a life circumstance [10]. It is because of these emotional and cognitive effects of hypoglycaemia, in addition to the potentially catastrophic effects of severe hypoglycaemia, that many people with diabetes try to avoid ever becoming hypoglycaemic even if it is at the expense of poor overall glycaemic control.

Impaired awareness of hypoglycaemia is thought to effect around 20-25% of all people with T1D (Figure 1), and is generally more prevalent in older subjects with longer duration of T1D (e.g.[11]). Interestingly, it is less clearly associated with overall glycaemic control [11], although may develop following a period of rapid improvement in HbA1c with intensification of therapy [12]. Clinically, it is important to recognise because most studies report a significantly higher risk of severe hypoglycaemia (up to x6-fold) in those subjects with IAH [11].

## Why does hypoglycaemia develop in Type 1 Diabetes?

In the main, glucose homeostasis is regulated by the fine balance between the glucose lowering action of insulin and the opposing actions of glucagon, both secreted in a co-ordinated manner by pancreatic beta- and alpha cells

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respectively. In T1D, the destruction of pancreatic beta cells results in the need for insulin replacement that is usually delivered to the circulation from a subcutaneous depot following injection or infusion. This creates two major physiological problems in the context of hypoglycaemia. Firstly, peripheral rather than portal delivery of insulin means that to reach insulin levels in the portal vein sufficient to suppress hepatic glucose production requires systemic hyperinsulinaemia. This provides an additional stimulus to enhance glucose uptake into peripheral insulin-sensitive tissues increasing the risk of hypoglycaemia. Secondly there is also a loss of feedback suppression of insulin secretion when glucose levels fall. Effectively, the T1D individual is unable to supress exogenous insulin release during developing hypoglycaemia and its glucose lowering action therefore continues.

In addition, alpha-cell glucagon secretion is also fundamentally disturbed in T1D [13]. People with T1D do not secrete glucagon, the primary counterregulatory hormone in response to a hypoglycaemic challenge [13], a defect that is present in nearly all people with T1D by 5 years disease duration [14]. The reason for this remains unknown though is thought to reflect loss of an intra-islet signal that follows beta-cell destruction (for review see [4]). Interestingly, basal and post-prandial glucagon levels are increased in T1D implying that the alpha-cell is in some ways behaving like a beta-cell, i.e. classical stimulus-secretion coupling occurs and high glucose stimulates glucagon release whereas low glucose supresses it. This profound disturbance in islet structure and function explains why hypoglycaemia is far more likely to occur in people with T1D but it does not explain why the symptom-complex of hypoglycaemia changes overtime. For this we now turn to a series of very elegant studies performed in in the late 1980s and early 1990's.

**We Are What We Repeatedly Do**

Early studies using insulin infusions in people with T1D showed that with longer duration of T1D in addition to abnormal glucagon secretion, many subjects also produced a less robust catecholaminergic (adrenaline and noradrenaline) response to hypoglycaemia [15]. In examining why this latter defect developed,

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Amiel [12] discovered that intensification of therapy raised the threshold (lowered the glucose level) at which the counterregulatory catecholamines were released in response to hypoglycaemia (Figure 2), and this was followed by the seminal work of Heller [16] demonstrating that prior exposure to hypoglycaemia itself was the principal reason for this. Thus, increasing duration of disease and in particular exposure to recurrent episodes of hypoglycaemia appeared to result in further suppression of the hormonal (catecholaminergic) and related autonomic symptomatic response to subsequent hypoglycaemia (Figure 3).

This latter work in particular led to the proposal by Cryer and the subject of his 1994 Banting Lecture to the American Diabetes Association [17] that antecedent iatrogenic hypoglycaemia was the major factor that lead to the development of impaired awareness of hypoglycaemia. He coined the term Hypoglycaemia-Associated Autonomic Failure (HAAF), to describe the constellation of physiological responses to recurrent hypoglycaemia, namely defective glucose counterregulation (the result of combined deficiencies of the glucagon and adrenaline responses to falling glucose levels), impaired awareness of hypoglycaemia (loss of the warning, neurogenic symptoms of developing hypoglycaemia), and elevated glycaemic thresholds (lower glucose levels required) for autonomic activation and symptoms during effective intensive therapy [17]. He would also later add exercise [18] and sleep [19] as related parts of HAAF because both led independently to impaired counterregulation. Cryer considered HAAF to be 'maladaptive', because it increased an individual's risk of severe hypoglycaemia. However, HAAF really only describes a series of physiological responses to recurrent hypoglycaemia in humans and provides little information on why this develops. To try and answer this question the 1990s and naughties saw a series of studies, largely in animal models that attempted to understand a little more of the molecular biology that underpinned hypoglycaemia detection and response to recurrent hypoglycaemia.

**How and where do we detect hypoglycaemia?**

In order to understand the mechanisms underpinning HAAF, the next two decades saw a series of studies in animal models that examined where the body

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detected a falling glucose and what mechanisms were employed to detect hypoglycaemia. This area is reviewed elsewhere and will not be addressed in any detail in this article (see [4] for a recent review). What we have learnt from this work is that the detection of hypoglycaemia actually takes place in many distinct regions of the body that together form a network of specialised glucose sensing neurons [20]. Of these regions, the brain is perhaps most critical given its high energy demands relative to the body as a whole and the near absolute requirement of the brain for glucose as a fuel source.

In the brain, glucose-sensing regions contain specialized neurons that sense fluctuations in the glucose levels to which they are exposed and, critically, translate this into a change in membrane potential and action potential activity. Changes in neural activity then signal to effector organs such as liver or muscle where subsequent changes in glucose uptake or output help maintain or restore glucose homeostasis. Interestingly, like the pancreatic beta-cell, these neurons use the pancreatic isoform of glucokinase (GK) as a rate limiting step in glucose sensing and SUR-1 selective ATP-sensitive potassium channel ($K_{ATP}$) to translate the ATP-signal into a change in $K^+$ flux and hence a change in membrane potential, firing frequency and network activity [4]. Although, this research is mainly cell or animal based, a recent study from our laboratory have confirmed that these same mechanisms are also integral to hypoglycaemia detection in people with type 1 diabetes [21].

**Why does exposure to recurrent hypoglycaemia lead to impaired glucose sensing?**

This basic and preclinical research performed in animal models led to the recognition that changes within key glucose sensing regions of the brain such as the hypothalamus were instrumental in the development of impaired hypoglycaemia sensing in type 1 diabetes [22, 23]. What is less clear is why the glucose sensing properties of these neurons change following exposure to recurrent hypoglycaemia. Proposed mechanisms have included an increase in the uptake or metabolism of glucose and/ or the alternate fuel lactate, increased

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release of the inhibitory neurotransmitter, GABA, or an external signal such as glucocorticoid-mediated suppression of glucose sensing neurons (reviewed in [4, 24]). While these mechanisms probably all contribute at least in part to the development of impaired glucose sensing, as yet no clear understanding of the mechanism that drives defective counterregulation has emerged.

During insulin-induced hypoglycaemia glucose levels in the extra-cellular space in both rodents and humans fall dramatically and are approximately 10% of plasma levels [25, 26]. This degree of glucose (energy) deprivation represents a profound physiological challenge to the cell as is evident from the marked counterregulatory response hypoglycaemia stimulates in humans without diabetes. It therefore seems likely that in response to this challenge neurons will initiate a survival response that is designed to both prevent cell death and prepare to neuron so that it is better able to survive future episodes of profound glucose (energy) deprivation [24]. Consistent with this, rats exposed to repeated moderate hypoglycaemia show less neuronal cell death during subsequent severe hypoglycaemia that their controls [27]. This biological process is often referred to as pre-conditioning, a neuroprotective response that induces tolerance to the physiological stressor (in this case hypoglycaemia) [24]. Pre-conditioning is not unique to hypoglycaemia and is a highly conserved means by which cells respond to varied homeostatic challenges such as energy deprivation, ischaemia or temperature extremes. The problem in type 1 diabetes is that this biological adaptation occurs within the unphysiological context of high circulating insulin levels and ultimately no matter how well adapted neurons have become hypoglycaemia of sufficient degree will lead progressively to cognitive dysfunction and then cell death. Therefore, in essence a highly conserved biological adaptation to energy deprivation occurs but in an unphysiological context in type 1 diabetes (unregulated hyperinsulinaemia and absent glucagon responses) and this drives glucose down to levels that cannot sustain neuronal function so severe hypoglycaemia ensues.

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**Hypoglycaemia Habituation**

In the preceding discussion, I have proposed that at a biological level, recurrent hypoglycaemia may provide a pre-conditioning stimulus that initiates cellular adaptations designed to protect the cell during subsequent exposure to that same stimulus (tolerance). Hypoglycaemia in humans initiates far more than simple biological responses. Integrated physiological (counterregulatory hormonal), symptomatic (autonomic and neuroglycopenic symptoms), and behavioural (food seeking) responses are all activated by acute hypoglycaemia and can all be affected by recurrent hypoglycaemia. For instance, driving performance in T1D is significantly disrupted at relatively mild hypoglycaemia, yet subjects demonstrate a hesitation to take corrective action, i.e. judgement is impaired and the appropriate behavioural response not initiated [28].

In the 1940s scientists were looking for simple models in which they could try to gain some understanding of the neuronal mechanisms underlying more complex behavioural change. Thompson and Spencer provided a detailed and informative review of this area in the 1960's by considering the phenomenon of 'habituation' that had been studied through simple responses such as the sensory-motor reflex in cats [29]. Eric Kandel, who received the Nobel Prize in Physiology or Medicine in 2000, exemplified this approach through his pioneering research into the gill and siphon withdrawal reflex (GSWR) of Aplysia californica (a large shell-less sea snail or sea slug), in the 1960s and 1970s. The GSWR is an involuntary, defensive reflex that causes the sea hare's delicate siphon and gill to be retracted when the animal is disturbed. Repeated stimulation of the GSWR eventually results in a progressive decrease in the response; the process referred to as habituation.

Thompson and Spencer, proposed that habituation was a 'reduction of the psychological, behavioural or physiological response to a stimulus as a result of repeated or prolonged exposure' [29]. They described nine features that were characteristic of habituation, and we will consider these now in the context of recurrent hypoglycaemia.

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1. *Given that a particular stimulus elicits a response, repeated applications of that stimulus result in a decreased response (Habituation).* For hypoglycaemia this means that for habituation to have occurred repeated hypoglycaemia should result eventually in a diminished response to hypoglycaemia in that individual. This is in fact a hallmark of recurrent hypoglycaemia where repeated exposure to hypoglycaemia in people with or without Type 1 diabetes leads to decreased symptom and hormonal counterregulatory responses during a subsequent episode of controlled hypoglycaemia (Figure 4) [16, 30].
2. *If the stimulus is withheld, the response tends to recover over time (spontaneous recovery).* This means that if the stimulus (hypoglycaemia) is withheld the response (counterregulation) should being to recover. Again, this has been demonstrated clinically where strict hypoglycaemia avoidance lead to recovery of counterregulatory responses to subsequent hypoglycaemia (Figure 5) [31].
3. *If repeated series of habituation training and spontaneous recovery are given, habituation becomes successively more rapid.* This has not been tested in animal or human models, but implies that a memory of the stress response is retained. If the thesis of this lecture is correct and impaired hypoglycaemia awareness does reflect a form of habituation then any intervention designed to prevent hypoglycaemia, educational or pharmacological, needs to be reinforced because impaired awareness will develop more rapidly if that individual is exposed to hypoglycaemia again in the future.
4. *The more rapid the frequency of stimulation the more rapid and/ or pronounced its habituation.* In a study involving rodents, it was shown that more frequent daily hypoglycaemia over 6 weeks lead to a greater counterregulatory defect than intermediate exposure to hypoglycaemia over this period [32]. Whether repeated brief episodes of hypoglycaemia compared with more prolonged but less frequent episodes over an equivalent time period lead to a greater counterregulatory defect is unknown, but may explain the development of impaired awareness of hypoglycaemia seen with intensification of therapy.

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5. *The weaker the stimulus, the more rapid and/or pronounced is habituation. Strong stimuli may yield no significant habituation.* This criteria does not appear at first to be consistent with published research where a greater depth of prior hypoglycaemia was shown to have more widespread effects on the subsequent counterregulatory response [33]. However, more recently, habituation to physiological stressors has been shown to relate more closely to the severity of the stimulus, which means it is likely that habituation may to some extent depend on the nature of the stimulus [34, 35].
6. *The effects of habituation may proceed beyond the zero or asymptotic level.* This means that even when the habituated response is minimal it does not spontaneously recover if the habituating stimulus continues, i.e. that if people with IAH continue to experience hypoglycaemia, then even if no CRR is initiated their condition will not spontaneously resolve. In the study performed by Powell and Colleagues in Yale University in rodents daily hypoglycaemia over 6 weeks profoundly suppressed the counterregulatory response to subsequent hypoglycaemia and there was no evidence of spontaneous recovery while animals were still exposed to hypoglycaemia [32]. Indirect evidence in support of this feature is also found in an early clinical study reporting that asymptomatic nocturnal hypoglycaemia lead to decreased responses to hypoglycaemia induced the following day in subjects without diabetes [36]. This means that humans, even if they do not produce a counterregulatory response to hypoglycaemia will still habituate as long as they are exposed to low glucose levels.
7. *Habituation of a response to a given stimulus exhibits stimulus generalization to other stimuli.* This has also been shown for hypoglycaemia and might also be considered a form of cross-tolerance. An example of this came from the laboratory of Davis in Vanderbilt University where they were able to demonstrate that a prior prolonged moderate exercise exposure (alternate physiological stimulus) was shown to suppress counterregulatory responses to subsequent hypoglycaemia [18]. Conversely, prior hypoglycaemia was shown to

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supress the physiological stress response to subsequent moderate exercise [37]. Similarly, in people with well–controlled T1D, catecholaminergic responses to a cold pressor test are reduced when compared to people without diabetes [38].

8. *Presentation of another (usually strong) stimulus results in recovery of the habituated response.* This is a process referred to as 'dishabituation', where there is fast recovery of a habituated response as a result of the presentation of a novel, strong or sometimes noxious stimulus. In a recent study in my laboratory we tried to address this is a study in rats [39]. We exposed two groups of rats to recurrent episodes of moderate (approx. 3.0 mmol/l) hypoglycaemia over 4 weeks (12 episodes in total). One group subsequently underwent a low intensity exercise regimen [15 mins "walking" pace of 5m/min with 10% incline] while the other underwent a high intensity regimen exercise (5 mins at walking pace followed by an incremental increase in speed from 5 m/min to 15m/min "running"). The following day all rats then underwent a controlled hyperinsulinaemic hypoglycaemic clamp, where glucose levels were maintained at 2.8 mmol/l for 90 minutes and CRR responses measured. We found that in comparison to a control group who had not been exposed to antecedent hypoglycaemia, recurrent hypoglycaemia followed by low intensity exercise resulted, as expected, in a significant suppression of CRR . In contrast, following a single bout of high intensity exercise those rats who had undergone 4-weeks of recurrent hypoglycaemia now showed a normal CRR during the clamp study [39]. This very much supports the concept that recurrent hypoglycaemia is a habituated response; in this case high intensity exercise acting as the dishabituating stimulus.
9. *Upon repeated application of the dishabituatory stimulus the amount of dishabituation produced habituates (habituation of dishabituation).* This final criteria has not been tested but would suggest that any therapeutic intervention using a single novel or strong stimulus such as high-intensity exercise would, in the long-term, be insufficient to restore hypoglycaemia awareness in the long term, although may be effective in the short-term.

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**Summary**

Hypoglycaemia remains a real and continuing problem for people with diabetes. Developing strategies or therapies designed to prevent or minimise hypoglycaemia risk is of outmost importance. Newer technologies, better insulin and better educational methods should all help in this respect, but limitations to all these approaches means we may need to consider alternative strategies and these will need to be based on a clear understanding of the mechanisms underpinning the development of IAH in diabetes. In this lecture, I propose that IAH may represent a form of learning called habituation; a response that at a cellular level may represent a biological adaptation designed to protect the organism from future exposure to that stressor. The problem that results from this biological adaptation to recurrent hypoglycaemia is that hypoglycaemia in types 1 and 2 diabetes develops under conditions of high systemic insulin, which can result in a continuing drive to lower glucose further until severe disabling hypoglycaemia develops. However, recognition of the underlying processes that are set in motion by recurrent exposure to hypoglycaemia means that the development of IAH can perhaps be better understood and explained to individuals with diabetes and novel therapeutic approaches such as dishabituation and cognitive behavioural therapies can be tested.

**Acknowledgements:** I am indebted to the many colleagues and mentors that have encouraged and influenced my research endeavours over the years and to the many organisations, such as Diabetes UK and the Juvenile Diabetes Research Foundation, that have supported this work. I am especially grateful to the mentorship of Dr's Robert Sherwin, Brian Frier and Ian Deary. I am also reliant on the hard work and dedication of post-doctoral, doctoral and technical staff who all contribute hugely to the development of our research programme. In particular, Dr Alison McNeilly and Jennifer Gallagher undertook the recent research on dishabituation.

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## Figure legends

**Figure 1.** Prevalence of impaired awareness of hypoglycaemia in T1DM. *Hepburn, Diabetic Med 1990; Muhlauser, Diabetes Care 1991, Pramming, Diabetic Med 1991, Orchard, Diabetic Med 1991, ter Braak, Diabetes Care 2000, Pedersen-Bjergaard, Diabetologia 2003, Geddes, Diabetic Med 2008, A'Campo, Diabetologia* 2010 [5, 11, 40-43].

**Figure 2.** Intensive insulin therapy and type 1 diabetes result in suboptimal counterregulatory responses during hypoglycaemia. This study performed by Amiel and colleagues [12] involved infusing a low fixed dose of insulin continuously into two groups of people with type 1 diabetes (T1D). For those T1D subjects who were on conventional insulin therapy (once or twice daily insulin and HbA1c approx. 75 mmol /mol (9%)) the effect on plasma glucose is shown by the series of white circles. In contrast, for those T1D undergoing intensive insulin therapy (multiple daily insulin injections of CSII with HbA1c approx. 55 mmol/mol (7%)) the glucose levels fall much further in response to the same dose of insulin (black circles). As a further comparison the likely response of people without diabetes to the same insulin infusion is shown by the grey circles (this response was not part of the study and is inserted for illustrative purposes only). The difference between people with and without diabetes is thought to primarily reflect loss of alpha cell glucagon secretion in T1D, while the difference between conventional and intensively-treated T1D results mainly from defective catecholamine secretion.

**Figure 3.** This illustrates the counterregulatory response to low glucose in an individual who does not have diabetes in comparison to someone with Type 1 diabetes and impaired awareness of hypoglycaemia (IAH). The individual without diabetes when exposed to low glucose responds in a step-wise manner, first switching of endogenous insulin secretion then hypoglycemia stimulates glucagon and other counterregulatory hormone release followed by symptom awareness of hypoglycemia and then cognitive dysfunction. In contrast in those subjects with type 1 diabetes and IAH, insulin cannot be switched off and the

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counterregulatory and symptom responses are both diminished in intensity and occur at lower glucose levels. The net effect is that the window between recognition of hypoglycemia and the ability to take action before cognition is significantly impaired is reduced leading to a much higher risk of severe hypoglycemia.

**Figure 4.** Habituation: Criteria ♯1. Given that a particular stimulus elicits a response, repeated applications of that stimulus result in a decreased response (Habituation). This figure shows the seminal work of Heller in 1991 [16] demonstrating how antecedent hypoglycemia (light grey bars), compared to antecedent euglycaemia (dark grey bars) led to a marked suppression of the adrenaline response to hypoglycaemia induced 24h hrs later. This is characteristic of a habituated response.

**Figure 5.** Habituation: Criteria ♯2. If the stimulus is withheld, the response tends to recover over time (spontaneous recovery). In this study by Cranston and colleagues a small group of subjects with type 1 diabetes were studied before and after 4.1 (1.1) months' of strict hypoglycaemia avoidance [31]. Responses to the initial hypoglycaemia challenge were small and only started when plasma glucose was significantly lower than the 2.8 (0.1) mmol/L. After hypoglycaemia avoidance hormone responses were greater and initiated at higher glucose levels.

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