



University of Dundee

Saxagliptin co-therapy in C-peptide negative Type 1 diabetes does not improve counter-regulatory responses to hypoglycaemia

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3 Saxagliptin co-therapy in c-peptide negative Type 1 diabetes does not improve counterregulatory
4 responses to hypoglycaemia.
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41 Word count; Abstract- 211 Main text- 2994
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- 44 • This study tested the novel hypothesis that DPP4i co-therapy in type 1 diabetes would act
45 indirectly to improve symptom and hormonal responses to hypoglycaemia.
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- 48
49 • The hypothesis was rejected and no significant impact of DPP4i was seen on measures of
50 glucose variability, hypoglycaemia counterregulation or glycaemic control.
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54
- 55 • These findings do not support the use of DPP4i in the management of c-peptide negative
56 T1D.
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Abstract

Aims and hypothesis: Therapies targeted at secondary physiological abnormalities in type 1 diabetes such as dysregulated glucagon secretion may have additional therapeutic benefits. This study tested the hypothesis that Dipeptidyl DiPeptidase 4 inhibition (DPP4i) in c-peptide negative type 1 diabetes would reduce glucose variability and exposure to hypoglycaemia and therefore may indirectly enhance counterregulatory responses to subsequent hypoglycaemia.

Methods: 12-week Double blind, randomized, placebo controlled crossover study. The study was conducted in a tertiary hospital outpatient clinic, with additional studies performed in a Clinical Research Centre. Upon informed consent we recruited 14 subjects with moderately well controlled (HbA1c 64 ± 2 mmol/mol) type 1 diabetes of long-standing (20.5 ± 2.7 years). Subjects received 12-weeks therapy with oral saxagliptin (5mg) or placebo. Glucose-variability assessed via continuous glucose monitoring, frequency of hypoglycaemia, hypoglycaemia awareness, and symptomatic, cognitive and counterregulatory hormone responses to experimental hypoglycaemia were all assessed. Additional outcome measures included HbA1c, weight, total daily insulin dose, and adverse events.

Results: Saxagliptin co-therapy did not reduce glucose variability (Low Blood Glucose Index, Average Daily Risk Range), hypoglycaemia frequency or awareness and did not improve counterregulatory hormonal responses during experimental hypoglycaemia (AUC adrenaline, 25,775 vs. 24,454, placebo vs. saxagliptin, respectively, $p=0.76$).

Conclusions: No additional benefit of DPP4i co-therapy with Saxagliptin in the management of type 1 diabetes was found.

This trial is registered with www.clinicaltrials.gov (NCT 01922817).

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6-7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a

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5	Sample size	7a	How sample size was determined	9
6				
7		7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
8				
9	Randomisation:			
10				
11	Sequence	8a	Method used to generate the random allocation sequence	7
12	generation			
13		8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
14				
15	Allocation	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any	7-8
16	concealment		steps taken to conceal the sequence until interventions were assigned	
17	mechanism			
18				
19	Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7-8
20				
21	Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing	7-8
22			outcomes) and how	
23		11b	If relevant, description of the similarity of interventions	n/a
24				
25	Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9
26				
27		12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9
28				
29	Results			
30				
31	Participant flow (a	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed	Sup fig 1
32	diagram is strongly		for the primary outcome	
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5	recommended)	13b	For each group, losses and exclusions after randomisation, together with reasons	Sup fig 1
6				
7	Recruitment	14a	Dates defining the periods of recruitment and follow-up	10
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9		14b	Why the trial ended or was stopped	n/a
10				
11	Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Desc. In 10
12				
13	Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Sup fig 1
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18	Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-11
19				
20		17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	n/a
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23	Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	n/a
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27	Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11
28				
29	Discussion			
30				
31	Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14
32				
33	Generalisability	21	Generalisability (external validity, applicability) of the trial findings	12-13
34				
35	Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-13
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37	Other information			
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5	Registration	23	Registration number and name of trial registry	2
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7	Protocol	24	Where the full trial protocol can be accessed, if available	n/a
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9	Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	14
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For Peer Review

Introduction

Long-term follow-up of people with type 1 diabetes (T1D) has demonstrated convincingly that achieving near-normal glucose control through intensive insulin therapy will markedly reduce an individual's risk of both micro- and macrovascular complications [1]. However, despite major improvements in insulin preparations and delivery systems, glycaemic targets are not achieved in the majority of individuals with T1D [1]. A major limitation to achieving glycaemic targets in T1D is the fear of hypoglycaemia. Hypoglycaemia in T1D develops because of profound defects in the normal counterregulatory response, cardinal features of which are; (i) the inability to suppress exogenous insulin, (ii) loss of pancreatic alpha-cell hypoglycaemia-sensing leading to a failure to release the primary counterregulatory hormone, glucagon, and (iii) markedly suppressed catecholaminergic and symptomatic counterregulatory responses to hypoglycaemia (reviewed in [2, 3]). The first two of these defects is present in all individuals with T1D by 5-years from disease diagnosis, while subnormal symptom and catecholamine responses to hypoglycaemia are present in the majority by 10 years disease duration [4]. Collectively, suppressed catecholaminergic and symptomatic responses to hypoglycaemia, as well as higher thresholds (lower glucose levels) for triggering these responses is referred to as impaired awareness of hypoglycaemia (IAH), which conservatively affects around 25% of people with T1D [5]. IAH is associated with up to a 6-fold increase in the frequency of severe hypoglycaemia in T1D [5].

The major risk factor leading to the development of IAH is hypoglycaemia itself, with repeated exposure to hypoglycaemia leading to suppression of subsequent counterregulatory responses, while conversely strict hypoglycaemia avoidance restores counterregulatory responses [3]. Clinical interventions aimed at improving hypoglycaemia awareness have therefore largely focused on educational strategies that minimize exposure to hypoglycaemia [2]. Although promising results are being achieved through these approaches, none have to date been able to fully restore hypoglycaemia awareness and as such it seems likely that in addition to educational and behavioural programmes, pharmacological interventions will be required to minimize hypoglycaemia exposure in T1D. Non-insulin adjunct therapies, particularly those targeting pancreatic α -cell glucagon production, have been the subject of recent interest in T1D therapeutics [6, 7]. In T1D there is a failure to release glucagon in response to hypoglycaemia [8], and a paradoxical increase in both basal and meal-stimulated glucagon release [9]. This in part explains why higher doses of exogenous insulin are required in T1D to achieve glucose levels within the normal physiological range and hyperinsulinaemia contributes directly to the increased hypoglycaemia risk in T1D. Dipeptidyl-peptidase 4 inhibitors (DPP4i), a class of orally active compounds that increase circulating levels of glucagon-like peptide 1 (GLP-1) and gastrointestinal peptide (GIP) [10], have been shown to suppress basal and post-prandial glucagon

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3 in T1D [10-13], but do not appear to further suppress glucagon secretion during hypoglycaemia [13].
4 This raises the intriguing possibility that DPP4i co-therapy in T1D through restoring basal and meal-
5 related glucagon secretion will reduce insulin requirements, which together reduce glucose variability
6 and subsequently reduce exposure to mild or moderate hypoglycaemia. The indirect effect of this will
7 be to improve CNS (hypothalamic) glucose sensing leading to improved hypoglycaemia
8 counterregulation and awareness. To directly test this hypothesis, we designed a 12-week double
9 blind, randomized, crossover study in individuals with established c-peptide negative T1D. The
10 primary outcome measure was the magnitude of the counterregulatory symptom and hormone
11 responses during a subsequent hyperinsulinaemic hypoglycaemic clamp study, the gold standard for
12 assessing hypoglycaemia responses T1D.
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18 **Methods**

19 **Study population**

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22 This was a single-centre, double blind, placebo-controlled randomized trial. Ethical approval was
23 obtained from an independent research ethics committee and the Medicines Healthcare Products
24 Regulatory Agency (MHRA)). The study was carried out in accordance with the Declaration of
25 Helsinki, and written informed consent obtained from all participants before inclusion in the study.
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30 Adult subjects (N=14) with C-peptide negative T1D with disease duration >5yrs were recruited and
31 underwent medical screening (Supplementary Figure 1). Exclusion criteria were; previous history of
32 pancreatic or liver disease, significant microvascular disease, taking drugs that affect CYP3A4
33 metabolism, pregnancy/breast feeding or a history of seizures. Baseline demographic and
34 information on current diabetes management was collated. All patients had assessment of their
35 hypoglycaemic awareness through utilization of the Gold questionnaire[14].
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40 Consenting participants had an initial 3-4 week baseline period where they underwent two blinded
41 continuous glucose-monitoring (CGM) periods for at least 5 days (one at the start and one at the end).
42 The first blinded CGM (iPRO) was used for education purposes – following this each participant had
43 their insulin, dietary and exercise regimes completely reviewed by a single investigator for
44 consistency and carbohydrate ratios reviewed by a single dietician. Treatment of hypoglycaemia was
45 re-iterated with an emphasis on quick recognition and treatment of all hypoglycaemic episodes. These
46 were all done in a one-to-one manner. A second blinded CGM was performed after a minimum of 3-4
47 weeks and the data from this was used as a baseline for calculation of glycaemic variability (GV)
48 indices prior to entry into the drug treatment phase. During each CGM, participants were required to
49 fill in the iPRO blood glucose-recording diary for calibration purposes during the 5-7 day monitoring
50 period. This involved self-monitoring of blood glucose (SMBG) at least 3 times a day prior to meals
51 and an additional reading prior to bed. In addition, participants were also encouraged to perform
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3 SMBG during all symptomatic hypoglycaemia episodes, and to record all levels below 3.5mmol/L
4 (frequency of hypoglycaemia measures). The data sheet from the iPRO web based software was
5 exported to EasyGV [15], an excel-enabled workbook. This program uses macros to calculate 10
6 different measures of glycaemic variability from continuous glucose monitoring data using a simple
7 interface. For the purposes of this study, we focused on Low Blood Glucose Index (LBGI) and
8 Average Daily Risk Range (ADRR). LBGI [16] is a measure of the burden of hypoglycaemia during a
9 period of measurement. Unlike other measures of glycaemic variability, it corrects for the degree of
10 skewness of the glucose range. ADDR [17] has been designed to equally be sensitive to
11 hypoglycaemia and hyperglycaemia, being shown to be the best predictor for extremes of the glucose
12 range. These measures are thought to be the best predictors of glucose variability and have been
13 shown to be strongly associated with severe hypoglycaemia risk [17, 18]. HbA1c, insulin doses and
14 weight were also recorded prior to the first treatment phase.

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22 Subsequently, subjects were enrolled into 2 groups using a randomized block design. Subjects were
23 randomized in blocks of 4 using a computer generated randomization sequence generator. The
24 research team issued a prescription to clinical trials pharmacy located at Ninewells Hospital. The
25 capsules were then dispensed to the participant for each 3-month treatment period (one bottle of
26 capsules for each month's treatment). Both participant and research team member were blinded to the
27 dispensing.

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32 Seven subjects were in each treatment sequence. Sequence A received placebo for the first 12 weeks,
33 before receiving the DPP4i for the second arm. Sequence B was in reverse order to Sequence A. All
34 subjects were advised to continue their usual diabetes, dietary and exercise regime during the entire
35 trial. Subjects were contacted on a weekly basis for the first month, and then monthly thereafter.
36 During each contact, adverse events were recorded and advice provided as required on insulin dose
37 adjustment.

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42 Subjects were provided with a single daily oral 5mg dose of the DPPi inhibitor saxagliptin (Onglyza
43 ®, Bristol Myers Squibb) or placebo for 12 weeks. Both placebo and Saxagliptin were encapsulated to
44 ensure they were identical in appearance. At the end of each 12-week period the subjects underwent a
45 further period of blinded CGM (at least 5 days), blood samples were taken and each subject
46 underwent a hyperinsulinaemic hypoglycaemic clamp study to assess the magnitude of their counter-
47 regulatory responses. Participants had at least a 2-week washout period before entering the second
48 arm of the trial.

52 53 **Hyperinsulinaemic hypoglycaemic clamp study**

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55 Overnight-fasted subjects reported to the Clinical Research Centre, Dundee at 8.00am. All subjects
56 were asked to avoid hypoglycaemia in the 48 hrs prior to the clamp study and this was subsequently
57 confirmed via CGM. A cannula was inserted into the non-dominant hand, and placed in a heated box
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(50-55°C) to obtain arterialized venous blood. A further cannula was inserted into the dominant antecubital vein of the contralateral arm. Insulin was started at a priming dose of 50 Units/hr, until a blood glucose of 7mmol/L was reached, and then insulin was maintained at a dose of 1.5mUnits/kg/min. Glycaemic plateaus were achieved through bedside measurement of blood glucose (Analox GM9D, Analox instruments, London, UK) every 5-10 minutes, and using a variable 20% dextrose infusion. Subjects were initially maintained in the euglycaemic range (between 4-6mmol/L) for 40 minutes, prior to hypoglycaemia (2.5mmol/l) being induced and subsequently maintained for 85mins. Blood samples for determination of insulin, adrenaline, noradrenaline, and glucagon were drawn in triplicate during the baseline period, and then every 20 minutes during the hypoglycaemic phase. Blood pressure and pulse rate were measured every 10 minutes (Accutorr Plus Monitor, Datascope Corp., New Jersey, USA).

Blood sampling and analyses

Samples were centrifuged to separate the plasma within 2 hours, and then stored at -80°C prior to assay. Hormones (Insulin-RIA-Diasorin; CV inter -6.7%, intra -5.8%), (Glucagon-RIA-MilliporeUK; CV inter 4.9%, intra 8.8%), (Adrenaline-EIA-Alpco; CV inter 22%, intra 16%), (Noradrenaline-EIA-Alpco; CV inter 16%, intra 22%) were measured by ELISA, and samples were analysed in duplicate according to the manufacturer's instructions.

Symptoms and cognitive function tests

Subjects rated hypoglycaemia symptoms three times over the 40-minute euglycaemic period and every 20mins during the hypoglycaemic plateaus. Symptoms were scored on a validated questionnaire, the Edinburgh Hypoglycaemia Scale, scoring from 1 (not at all) to 7 (very severe) on a visual analogue scale [19].

Cognitive function was assessed using Trail Making B(TMB)[20] and Digit symbol substitution (DSS) tasks, which are known to be sensitive to hypoglycaemia [21]. To minimize learning effects, all subjects had practiced both tasks. (5-7 days prior to the clamp study and also twice at the start of the clamp study)

Statistical analysis

The hypothesis predicted that DPP4i would reduce exposure to hypoglycaemia leading to improved CNS hypoglycaemia detection and subsequently enhanced adrenaline responses to subsequent hypoglycaemia. This was therefore the pre-specified primary outcome measure. Prior power calculations indicated that 12 subjects were needed for a matched analysis, with 80% power to detect a difference in change of 450pmol/L with SD of 500 and an alpha of 0.05, two sided. This difference in the adrenaline response was chosen based on previous published work [22]. Additional subjects were recruited to account for a potential 25% dropout rate. Secondary outcomes included insulin

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3 requirements, HbA1c, glucose variability indices, frequency of hypoglycaemia, hypoglycaemic
4 awareness, and glucagon response during hypoglycaemia. Statistical analyses were conducted using
5 Graphpad Prism 6 and $p < 0.05$ was considered statistically significant. Normally distributed data were
6 compared using paired samples t tests, while non-normally distributed data were compared using the
7 Wilcoxon signed rank test. Repeated measures ANOVA was used to determine differences in other
8 parameters measured over time, with t-testing used to localise effects where indicated. No order
9 effects were noted in any of the subsequent analyses.
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14 **Results**

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16 Recruitment was between September 2012-July 2013. Eighteen subjects with T1D were screened,
17 with 14 (8 male, 6 female) Caucasian subjects completing the two arms of the trial. The consort
18 diagram is shown in Supplemental Figure 1. Median (IQR) age of participants was 45 (35-53) years.
19 All participants had C-peptide -ve (< 0.10 nmol/L) T1D, with a median (IQR) duration of disease of 18
20 (12-31) years. Mean (\pm SD) glycaemic control at trial entry was HbA1c 64 (± 2) mmol/mol. Mean
21 weight was 74.1 (± 3) kg, BMI 26 (± 0.8) kg/m². Mean total daily insulin dose at baseline was 55 (± 4)
22 IU of human insulin [27 (± 4) IU of basal insulin, 28 (± 4) IU of short-acting insulin]. Median (IQR)
23 baseline Gold Score was 3.0 (2.0-4.0) (see table 1). Compliance with study drug was high in both
24 arms of the trial (placebo and saxagliptin arms, 94.4 and 91.8% respectively).
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31 **Hyperinsulinaemic hypoglycaemia studies**

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33 Glucose profiles during the hyperinsulinaemic clamps studies were well matched with no effect of
34 treatment [$F(1,26)=0.00$ $p=0.96$] (Figure 1A). Glucose infusion rates (GIR) required to maintain the
35 hypoglycaemia plateau were also comparable in the two treatment groups [$F(1,26)=0.23$ $p=0.64$]
36 (Figure 1B).
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40 Plasma adrenaline increased with time over the clamp period [main effect of time, $F(6, 156)=40.36$
41 $p < 0.0001$]. However there was no effect of treatment [$F(1,26)=0.02$ $p=0.89$] and there was no time X
42 treatment interaction [$F(6,156)=0.17$ $p=0.98$]. The AUC of the adrenaline responses were also
43 similar between groups [25,775 vs. 24,454, Placebo vs. saxagliptin, respectively, $p=0.76$] (Figure
44 1C). No significant effect of either hypoglycaemia or treatment was seen on the glucagon response to
45 hypoglycaemia ($p=ns$; Figure 1D)
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49 Consistent with the hormonal responses, subjects did not report any differences in their total symptom
50 scores during hypoglycaemia between the two treatment arms [26 (± 4) vs. 28 (± 3), Placebo vs.
51 Saxagliptin; $p=0.38$], or between autonomic symptoms [12 (± 1) vs. 13 (± 1), Placebo vs. Saxagliptin;
52 $p=0.36$] (see Figure 1E). The two groups also performed similarly on cognitive tasks during
53 hypoglycaemia: TMB [37 (± 6) vs. 37 (± 8)s; $p=0.96$] or DSS [67 (± 4) vs. 62 (± 4); $p=0.16$] both
54 Placebo vs. Saxagliptin, respectively (see Figure 1F).
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Glucose variability, hypoglycaemia frequency, hypoglycaemia awareness

No significant effect of saxagliptin adjunct therapy was seen on CGM measures of mean or standard deviation of glucose or on the principal measures of LBG1 [F(1,9)=0.418 p=0.534] or ADRR [F(1,9)=0.365 p=0.365] (Table 2) (Figure 2A-C). Consistent with these findings, no overall effects of saxagliptin on self-reported hypoglycaemia frequency [F(1,11)=0.393 p=0.54] or hypoglycaemia awareness [F(1,11)=3.43 p=0.09] were seen.

Glycaemic control and body weight

There was no overall effect of saxagliptin on glycaemic control [HbA1c F(1,11)=2.49 p=0.14], or daily insulin dose [F(1,11)=0.069 p=0.80] (Table 2). During each treatment phase the change in HbA1c from pre-treatment levels was small (+0.3mmol/L with saxagliptin and -1.6mmol/L with placebo) and did not differ significantly between groups (p=0.61). There was no effect of saxagliptin on weight [mean increase of 0.24kg with saxagliptin and 0.07kg with placebo; F(1,11)=0.40 p=0.54].

Glucose variability

Adverse events

No serious adverse events were reported during the trial. Other adverse events reported were infrequent (<10%), mild and did not differ with placebo or Saxagliptin therapy.

Discussion

Antecedent hypoglycaemia is the major risk factor that leads to the development of IAH, which in turn markedly increases the risk of severe hypoglycaemia [3]. Conversely, hypoglycaemia avoidance strategies improve counterregulatory responses to subsequent hypoglycaemia when tested formally using the clamp technique [23, 24]. Hypoglycaemia in T1D results in a large part from non-physiological and unregulated hyperinsulinaemia as well as dysregulated glucagon secretion. As a consequence of this specialised glucose sensing neurons in the brain are exposed to repeated hypoglycaemia leading to a series of molecular adaptations that results in reduced catecholaminergic (adrenaline and noradrenaline) and symptom responses to subsequent hypoglycaemia; clinically referred to as IAH [3]. Therefore, by improving physiological glucagon secretion in T1D, it should be possible to both reduce insulin requirements and propensity to mild-moderate hypoglycaemia, which in turn should reduce the central drive to suppress catecholaminergic and symptom responses. Recent reports would appear to indicate that DPP4i in T1D can exert this effect on glucagon secretion [10-13], and consistent with the underlying hypothesis, Ellis et al. [12] reported that 4-weeks of sitagliptin adjunct therapy in T1D significantly improved glucose variability as assessed by M100, Glycaemic Risk Assessment Diabetes Equation and J-index. In contrast, the current study found no effect of

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3 DPP4i in any measure of GV, self-reported hypoglycaemia frequency or insulin-dose, and
4 subsequently no overall effect on hypoglycaemia counterregulation. Although a mixed-meal test was
5 not performed to examine whether 12-weeks DPP4i consistently suppressed basal and post-prandial
6 glucagon levels, it seems unlikely based on our data that any significant impact on alpha-cell
7 glucagon secretion would have been detected. Interestingly, in the recent LIBRA trial the GLP-1
8 receptor agonist liraglutide actually induced a paradoxical rise in post-prandial glucagon in T2D
9 subjects the first evidence of which emerged at around 12 weeks treatment duration [25]. Therefore,
10 and consistent with our findings, any benefit of DPP4i in T1D, at least in terms of alpha-cell
11 suppression, may be short-lived and unlikely to translate into significant improvements in glucose
12 variability.
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19 In addition, no benefit of DPP4i on glycaemic control was found in the current study. While the study
20 was not powered to detect anything other than large effects, the very small change in HbA1c seen
21 following 12-weeks co-therapy would suggest that any clinical benefit would be minimal. In contrast,
22 Farngren et al. [13] reported that 28 days prior therapy with the DPP4i vildagliptin in T1D had a small
23 benefit in terms of HbA1c reduction, and Ellis et al. [12] reported that 4-weeks of sitagliptin adjunct
24 therapy in T1D significantly improved HbA1c (-2.91 ± 1.16 mmol/l). However, the latter was a short
25 duration trial with no washout period, and there was a marked Hawthorne effect suggesting that
26 increased contact with health care personal and more frequent monitoring played a large part in the
27 improvements seen. Others have also reported no effect of DPP4i on glycaemic control in T1D [26].
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33 The main limitations of the study were that the assessment of glucose variability and hypoglycaemia
34 frequency was made during periods of CGM over the 6 days of measurement and longer periods of
35 assessment may have been more representative. However the robust methodology involved in the
36 clamp studies is very suggestive that hypoglycaemia frequency was not reduced. Finally, we were not
37 able to measure GLP-1, glucagon and c-peptide responses to a standard meal in the current trial so
38 cannot say for certain that saxagliptin therapy in T1D was ineffective at improving post-prandial
39 glucose and glucagon responses.
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45 In summary, in the current study we tested the hypothesis that adjunct therapy with oral DPP4i, by
46 reducing overall exposure to hypoglycaemia, would improve symptom and hormonal responses to
47 hypoglycaemia in T1D. Our study rejects this hypothesis, by failing to demonstrate a significant effect
48 on the primary outcome measure, the adrenaline response during a hyperinsulinaemic hypoglycaemic
49 clamp study, after 12-weeks DPP4i therapy when compared with placebo. In addition, no effect of
50 DPP4i was seen on secondary measures of symptom or cognitive responses to controlled
51 hypoglycaemia, self-reported hypoglycaemia frequency and awareness, glucose variability or
52 glycaemic control. These findings do not support the use of DPP4i in the management of c-peptide
53 negative T1D.
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Competing Interests

None declared

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All authors have contributed equally to the concept in, design, the acquisition of data and analysis and the development of the manuscript.

Disclosure summary

RJM has consulted for NovoNordisk, Bristol-Myers-Squibb, Astra-Zeneca, Janssen and Sanofi-Aventis and has received lecture fees from Eli Lilly Ltd.

Author's contribution

PSG conducted the trial and researched data and wrote manuscript

R.J.M conceived study wrote/edited the manuscript.

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Table 1. Baseline clinical and biochemical characteristics

Mean (SEM) age (yrs)	42.9 (3.3)
Mean (SEM) weight (kg)	74.1 (3)
Mean (SEM) duration of diabetes (yrs)	20.5 (2.7)
Median (range) Gold Score	3 (2-4)
Mean (SEM) HbA1c (mmol/mol)	64 (2)
Mean (SEM) insulin doses	
-long acting (units)	27 (4)
-short acting (units)	28 (4)

Table 2. Measures of glycaemic control and glucose variability following 12-weeks adjunct therapy with DPP4i (Saxagliptin) or placebo in subjects with type 1 diabetes. Glucose variability measures recorded using continuous glucose monitoring assessments in the final week of the trial. Values shown as mean (SEM).

	Variable	Placebo	Saxagliptin
Glycaemic Control	HbA1c (mmol/mol)	66(2)	65(2)
	Total Insulin dose (Units)	60(8)	56(7)
	Long acting (Units)	29 (4)	28(4)
	Short acting (Units)	31(3)	28 (4)
Glucose Variability			
	LBGI	6.1 (1.6)	6.1 (1.8)
	HBGI	12.8 (1.6)	13.5 (1.9)
	ADRR	12.3 (1.9)	12.3 (1.7)
	Mean Glucose (mmol/l)	9.7 (0.6)	10.2 (0.6)
	StDev Glucose(mmol/l)	3.6 (0.2)	3.7 (0.3)

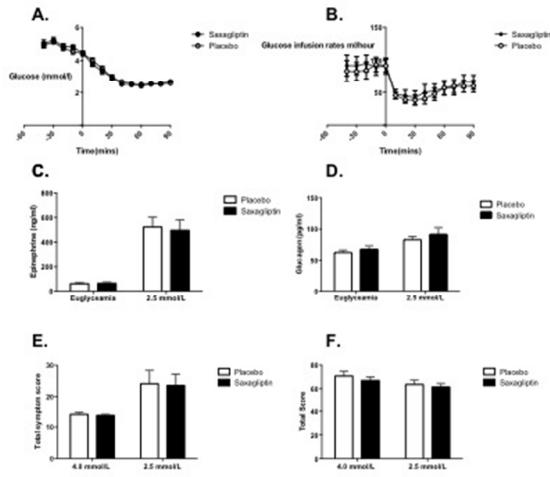
Figure Legends

Figure 1. Non-insulin adjunct therapy with Saxagliptin in c-peptide negative type 1 diabetes had no effect on hormonal, symptom and cognitive responses to acute hypoglycaemia. (A) Blood glucose profiles and (B) Glucose Infusion rates during hyperinsulinaemic glucose clamp. (C) Peak Adrenaline during hypoglycaemia, (D) Peak Glucagon during hypoglycaemia, (E) Total Hypoglycaemia Symptom Score during euglycaemic and hypoglycaemic plateaus (F) Digit Symbol Substitution Test,. Saxagliptin group shown by black bars or black circles, Placebo by white bars or circles. Values shown as Mean \pm SEM.

Figure 2. Non-insulin adjunct therapy with Saxagliptin in c-peptide negative type 1 diabetes had no effect on (A) Hypoglycaemia Awareness, (B) Low Blood Glucose Index (LBGI), (C) Average Daily Risk Range (ADRR). Saxagliptin group shown by black bars, Placebo by white bars. Values shown as Mean \pm SEM.

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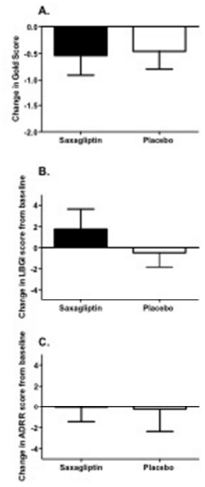
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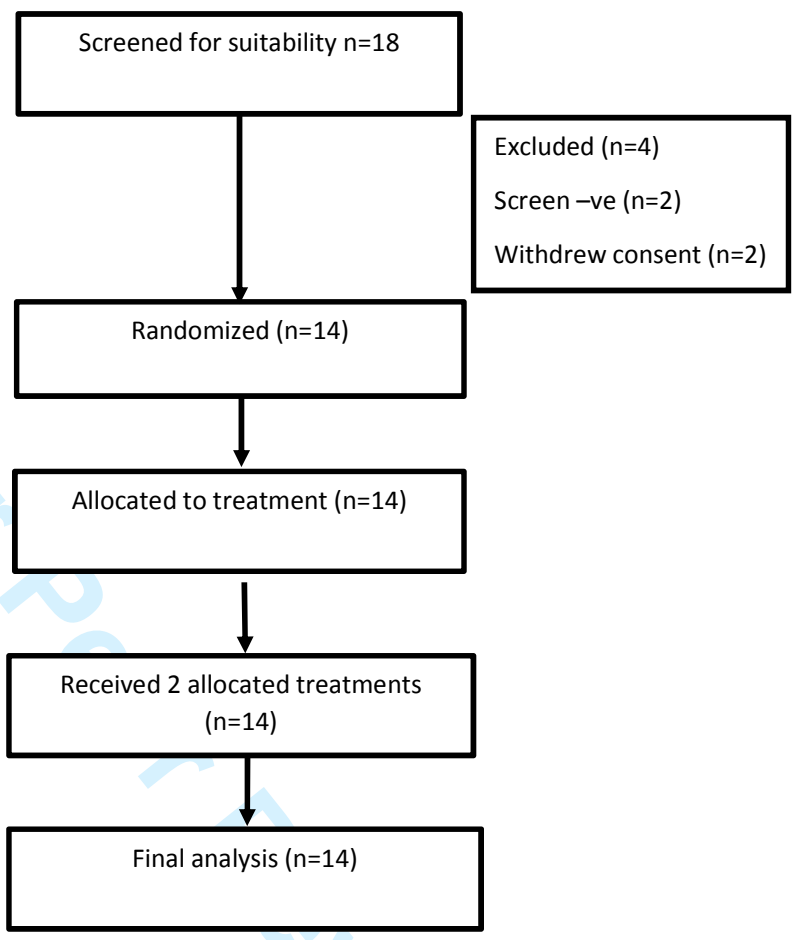


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Supplemental FIGURE 1 A. CONSORT DIAGRAM



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