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

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Published in:
Diabetic Medicine

DOI:
10.1111/dme.13046

Publication date:
2016

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Journal:	*Diabetic Medicine*
Manuscript ID:	DME-2015-00293.R2
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	George, Priya; Ninewells Hospital and Medical School, Medical Research Institute McCrimmon, Rory; Ninewells Hospital and Medical School, Medical Research Institute
Keywords:	clinical studies, HbA1c, type 1 diabetes, drug treatment, hypoglycaemia

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Word count; Abstract- 211 Main text- 2994

- This study tested the novel hypothesis that DPP4i co-therapy in type 1 diabetes would act indirectly to improve symptom and hormonal responses to hypoglycaemia.

- The hypothesis was rejected and no significant impact of DPP4i was seen on measures of glucose variability, hypoglycaemia counterregulation or glycaemic control.

- These findings do not support the use of DPP4i in the management of c-peptide negative T1D.

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).

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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

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

Methods

Study population

This was a single-centre, double blind, placebo-controlled randomized trial. Ethical approval was obtained from an independent research ethics committee and the Medicines Healthcare Products Regulatory Agency (MHRA)). The study was carried out in accordance with the Declaration of Helsinki, and written informed consent obtained from all participants before inclusion in the study.

Adult subjects (N=14) with C-peptide negative T1D with disease duration >5yrs were recruited and underwent medical screening (Supplementary Figure 1). Exclusion criteria were; previous history of pancreatic or liver disease, significant microvascular disease, taking drugs that affect CYP3A4 metabolism, pregnancy/breast feeding or a history of seizures. Baseline demographic and information on current diabetes management was collated. All patients had assessment of their hypoglycaemic awareness through utilization of the Gold questionnaire[14].

Consenting participants had an initial 3-4 week baseline period where they underwent two blinded continuous glucose-monitoring (CGM) periods for at least 5 days (one at the start and one at the end). The first blinded CGM (iPRO) was used for education purposes – following this each participant had their insulin, dietary and exercise regimes completely reviewed by a single investigator for consistency and carbohydrate ratios reviewed by a single dietician. Treatment of hypoglycaemia was re-iterated with an emphasis on quick recognition and treatment of all hypoglycaemic episodes. These were all done in a one-to-one manner. A second blinded CGM was performed after a minimum of 3-4 weeks and the data from this was used as a baseline for calculation of glycaemic variability (GV) indices prior to entry into the drug treatment phase. During each CGM, participants were required to fill in the iPRO blood glucose-recording diary for calibration purposes during the 5-7 day monitoring period. This involved self-monitoring of blood glucose (SMBG) at least 3 times a day prior to meals and an additional reading prior to bed. In addition, participants were also encouraged to perform

SMBG during all symptomatic hypoglycaemia episodes, and to record all levels below 3.5mmol/L (frequency of hypoglycaemia measures). The data sheet from the iPRO web based software was exported to EasyGV [15], an excel-enabled workbook. This program uses macros to calculate 10 different measures of glycaemic variability from continuous glucose monitoring data using a simple interface. For the purposes of this study, we focused on Low Blood Glucose Index (LBGI) and Average Daily Risk Range (ADRR). LBGI [16] is a measure of the burden of hypoglycaemia during a period of measurement. Unlike other measures of glycaemic variability, it corrects for the degree of skewness of the glucose range. ADDR [17] has been designed to equally be sensitive to hypoglycaemia and hyperglycaemia, being shown to be the best predictor for extremes of the glucose range. These measures are thought to be the best predictors of glucose variability and have been shown to be strongly associated with severe hypoglycaemia risk [17, 18]. HbA1c, insulin doses and weight were also recorded prior to the first treatment phase.

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

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

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

Hyperinsulinaemic hypoglycaemic clamp study

Overnight-fasted subjects reported to the Clinical Research Centre, Dundee at 8.00am. All subjects were asked to avoid hypoglycaemia in the 48 hrs prior to the clamp study and this was subsequently confirmed via CGM. A cannula was inserted into the non-dominant hand, and placed in a heated box

(50-55^{O}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

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

Results

Recruitment was between September 2012-July 2013. Eighteen subjects with T1D were screened, with 14 (8 male, 6 female) Caucasian subjects completing the two arms of the trial. The consort diagram is shown in Supplemental Figure 1. Median (IQR) age of participants was 45 (35-53) years. All participants had C-peptide –ve (<0.10nmol/L) T1D, with a median (IQR) duration of disease of 18 (12-31) years. Mean (±SD) glycaemic control at trial entry was HbA1c 64 (±2) mmol/mol. Mean weight was 74.1(±3) kg, BMI 26 (±0.8) kg/m2. Mean total daily insulin dose at baseline was 55 (±4) IU of human insulin [27 (±4) IU of basal insulin, 28 (±4) IU of short-acting insulin]. Median (IQR) baseline Gold Score was 3 .0 (2.0-4.0) (see table 1). Compliance with study drug was high in both arms of the trial (placebo and saxagliptin arms, 94.4 and 91.8% respectively).

Hyperinsulinaemic hypoglycaemia studies

Glucose profiles during the hyperinsulinaemic clamps studies were well matched with no effect of treatment [$F(1,26)=0.00$ $p=0.96$] (Figure 1A). Glucose infusion rates (GIR) required to maintain the hypoglycaemia plateau were also comparable in the two treatment groups [$F(1,26)=0.23$ $p=0.64$] (Figure 1B).

Plasma adrenaline increased with time over the clamp period [main effect of time, $F(6, 156)=40.36$ $p<0.0001$]. However there was no effect of treatment [$F(1,26)=0.02$ $p=0.89$] and there was no time X treatment interaction [$F(6,156)=0.17$ $p=0.98$]. The AUC of the adrenaline responses were also similar between groups [25,775 vs. 24,454, Placebo vs. saxagliptin, respectively, $p=0.76$] (Figure 1C). No significant effect of either hypoglycaemia or treatment was seen on the glucagon response to hypoglycaemia (p=ns; Figure 1D)

Consistent with the hormonal responses, subjects did not report any differences in their total symptom scores during hypoglycaemia between the two treatment arms [26 (±4) vs. 28 (±3), Placebo vs. Saxagliptin; $p=0.38$], or between autonomic symptoms [12 (±1) vs. 13 (±1), Placebo vs. Saxagliptin; $p=0.36$) (see Figure 1E). The two groups also performed similarly on cognitive tasks during hypoglycaemia: TMB [37 (±6) vs. 37 (±8)s; $p=0.96$] or DSS [67 (±4) vs. 62 (±4); $p=0.16$] both Placebo vs. Saxagliptin, respectively (see Figure 1F).

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 LBGI [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 supress 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

DPP4i in any measure of GV, self-reported hypoglycaemia frequency or insulin-dose, and subsequently no overall effect on hypoglycaemia counterregulation. Although a mixed-meal test was not performed to examine whether 12-weeks DPP4i consistently supressed basal and post-prandial glucagon levels, it seems unlikely based on our data that any significant impact on alpha-cell glucagon secretion would have been detected. Interestingly, in the recent LIBRA trial the GLP-1 receptor agonist liraglutide actually induced a paradoxical rise in post-prandial glucagon in T2D subjects the first evidence of which emerged at around 12 weeks treatment duration [25]. Therefore, and consistent with our findings, any benefit of DPP4i in T1D, at least in terms of alpha-cell suppression, may be short-lived and unlikely to translate into significant improvements in glucose variability.

In addition, no benefit of DPP4i on glycaemic control was found in the current study. While the study was not powered to detect anything other than large effects, the very small change in HbA1c seen following 12-weeks co-therapy would suggest that any clinical benefit would be minimal. In contrast, Farngren et al. [13] reported that 28 days prior therapy with the DPP4i vildagliptin in T1D had a small benefit in terms of HbA1c reduction, and Ellis et al. [12] reported that 4-weeks of sitagliptin adjunct therapy in T1D significantly improved HbA1c (-2.91± 1.16 mmol/l). However, the latter was a short duration trial with no washout period, and there was a marked Hawthorne effect suggesting that increased contact with health care personal and more frequent monitoring played a large part in the improvements seen. Others have also reported no effect of DPP4i on glycaemic control in T1D [26].

The main limitations of the study were that the assessment of glucose variability and hypoglycaemia frequency was made during periods of CGM over the 6 days of measurement and longer periods of assessment may have been more representative. However the robust methodology involved in the clamp studies is very suggestive that hypoglycaemia frequency was not reduced. Finally, we were not able to measure GLP-1, glucagon and c-peptide responses to a standard meal in the current trial so cannot say for certain that saxagliptin therapy in T1D was ineffective at improving post-prandial glucose and glucagon responses.

In summary, in the current study we tested the hypothesis that adjunct therapy with oral DPP4i, by reducing overall exposure to hypoglycaemia, would improve symptom and hormonal responses to hypoglycaemia in T1D. Our study rejects this hypothesis, by failing to demonstrate a significant effect on the primary outcome measure, the adrenaline response during a hyperinsulinaemic hypoglycaemic clamp study, after 12-weeks DPP4i therapy when compared with placebo. In addition, no effect of DPP4i was seen on secondary measures of symptom or cognitive responses to controlled hypoglycaemia, self-reported hypoglycaemia frequency and awareness, glucose variability or glycaemic control. These findings do not support the use of DPP4i in the management of c-peptide negative T1D.

Funding Sources

This work was supported by grants from Tenovus Scotland (T11/22).

Competing Interests

None declared

Acknowledgements

We gratefully acknowledge Lesley Grant, Dietician who provided dietetic support during this trial, Joanne Forbes and Caron Innes from the Clinical Research Centre, Ninewells Hospital who helped with the clamp studies, Gwen Kiddie who processed all the blood samples, Lesley McFarlane Biomarker and Drug Analysis Core Facility, University of Dundee who analysed the blood samples, Tayside Pharmaceuticals for their role in preparation of drug/matched placebo and Clinical Trials Pharmacy, Ninewells Hospital for their role in randomization, blinding and delivery of the drug to the research team.

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.

References

1. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ*, et al.* Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353**:2643-2653.
2. Amiel SA. Hypoglycemia: from the laboratory to the clinic. *Diabetes Care* 2009; **32**:1364-1371.
3. McCrimmon R. Glucose sensing during hypoglycemia: lessons from the lab. *Diabetes Care* 2009; **32**:1357-1363.
4. Mokan M, Mitrakou A, Veneman T, Ryan C, Korytkowski M, Cryer P*, et al.* Hypoglycemia unawareness in IDDM. *Diabetes Care* 1994; **17**:1397-1403.
5. Geddes J, Schopman JE, Zammitt NN, Frier BM. Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes. *Diabet Med* 2008; **25**:501-504.
6. George P, McCrimmon RJ. Potential role of non-insulin adjunct therapy in Type 1 diabetes. *Diabet Med* 2013; **30**:179-188.
7. Lebovitz HE. Adjunct therapy for type 1 diabetes mellitus. *Nat Rev Endocrinol* 2010; **6**:326-334.
8. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. *Science* 1973; **182**:171-173.
9. Dupre J. Glycaemic effects of incretins in Type 1 diabetes mellitus: a concise review, with emphasis on studies in humans. *Regul Pept* 2005; **128**:149-157.
10. Thornberry NA, Gallwitz B. Mechanism of action of inhibitors of dipeptidyl-peptidase-4 (DPP-4). *Best practice & research Clinical endocrinology & metabolism* 2009; **23**:479-486.
11. Dupre J, Behme MT, Hramiak IM, McFarlane P, Williamson MP, Zabel P*, et al.* Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* 1995; **44**:626-630.
12. Ellis SL, Moser EG, Snell-Bergeon JK, Rodionova AS, Hazenfield RM, Garg SK. Effect of sitagliptin on glucose control in adult patients with Type 1 diabetes: a pilot, double-blind, randomized, crossover trial. *Diabet Med* 2011; **28**:1176-1181.
13. Farngren J, Persson M, Schweizer A, Foley JE, Ahren B. Vildagliptin reduces glucagon during hyperglycemia and sustains glucagon counterregulation during hypoglycemia in type 1 diabetes. *J Clin Endocrinol Metab* 2012; **97**:3799-3806.
14. Gold AE, MacLeod KM, Frier BM. Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia. *Diabetes Care* 1994; **17**:697-703.
15. Hill NR, Oliver NS, Choudhary P, Levy JC, Hindmarsh P, Matthews DR. Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. *Diabetes Technol Ther* 2011; **13**:921-928.
16. Kovatchev BP, Cox DJ, Gonder-Frederick LA, Clarke W. Symmetrization of the blood glucose measurement scale and its applications. *Diabetes Care* 1997; **20**:1655-1658.
17. Kovatchev BP, Otto E, Cox D, Gonder-Frederick L, Clarke W. Evaluation of a new measure of blood glucose variability in diabetes. *Diabetes Care* 2006; **29**:2433-2438.
18. Kovatchev BP, Cox DJ, Gonder-Frederick LA, Young-Hyman D, Schlundt D, Clarke W. Assessment of risk for severe hypoglycemia among adults with IDDM: validation of the low blood glucose index. *Diabetes care* 1998; **21**:1870-1875.
19. Deary IJ, Hepburn DA, MacLeod KM, Frier BM. Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis. *Diabetologia* 1993; **36**:771-777.
20. Kortte KB, Horner MD, Windham WK. The trail making test, part B: cognitive flexibility or ability to maintain set? *Applied neuropsychology* 2002; **9**:106-109.
21. Wechsler D. Manual for the Wechsler Adult Intelligence Scale Revised. . *New York* 1981; **Psychological Corp**.
22. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes* 1988; **37**:901-907.
23. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA. Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. *Lancet* 1994; **344**:283-287.

24. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F*, et al.* Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM. *Diabetes* 1993; **42**:1683-1689.
25. Kramer CK, Zinman B, Choi H, Connelly PW, Retnakaran R. The Impact of Chronic Liraglutide Therapy on Glucagon Secretion in Type 2 Diabetes: Insight from the LIBRA Trial. *J Clin Endocrinol Metab* 2015:jc20152725.
26. Garg SK, Moser EG, Bode BW, Klaff LJ, Hiatt WR, Beatson C*, et al.* Effect of sitagliptin on post-prandial glucagon and GLP-1 levels in patients with type 1 diabetes: investigator-initiated, double-blind, randomized, placebo-controlled trial. *Endocr Pract* 2013; **19**:19-28.

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)

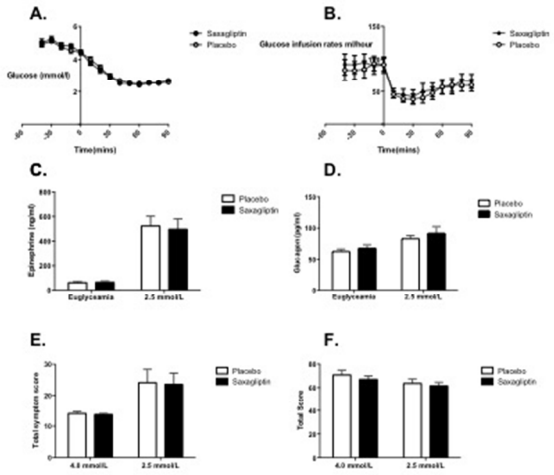
Table 2. Measures of glycaemic control and glucose variability following 12-weeks adjunct therapy with DPP4i (Saxagliptin) or placebo in subjects with type 1 diabetes. Glucose variability measures recorded using continuous glucose monitoring assessments in the final week of the trial. Values shown as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



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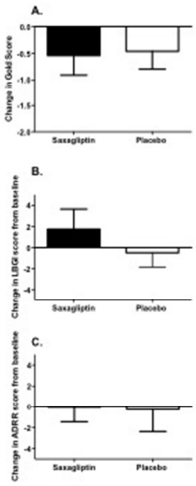

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Supplemental FIGURE 1 A. CONSORT DIAGRAM