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

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Abstract

Aims To test the hypothesis that dipeptidyl peptidase-4 inhibition in C-peptide negative Type 1 diabetes would reduce glucose variability and exposure to hypoglycaemia and therefore may indirectly enhance counter-regulatory responses to subsequent hypoglycaemia.

Methods We conducted a 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. After obtaining informed consent, we recruited 14 subjects with moderately well controlled Type 1 diabetes (HbA_{1c} 64 ± 2 mmol/mol) of long duration (20.5 ± 2.7 years). The subjects received 12 weeks' therapy with oral saxagliptin (5 mg) or placebo. Glucose variability, assessed via continuous glucose monitoring, together with frequency of hypoglycaemia, hypoglycaemia awareness and symptomatic, cognitive and counter-regulatory hormone responses to experimental hypoglycaemia, were assessed. Additional outcome measures included HbA_{1c} level, 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 counter-regulatory hormonal responses during experimental hypoglycaemia (area under the curve for adrenaline 25 775 vs. 24 454, for placebo vs saxagliptin, respectively; $P = 0.76$).

Conclusions No additional benefit of dipeptidyl peptidase-4 inhibition co-therapy with saxagliptin in the management of Type 1 diabetes was observed.

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Introduction

Long-term follow-up of people with Type 1 diabetes has shown 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 Type 1 diabetes [1]. A major challenge to achieving glycaemic targets in Type 1 diabetes is the fear of hypoglycaemia. Hypoglycaemia in Type 1 diabetes develops because of profound defects in the normal counter-regulatory response, cardinal features of which are: (1) the inability to suppress exogenous insulin; (2) loss of pancreatic α-cell hypoglycaemia-sensing, leading to a failure to release the primary counter-regulatory hormone, glucagon; and (3) markedly suppressed catecholaminergic and symptomatic counter-regulatory responses to hypoglycaemia [2,3]. The first two of these defects is present in all individuals with Type 1 diabetes by 5 years from disease diagnosis, while subnormal symptom and catecholamine responses to hypoglycaemia are present in the majority of patients 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, are referred to as impaired awareness of hypoglycaemia, which affects ~25% of people with Type 1 diabetes [5]. Impaired awareness of hypoglycaemia is associated with an up to sixfold increase

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What's new?

- This study tested the novel hypothesis that dipeptidyl peptidase-4 inhibitor 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 dipeptidyl peptidase-4 inhibitor therapy was seen on measures of glucose variability, hypoglycaemia counter-regulation or glycaemic control.
- These findings do not support the use of dipeptidyl peptidase-4 inhibitors in the management of C-peptide-negative Type 1 diabetes.

in the frequency of severe hypoglycaemia in Type 1 diabetes [5].

The major risk factor leading to the development of impaired awareness of hypoglycaemia is hypoglycaemia itself, with repeated exposure to hypoglycaemia leading to suppression of subsequent counter-regulatory responses, while, conversely, strict hypoglycaemia avoidance restores counter-regulatory 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 it therefore seems likely that, in addition to educational and behavioural programmes, pharmacological interventions will be required to minimize hypoglycaemia exposure in Type 1 diabetes. Non-insulin adjunct therapies, particularly those targeting pancreatic α-cell glucagon production, have been the subject of recent interest in Type 1 diabetes therapeutics [6,7]. In Type 1 diabetes 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 Type 1 diabetes to achieve glucose levels within the normal physiological range and hyperinsulinaemia contributes directly to the increased hypoglycaemia risk. Dipeptidyl-peptidase-4 (DPP-4) inhibitors, a class of orally active compounds that increase circulating levels of glucagon-like peptide-1 (GLP-1) and gastrointestinal peptide [10], have been shown to suppress basal and postprandial glucagon in Type 1 diabetes [10–13], but do not appear to further suppress glucagon secretion during hypoglycaemia [13]. This raises the intriguing possibility that DPP-4 inhibitor co-therapy in Type 1 diabetes, 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 central nervous system (hypothalamic) glucose sensing, leading to improved hypoglycaemia counter-regulation and awareness. To test this hypothesis directly, we designed a 12-week double-blind, randomized, crossover study in individuals with established C-peptide negative Type 1 diabetes. The primary outcome measure was the magnitude of the counter-regulatory symptom and hormone responses during a subsequent hyperinsulinaemic-hypoglycaemic clamp study, the 'gold standard' for assessing hypoglycaemia responses in Type 1 diabetes.

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

Adult subjects ($N = 14$) with C-peptide negative Type 1 diabetes and disease duration > 5 years were recruited and underwent medical screening (Fig. S1). Exclusion criteria were: previous history of pancreatic or liver disease; significant microvascular disease; taking drugs that affect CYP3A4 metabolism; pregnancy/breastfeeding; or history of seizures. Baseline demographic and information on current diabetes management was collated. All participants underwent assessment of their hypoglycaemic awareness through completion of the Gold questionnaire [14].

Consenting participants had an initial 3–4-week baseline period during which 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 period (iPRO CGM device; Medtronic Minimed Inc, Watford, UK) was used for education purposes; after 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. This was carried out in a one-to-one manner. A second blinded CGM was performed after a minimum of 3–4 weeks and the data from this were used as a baseline for calculation of glycaemic variability indices before 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 at least three times a day before meals and an additional reading before going to bed. In addition, participants were also encouraged to perform self-monitoring of blood glucose during all symptomatic hypoglycaemia episodes, and to record all levels < 3.5 mmol/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 CGM data using a simple interface. For the purposes of the present 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. The ADRR [17] has been designed to be equally sensitive to hypoglycaemia and hyperglycaemia, and has been shown to be the best predictor of 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]. HbA_{1c}, insulin doses and weight were also recorded before the first treatment phase.

Subsequently, participants were enrolled into two groups using a randomized block design. The participants were randomized in blocks of four using a computer-based randomization sequence generator. The research team issued a prescription to the 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 participants were allocated to each treatment sequence. Sequence A received placebo for the first 12 weeks, before receiving the DPP-4 inhibitor for the second arm. Sequence B received the treatments in reverse order to Sequence A. All participants were advised to continue their usual diabetes, dietary and exercise regime during the entire trial. The participants were contacted on a weekly basis for the 1st month, and then monthly thereafter. During each contact, adverse events were recorded and advice provided as required on insulin dose adjustment.

The participants were provided with a single daily oral 5-mg dose of the DPP-4 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 participants underwent a further period of blinded CGM (at least 5 days), blood samples were taken and each participant underwent a hyperinsulinaemic-hypoglycaemic clamp study to assess the magnitude of their counter-regulatory responses. Participants had a washout period of at least 2 weeks before entering the second arm of the trial.

Hyperinsulinaemic-hypoglycaemic clamp study

Overnight-fasted participants reported to the Clinical Research Centre, Dundee at 08:00 h. All subjects were asked to avoid hypoglycaemia in the 48 h before 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°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/h, until a blood glucose concentration of 7 mmol/l was reached, and then insulin was maintained at a dose of 1.5 mU/kg/min. Glycaemic plateaus were achieved through bedside measurement of blood glucose (Analox GM9D; Analox Instruments, London, UK) every 5–10 min, and using a variable 20% dextrose infusion. The participants were initially maintained in the euglycaemic range (4–6 mmol/l) for 40 min, before hypoglycaemia (2.5 mmol/l) being induced and subsequently maintained for 85 min. Blood samples for determination of insulin, adrenaline, noradrenaline and glucagon were drawn in triplicate during the baseline period, and then every 20 min during the hypoglycaemic phase. Blood pressure and pulse rate were measured every 10 min (Accutorr Plus Monitor, Datascope Corp., NJ, USA).

Blood sampling and analyses

Samples were centrifuged to separate the plasma within 2 h, and then stored at −80°C prior to assay. Hormone levels [insulin radioimmunoassay (DiaSorin, Dartford, UK): interassay coefficient of variation (CV) −6.7%, intra-assay CV −5.8%; glucagon radioimmunoassay (Millipore, Billerica, MA, USA): interassay CV 4.9%, intra-assay CV 8.8%; adrenaline ELISA assay kit (Alpco, Salem, NH, USA): interassay CV 22%, intra-assay CV 16%; noradrenaline-EIA (Alpco): interassay CV 16%, intra-assay CV 22%] were measured using an ELISA, and samples were analysed in duplicate, according to the manufacturer's instructions.

Symptoms and cognitive function tests

The participants rated hypoglycaemia symptoms three times during the 40-min euglycaemic period and every 20 min 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 [20] and Digit Symbol Substitution tasks, which are known to be sensitive to hypoglycaemia [21]. To minimize learning effects, all subjects had practised both tasks (5–7 days before the clamp study and also twice at the start of the clamp study).

Statistical analysis

The hypothesis predicted that DPP-4 inhibition would reduce exposure to hypoglycaemia, leading to improved central nervous system hypoglycaemia detection and subsequently enhanced adrenaline responses to subsequent hypoglycaemia.

This was therefore the prespecified primary outcome measure. Prior power calculations indicated that 12 participants were needed for a matched analysis, with 80% power to detect a difference in change of 450 pmol/l, with a standard deviation of 500 and a two-sided α value of 0.05. This difference in the adrenaline response was chosen based on previous published work [22]. Additional participants were recruited to account for a potential 25% dropout rate. Secondary outcomes included insulin requirements, HbA_{1c} values, glucose variability indices, frequency of hypoglycaemia, hypoglycaemic awarenes, and glucagon response during hypoglycaemia. Statistical analyses were conducted using GRAPHPAD PRISM 6 and a P value < 0.05 was taken to indicate statistical significance. 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 variables measured over time, with t-testing used to localize effects where indicated. No order effects were noted in any of the subsequent analyses.

Results

Recruitment was between September 2012 and July 2013. A total of 18 participants with Type 1 diabetes were screened, with a total of 14 white participants (eight men, six women) completing the two arms of the trial. The consort diagram is shown in Fig. S1. The median (interquartile range) age of participants was 45 (35–53) years. All participants had C-peptide negative (< 0.10 nmol/l) Type 1 diabetes, with a median (interquartile range) duration of disease of 18 (12–31) years. The mean (± SD) glycaemic control at trial entry was HbA_{1c} 64 (±2) mmol/mol. The mean weight was 74.1(±3) kg and mean BMI 26 (±0.8) kg/m^2. The 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]. The median (interquartile range) baseline Gold score was 3 .0 (2.0–4.0; 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 (Fig. 1a)]. The glucose infusion rates required to maintain the hypoglycaemia plateau were also similar in the two treatment groups [F(1,26) = 0.23, P = 0.64 (Fig. 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 × treatment interaction [F (6,156) = 0.17, P = 0.98]. The area under the curve for the adrenaline responses were also similar between groups [25 775 vs 24 454 for placebo vs saxagliptin, respectively; P = 0.76 (Fig. 1c)]. No significant effect of either hypoglycaemia or treatment was seen on the glucagon response to hypoglycaemia [P = non-significant (Fig. 1d)].

Consistent with the hormonal responses, the participants did not report any differences in their total symptom scores during hypoglycaemia between the two treatment arms [26 (±4) vs 28 (±3) for placebo vs saxagliptin; P = 0.38], or between autonomic symptoms [12 (±1) vs 13 (±1) for placebo vs saxagliptin; P = 0.36 (Fig. 1e)]. The two groups also performed similarly on cognitive tasks during hypoglycaemia: Trail Making B [37 (±6) vs 37 (±8) s for placebo vs saxagliptin; P = 0.96] or Digit Symbol Substitution [67 (±4) vs. 62 (±4) for placebo vs saxagliptin; P = 0.16 (Fig. 1f)].

Table 1 Baseline clinical and biochemical characteristics

Mean (SEM) age, years	42.9 (3.3)
Mean (SEM) weight, kg	74.1 (3)
Mean (SEM) duration of diabetes, years	20.5 (2.7)
Median (range) Gold score	3 (2–4)
Mean (SEM) HbA_{1c}, mmol/mol	64 (2)
Mean (SEM) insulin doses, units	
Long-acting	27 (4)
Short-acting	28 (4)

Glucose variability, hypoglycaemia frequency and 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 and Fig. 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 [HbA_{1c} 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 HbA_{1c} from pretreatment levels was small (+0.3 mmol/l with saxagliptin and −1.6 mmol/l with placebo) and did not differ significantly between groups (P = 0.61). There was no effect of saxagliptin on weight [mean increase of 0.24 kg with saxagliptin and 0.07 kg with placebo; F(1,11) = 0.40 P = 0.54].

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.

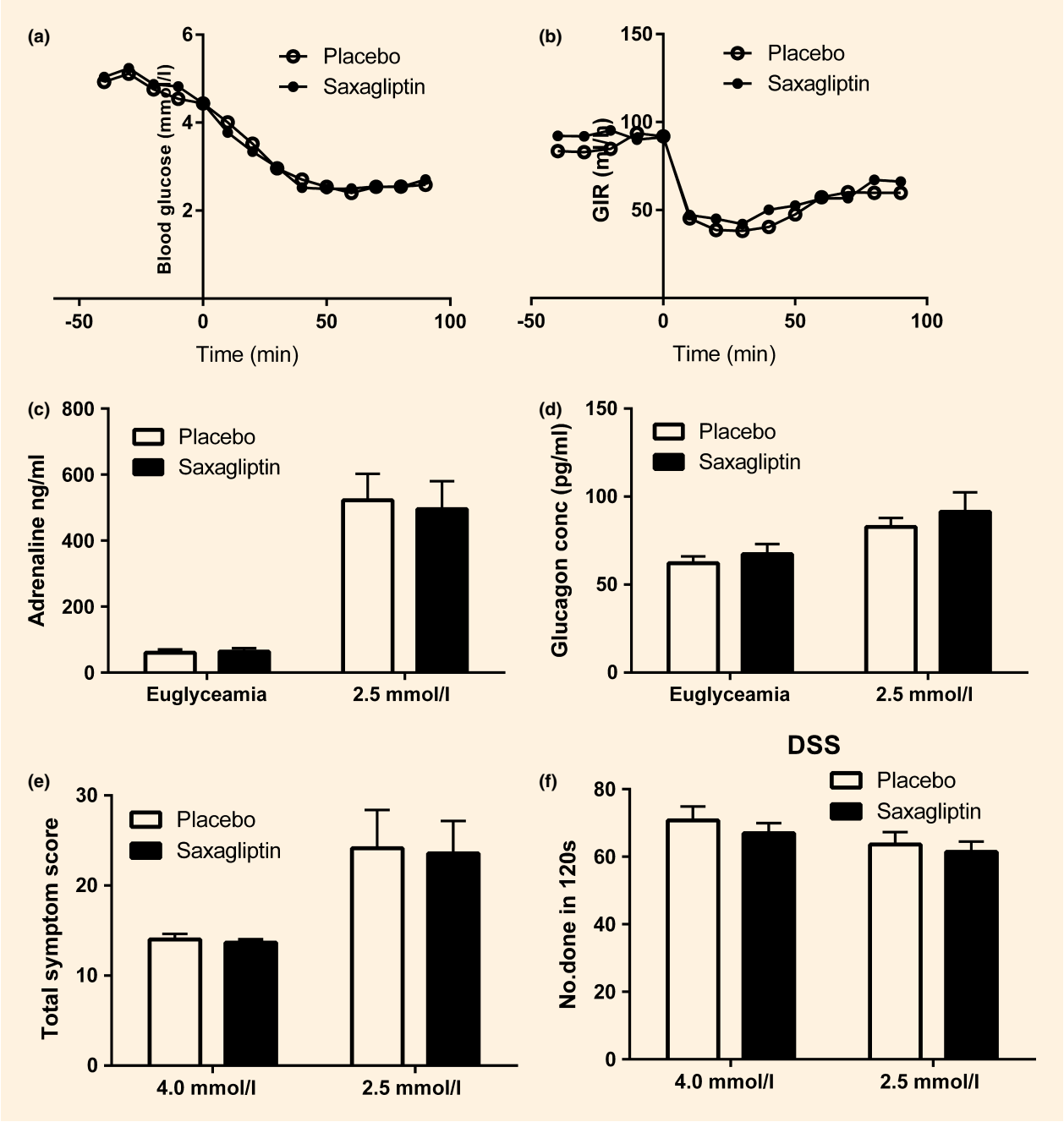

FIGURE 1 Non-insulin adjunct therapy with saxagliptin in subjects with C-peptide negative Type 1 diabetes had no effect on hormonal, symptom and cognitive responses to acute hypoglycaemia. (a) Blood glucose profiles and (b) glucose Infusion rates during hyperinsulinaemic glucose clamp. (c) Peak adrenaline 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.

Discussion

Antecedent hypoglycaemia is the major risk factor that leads to the development of impaired awareness of hypoglycaemia, which in turn markedly increases the risk of severe hypoglycaemia [3]. Conversely, hypoglycaemia avoidance strategies improve counter-regulatory responses to subsequent hypoglycaemia when tested formally using the clamp technique [23,24]. Hypoglycaemia in Type 1 diabetes results in a large part from non-physiological and unregulated hyperinsulinaemia as well as dysregulated glucagon secretion. As a consequence of this, specialized glucose sensing neurons in

Table 2 Measures of glycaemic control and glucose variability after 12 weeks' adjunct therapy with a dipeptidyl peptidase inhibitor (saxagliptin) or placebo in subjects with Type 1 diabetes

Variable	Placebo	Saxagliptin
Glycaemic control		
HbA_{1c}, mmol/mol	66 (2)	65 (2)
Total insulin dose, units	60 (8)	56 (7)
Long-acting	29 (4)	28 (4)
Short-acting	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)
Standard deviation glucose, mmol/l	3.6 (0.2)	3.7 (0.3)

ADRR, average daily risk range; LBGI, low blood glucose index; HBGI, high blood glucose index.
Glucose variability measures recorded using continuous glucose monitoring assessments in the final week of the trial. Values shown as mean (SEM).

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 impaired awareness of hypoglycaemia [3]; therefore, by improving physiological glucagon secretion in Type 1 diabetes, it should be possible to both reduce insulin requirements and propensity to mild to moderate hypoglycaemia, which in turn should reduce the central drive to supress catecholaminergic and symptom responses. Recent reports would appear to indicate that DPP-4 inhibition in Type 1 diabetes 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 Type 1 diabetes significantly improved glucose variability as assessed by M100, Glycaemic Risk Assessment Diabetes Equation and J-index. In contrast, the present study found no effect of DPP-4 inhibition in any measure of glycaemic variability, self-reported hypoglycaemia frequency or insulin dose, and subsequently no overall effect on hypoglycaemia counter-regulation. Although a mixed-meal test was not performed to examine whether 12 weeks of DPP-4 inhibitor therapy consistently supressed basal and postprandial glucagon levels, it seems unlikely based on our data that any significant impact on α-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 postprandial glucagon in subjects with Type 2 diabetes, the first evidence of which emerged at ~12 weeks' treatment duration [25]; therefore, and consistent with our findings, any benefit of DPP-4 inhibition in Type 1 diabetes, at least in terms of α-cell suppression, may be short-lived and unlikely to translate into significant improvements in glucose variability.

In addition, no benefit of DPP-4 inhibition on glycaemic control was found in the present study. Although the study was not powered to detect anything other than large effects, the very small change in HbA_{1c} levels observed after 12 weeks of 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 DPP-4 inhibitor vildagliptin in Type 1 diabetes had a small benefit in terms of HbA_{1c} reduction, and Ellis *et al.* [12] reported that 4 weeks of sitagliptin adjunct therapy in Type 1 diabetes significantly improved HbA_{1c} (-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 healthcare staff and more frequent monitoring played a large part in the improvements seen. Others have also reported no effect of DPP-4 inhibitors on glycaemic control in Type 1 diabetes [26].

The main limitation of the present study was 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. In addition, we were not able to measure GLP-1, glucagon and C-peptide

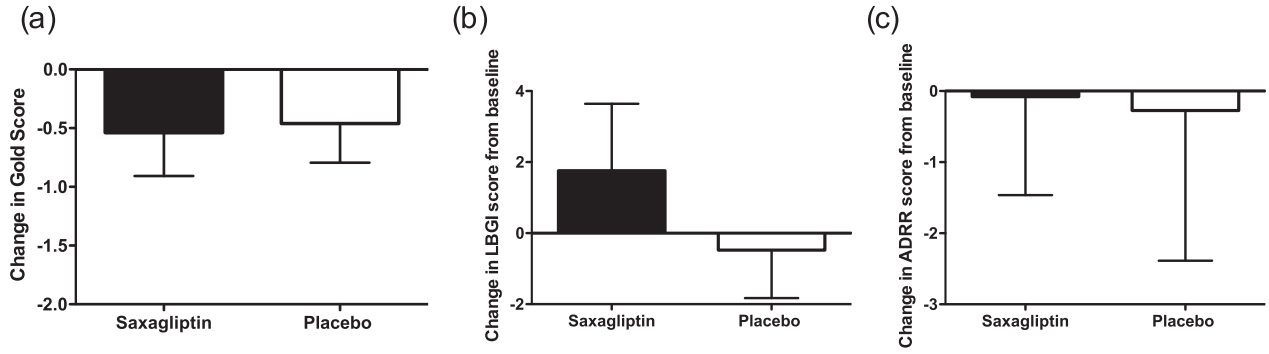

FIGURE 2 Non-insulin adjunct therapy in subjects with saxagliptin in C-peptide negative Type 1 diabetes had no effect on (a) hypoglycaemia awareness, (b) low blood glucose index (LBGI) or (c) average daily risk range (ADRR). Saxagliptin group shown by black bars, Placebo by white bars. Values shown as mean ± SEM.

responses to a standard meal in the present study so cannot say for certain that saxagliptin therapy in Type 1 diabetes was ineffective at improving postprandial glucose and glucagon responses.

In summary, we tested the hypothesis that adjunct therapy with oral DPP-4 inhibitor therapy, by reducing overall exposure to hypoglycaemia, would improve symptom and hormonal responses to hypoglycaemia in Type 1 diabetes. The results of the study lead us to reject this hypothesis, as we failed to show a significant effect on the primary outcome measure, the adrenaline response during a hyperinsulinaemic hypoglycaemic clamp study, after 12 weeks' DPP-4 inhibitor therapy when compared with placebo. In addition, no effect of DPP-4 inhibitor therapy 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 DPP-4 inhibitor therapy in the management of C-peptide negative Type 1 diabetes.

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Competing interests

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

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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 Pract Res Clin Endocrinol Metab* 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? *Appl Neuropsychol* 2002; **9**: 106–109.
21 Wechsler D. *Manual for the Wechsler Adult Intelligence Scale Revised*. New York: Psychological Corp, 1981.
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.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. CONSORT diagram.