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Chronic Treatment with the Dipeptidyl Peptidase-4 Inhibitor (*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356) Increases Basal Glucagon-Like Peptide-1 and Improves Glycemic Control in Diabetic Rodent Models

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ANOVA, analysis of variance; AUC, area under the curve; BI 1356, (*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione; DPP, dipeptidyl peptidase; GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide; HFD, high fat diet; NVP-DPP728, 1-[2-[(5-cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(*S*)-pyrrolidinecarbonitrile; OGTT, oral glucose tolerance test; STZ, streptozotozin; ZDF, Zucker Diabetic Fatty.

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Abstract

Antidiabetic effects of dipeptidyl peptidase (DPP)-4 inhibitors are exerted by potentiation of the biological activity of incretin hormones like glucagon-like peptide (GLP)-1. BI 1356 [proposed trade name ONDERO; (*R*)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione] is a novel competitive, selective, potent and long-acting DPP-4 inhibitor under clinical development for the treatment of type 2 diabetes. The effect of 1-2 months of chronic dosing of BI 1356 in two different animal models is investigated. The first is a primarily genetic model (Zucker Diabetic Fatty (ZDF) rats) and the second is a non-genetic model (mice with diabetes induced by a combination of high fat diet (HFD) and a low-dose streptozotocin (STZ)). BI 1356 was shown to lower HbA1c after multiple dosing in both models. The improvement of glycemic control achieved in disease models of different etiology suggests that BI 1356 would also be efficacious in treating a broad spectrum of type 2 diabetic patients. In addition, multiple dosing of BI 1356 leads to a sustained increase in basal levels of active GLP-1 in the systemic circulation with expected long-term benefits on pancreatic α- and ß-cells. The effects on HbA1c, as well as, GLP-1 were superior to the short-acting DPP-4 inhibitor vildagliptin, demonstrating the potential of BI 1356 as a once-daily treatment for type 2 diabetes at low therapeutic doses.

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Introduction

The global prevalence of type 2 diabetes appears to be increasing dramatically, possibly as a consequence of a more sedentary lifestyle and the adoption of Western diets (King et al., 1998). The World Health Organization estimates the number of people with diabetes to be approximately 180 million and this figure is projected to more than double by the year 2030. Furthermore, there is convincing evidence that the risk of developing microvascular complications, such as retinopathy, nephropathy, and neuropathy, is related to the degree of hyperglycemia (Palumbo, 2001). In addition, patients with type 2 diabetes have a higher cardiovascular morbidity and mortality and increased all-cause mortality compared with non-diabetic subjects (Manson et al., 1991; Balkau and Eschwège, 1999).

Type 2 diabetes is a progressive disease the underlying pathology of which comprises impaired insulin secretion and insulin resistance in target tissues (Taylor, 1999). The available therapies have only limited long-term efficacy given the progressive nature of the disease and tolerability issues arise during chronic treatment (Bolen et al., 2007). A novel approach targets the incretin hormones glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) that are released from the intestine in response to a meal (Meier and Nauck, 2006; Todd and Bloom, 2007). GLP-1 exerts a glucose-dependent insulinotropic action in ß-cells and suppresses glucagon secretion from α-cells (Nauck et al., 1993; Rachman et al., 1996). Furthermore, GLP-1 has been shown to restore ß-cell mass in rodents by stimulation of ß-cell neogenesis and proliferation, as well as, inhibiting ß-cell apoptosis (Xu et al., 1999; Tourrel et al., 2001; Farilla et al., 2002; Mu et al., 2006). GLP-1 is rapidly truncated and inactivated by dipeptidyl peptidase (DPP)-4 which possesses N-terminal dipeptidyl exopeptidase activity and exists as both a membrane-bound protein and as a soluble protein in plasma (Pauly et al., 1996). Inhibitors of DPP-4 potentiate the biological activity of

GLP-1 by preventing its inactivation and have demonstrated glucose-lowering efficacy in patients with type 2 diabetes (Pratley and Salsali, 2007).

BI 1356 (proposed trade name ONDERO) is a novel DPP-4 inhibitor under clinical development for the treatment of type 2 diabetes. The compound is derived from the xanthine class (Eckhardt et al., 2007) and is structurally different from other DPP-4 inhibitors that are currently available or in late stage clinical development. BI 1356 is a competitive inhibitor with a high potency (K_i = 1 nM) and a long off-rate from the DPP-4 enzyme that also shows a high selectivity against a wide range of peptidases including DPP-8 and DPP-9. After single dosing in preclinical models, the inhibition of DPP-4 and the duration of action on glucose tolerance are longer lasting with BI 1356 than with other DPP-4 inhibitors (sitagliptin, saxagliptin, vildagliptin, or alogliptin). Furthermore, BI 1356 demonstrated an increase in basal active GLP-1 even 24 h after single dosing (Thomas et al., 2008). The long duration of action of BI 1356 in vivo is in agreement with its long terminal half-life (35.5 h in rat) (Eckhardt et al., 2007).

In the current study, the efficacy of BI 1356 after chronic once-daily dosing in diabetic animal models of various disease etiologies is described and compared with the short acting DPP-4 inhibitor vildagliptin.

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Methods

Animals. This series of investigations looked at the *in vivo* profile of BI 1356 after chronic treatment in male high-fat diet (HFD)/streptozotocin (STZ)-induced diabetic ICR mice (Crl:CD1(ICR); obtained from Charles River Laboratories, Sulzfeld, Germany) and in male and female Zucker Diabetic Fatty (ZDF) rats (ZDF-*Lepr*^{fa}/Crl; obtained from Charles River Laboratories, Wilmington, MA). Animal procedures were approved by the local animal ethics committee and complied with National Institutes of Health guidance (Institute of Laboratory Animal Resources, 1996).

Animals were housed singly (male ZDF rats) or in groups (female ZDF rats, ICR mice) at controlled temperature and humidity conditions with a 12 h/12 h light/dark cycle (lights out between 6 PM and 6 AM). The animals had access to diet and water ad libitum. ICR mice were fed diet 824055 (Special Diets Services, Witham, England; 60% of kcal from fat). Male ZDF rats were on diet 5008 (Purina LabDiet, London, England; containing 2.6% sucrose) and female ZDF rats were on diet 58NX (Purina TestDiet, Richmond, IN; 48% of kcal from fat).

Compounds and dosing. BI 1356 (Eckhardt et al., 2007), vildagliptin (Villhauer et al., 2003) and NVP-DPP728 (Villhauer et al., 2002) were provided by the Department of Chemical Research (Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany). During chronic *in vivo* studies, compound administration was by oral gavage using 0.5% aqueous hyroxyethylcellulose as a vehicle. Compounds were administered once daily in the afternoon, 2-3 h before the onset of the dark phase. Control animals received vehicle only.

Analytical procedures. Blood glucose was measured with a glucometer (OneTouch Ultra; Lifescan, Milpitas, CA) or using an automated analyzer (Cobas Integra 400 Plus; Roche

Diagnostics; Indianapolis, IN), which was also used for measuring glycated hemoglobin A1c (HbA1c) in whole blood. Commercially available enzyme-linked immunosorbent and colorimetric assay kits were used to measure GLP-1-(7-36)amide (Linco Research Inc., St. Charles, MO), insulin (Crystal Chem Inc., Downers Grove, IL) and free fatty acids (Wako Chemicals GmbH, Neuss, Germany) in EDTA plasma. Blood samples were immediately supplemented with 100 µM DPP-4 inhibitor NVP-DPP728 to prevent degradation of active GLP-1.

***Ex vivo* DPP-4 inhibition assay.** Blood samples were drawn from the retrobulbal venous plexus under isoflurane anesthesia 21 h after compound administration. EDTA plasma was frozen for *ex vivo* measurement of DPP-4 activity which was assayed in 5-fold diluted plasma after 10 min incubation with substrate as described (Thomas et al., 2008).

Chronic study in diabetic mice. Diabetes was induced in male ICR mice by a combination of HFD feeding starting at the age of 4 weeks and a single low-dose intraperitoneal injection of 100 mg/kg streptozotocin (Sigma-Aldrich, St. Louis, MO) at the age of 7 weeks. Once daily compound treatment in diabetic animals was started at the age of 12 weeks after groups had been randomized for HbA1c and glucose. Treatment was continued for 4 weeks. Blood glucose measurements in fed animals (treatment day 27) and an oral glucose tolerance test (OGTT; treatment day 17) in overnight (16 h) fasted animals with a glucose challenge of 2 g/kg were performed during the study 16 h after compound administration. Blood samples for glucose measurement were obtained by tail bleed. Blood samples for measurement of HbA1c (treatment days 14 and 27), GLP-1 and insulin (treatment day 27) were drawn from the retrobulbal venous plexus under isoflurane anesthesia.

Chronic studies in diabetic rats. Once daily compound treatment was started in male ZDF rats at the age of 6 weeks after randomization for HbA1c values. Treatment was continued for 5 weeks. In this study, all blood samples were obtained by tail bleed. During the study, a 24 h glucose profile was measured by drawing blood samples every 4 h (treatment day 21 and 22). Animals had free access to food and water throughout the 24 h sampling period. At the end of the study (treatment day 34), an OGTT was performed in rats that were mildly fasted (60% of normal food intake resulting in an approximately 8 h fasting) before the test. On the day of the OGTT, animals received a compound administration 15 min before an oral glucose load of 1 g/kg. Plasma glucose, GLP-1 and insulin were measured before the administration of the compound, before the glucose load and at serial time points after the glucose challenge. In this study, plasma glucose was measured on an Vitros DT60II automated analyzer (Ortho Clinical Diagnostics, Inc., Rochester; NY), and GLP-1 and insulin were measured using a Rat Endocrine Immunoassay Panel (LINCOplex, analyzed using a Luminex100TM system; LINCO Research Inc., St. Charles, MO).

In female ZDF rats, HFD feeding was started at the age of 7 weeks and once daily compound treatment began one week later after randomization for HbA1c values. Treatment was continued for 7.5 weeks. An OGTT was performed during the study (treatment day 32) in overnight (16 h) fasted animals with a glucose challenge of 2 g/kg given 16 h after compound administration. Blood samples for glucose measurement were obtained by tail bleed and blood samples for measurement of HbA1c by puncture of the retrobulbal venous plexus under isoflurane anesthesia.

Data Analysis. Statistical comparisons were conducted by Student's *t* test or one-way ANOVA followed by Bonferroni's post test for group-wise comparisons, as specified in the legends for figures. In the female ZDF study, statistical comparison between the HbA1c

values of control and active groups was conducted by repeated measures two-way ANOVA followed by one-sided decreasing *t* tests for group-wise comparisons. A p value < 0.05 was considered to show a statistically significant difference, and a p value < 0.1 was regarded as a trend.

Results

Effects of BI 1356 on glycemic control in high-fat diet/streptozotocin-induced diabetic mice. BI 1356 is a novel competitive, selective, potent and long-acting inhibitor of DPP-4 after single dosing. To investigate the effects of BI 1356 on glycemic control after multiple dosing, a 4-week study was carried out in a non-genetic rodent model of diabetes (Luo et al., 1998). Male ICR mice were fed a high-fat diet (HFD) for 3 weeks and then received a single low-dose injection of streptozotocin (STZ). After STZ administration, HFD feeding was continued. Mean body weight (45 g), blood glucose (18 mM) and plasma insulin (0.75 ng/mL) at the start of the treatment with BI 1356 were comparable to published values (Mu et al., 2006) confirming the hyperglycemic and hyperinsulinemic state of the animals. The HFD/STZ mice then received once-daily oral administrations of BI 1356 at 1, 3, or 10 mg/kg for a period of 4 weeks.

DPP-4 activity in plasma was measured 21 h post-dose (close to trough) on treatment day 27. Treatment with BI 1356 resulted in a dose-dependent inhibition of plasma DPP-4 by 59%, 78%, and 87%, compared with the baseline values on day 0 (Figure 1A). Virtually the same inhibition of plasma DPP-4 activity was already seen after 14 days of treatment, indicating that a steady state of enzyme inhibition had been reached (data not shown). There was no difference in food intake and body weight development during the course of the study between the vehicle-treated control group and the groups treated with the DPP-4 inhibitor (data not shown).

All doses of BI 1356 equally improved oral glucose tolerance after 17 days of treatment (Figure 1, B and C), indicating that in all cases the achieved inhibition of DPP-4 was sufficient to result in a maximal reduction of glucose excursion. Since the test was performed

16 h post-dose, the DPP-4 inhibition is expected to be more than 60% even with the low-dose of 1 mg/kg (compare Figure 1A). In accordance with the improved glucose tolerance, postprandial glucose (measured after beginning of the light phase) was also reduced (Figure 1D). The lowering of blood glucose is reflected in a significant decrease of HbA1c over the course of the study (Figure 2). The change in HbA1c after 4 weeks compared with vehicle-treated animals was 0.8%, 0.9%, and 1.0% with BI 1356 doses of 1, 3, and 10 mg/kg, respectively.

Basal GLP-1 levels 16 h after compound administration were also examined. Active GLP-1 was dose-dependently elevated after multiple dosing of BI 1356 on day 28 of the study (Figure 3A). This rise in active GLP-1 is an increase in basal levels and not glucose-dependent since glucose levels were even lower in animals treated with BI 1356 than in the controls (Figure 1D). In contrast to GLP-1, insulin levels were not different between the groups (Figure 3B).

Effects of BI 1356 on glycemic control in Zucker Diabetic Fatty rats. Next, the effect of BI 1356 on glucose homeostasis after multiple dosing in a more extreme diabetic animal model, the male ZDF rat, was investigated. This model is characterized by very severe insulin resistance in young animals and a progressive ß-cell failure with increasing age (Peterson RG, 2001). The ZDF rats received once-daily oral administrations of 3 mg/kg BI 1356 for 5 weeks and this treatment was compared to another DPP-4 inhibitor, vildagliptin, at the same dose and regimen. No change in body weight development was observed between the different groups during the course of the study (Figure 4A). However, BI 1356 reduced cumulative food intake at the end of the study by 6% (Figure 4B). Vildagliptin had no significant effect on food intake.

An OGTT performed on treatment day 34 demonstrated that BI 1356 and vildagliptin both reduced glucose excursion by about 50% (Figure 5, A and B). Both compounds had been administered 15 min before the glucose load, indicating that under these acute conditions they are equally effective on glucose homeostasis. In the case of vildagliptin, levels of active GLP-1 in plasma were not different from control animals before compound administration. In contrast, active GLP-1 was increased about 2.5-fold before dosing with BI 1356 (Figure 5C). Since the last compound administration had been 16 h previously, these data show that treatment with BI 1356, but not vildagliptin results in a long-lasting increase in basal levels of active GLP-1. BI 1356 and vildagliptin were both shown to further increase active GLP-1 after administration and before glucose load, which was followed by a peak level 5 min after the glucose load. Levels of active GLP-1 were still elevated 2 h after glucose challenge, but at a higher level for BI 1356 compared to vildagliptin. Basal insulin levels before and after the glucose tolerance test were equally high between all the groups (Figure 5D). An insulin peak was observed 15 min after the glucose load which was particularly prominent with BI 1356.

On days 21 and 22 of the study, a 24 h glucose profile in the fed state was measured by taking blood samples every 4 h (Figure 6). In contrast to vildagliptin, animals tended to have lower blood glucose levels after chronic treatment with BI 1356 and total 24 h glucose AUC was significantly reduced by 10%.

The control animals showed a marked increase in HbA1c from 3.2% to 6.4% during the 5-week study (Figure 7A), underlining the severe progression of diabetes in the male ZDF rats. Vildagliptin appeared to have no effect on the development of HbA1c, whereas BI 1356 showed an, albeit insignificant, tendency for lowering HbA1c by 0.4%. The reduction of HbA1c by BI 1356 is in accordance with its superior effect on the 24 h glucose profile as compared to vildagliptin.

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In order to confirm the effect of BI 1356 on HbA1c in the ZDF model, multiple dosing was repeated in the less severe diabetic female animals of the same strain and the duration of the treatment was extended to 7.5 weeks. Glucose tolerance was improved after multiple dosing (Figure 8). HbA1c in the control animals increased from 2.1% to 3.5% after 4 weeks and to 4.7% after 7.5 weeks (Figure 7B). Again, BI 1356 showed a dose-dependent trend to reduce HbA1c versus control by 0.3% after 4 weeks and by 0.7% after 7.5 weeks ($p < 0.1$ for the 3 mg/kg dose of BI 1356), thus supporting the effect observed in male ZDF rats.

Discussion

DPP-4 inhibitors augment the effects of incretin hormones by prolonging their half-life and represent a new therapeutic approach for the treatment of type 2 diabetes (Pratley and Salsali, 2007). Currently, in late stage clinical development BI 1356 is a potent, selective and long-acting DPP-4 inhibitor from a novel structural class (Thomas et al., 2008). In the current study, BI 1356 was tested after chronic once-daily dosing in several preclinical models of diabetes. The ZDF rat is a well-characterized genetic model of obesity and type 2 diabetes. Male ZDF rats develop diabetes rapidly between 6 and 10 weeks of age (Peterson RG, 2001).

It has previously been shown that BI 1356 increases basal GLP-1 levels even 24 h after single dosing (Thomas et al., 2008). This long-lasting rise in basal GLP-1 was shown to be sustained in diabetic male ZDF rats after multiple dosing, demonstrating that the effect does not deteriorate with time. Since the basal GLP-1 rise is not dependent on elevated glucose levels, it is hypothesized that small amounts of active GLP-1 are permanently secreted by intestinal cells and that BI 1356 causes these minor amounts to accumulate by preventing their degradation. It has also previously been demonstrated that another DPP-4 inhibitor, vildagliptin, is a short-acting compound in contrast to BI 1356 (Thomas et al., 2008). Indeed, basal GLP-1 levels were not increased by vildagliptin indicating that a long-lasting inhibition of the DPP-4 enzyme is required for this to occur. Increased basal GLP-1 levels might therapeutically result in stimulation of ß-cell regeneration and increased ß-cell mass (Baggio and Drucker, 2006; Mu et al., 2006), as well as, attenuate the exaggerated glucagon secretion from α-cells that is characteristic of diabetes (Franklin et al., 2005). Furthermore, GLP-1 is known to have extrapancreatic actions which include inhibition of food intake and gastric empyting (Drucker 2006). The reduction in cumulative food intake during the study in male ZDF rats seen with BI 1356 but not vildagliptin is in agreement with the elevated basal GLP-1

levels elicited by BI 1356. This reduced food intake over the course of our study (5 weeks) did not result in body weight loss which may be due to severe hyperphagia and/or secondary mechanisms in this model, e.g. a compensatory decrease in energy expenditure.

Irrespective of the different basal GLP-1 levels, the time course of active GLP-1 during an OGTT carried out immediately after administration of BI 1356 or vildagliptin revealed that both DPP-4 inhibitors resulted in a comparable increase in GLP-1, which was released glucose-independently (i.e. before the glucose bolus) and putatively (at least in part) in a glucose-dependent manner after the glucose bolus. The glucose-dependent increase by BI 1356 in active GLP-1 on top of an elevated basal GLP-1 level has already been demonstrated in acute studies 16 h after dosing of the DPP-4 inhibitor (Thomas et al., 2008). However, while there was only a very minor increase in glucose-dependent insulin secretion by vildagliptin, the insulin peak was much more profound with BI 1356. This finding indicates that either the higher active GLP-1 on top of the elevated basal levels has a superior effect on insulin secretion or that the long-term elevated basal levels bona fide exert a trophic effect on ß-cells, thus increasing their glucose competence. It is noteworthy that the elevated basal GLP-1 levels did not further enhance the basal hyperinsulinemia in these animals, signifying that the insulinotropic effect of GLP-1 is still glucose-dependent. Furthermore, the high fasting hyperinsulinemia (about 4-5 ng/mL) reminiscent of the high peripheral insulin resistance in this animal model probably explains why the acute effects on glucose excursion are similar for BI 1356 and vildagliptin despite clearly divergent mechanistic effects on active GLP-1 and insulin. These data indicate that the maximal achievable effect seems to be limited by the degree of insulin resistance in this animal model.

The results of a glucose homeostasis analysis, acutely after dosing with either BI 1356 or vildapliptin failed to show any significant difference in lowering glucose excursion between

the two DPP-4 inhibitors. However, when studying the full 24 h glucose profiles in the fed state the long duration of action for BI 1356 over vildagliptin became very evident. Only chronic treatment with the long-acting BI 1356 (and supposedly also any other long-acting DPP-4 inhibitor) resulted in an overall glucose lowering effect, but not the treatment with the short-acting vildagliptin. The difference in the duration of action between BI 135 and vildagliptin has previously been shown in acute studies (Thomas et al., 2008). The glucose lowering seen with BI 1356 in the chronic study translated into a numerical reduction of HbA1c by 0.4% after 5 weeks of treatment. No such reduction was seen for vildagliptin. These data emphasize that the glycemic control achievable via the DPP-4 mechanism depends not only on the degree of DPP-4 inhibition but on the duration of DPP-4 inhibition during a 24 h period.

To further substantiate the minor effect of BI 1356 on HbA1c in the severely diabetic male ZDF rats, an additional chronic study in female animals of the same strain was performed. Female obese ZDF rats have a somewhat less severe diabetic phenotype with a slower progression than males and they develop this phenotype only when consuming a diabetogenic diet (Peterson, 2001). Although female ZDF rats also show a deterioration of diabetes with time, the progression is slower in comparison to the males. BI 1356 showed a dose- and time-dependent reduction of HbA1c in the female ZDF model that had higher statistical significance when compared to the males. Together, these data demonstrate that a potent and long acting DPP-4 inhibitor such as BI 1356 improves glycemic control even in disease models, which are hampered by genetically driven severe insulin resistance and progressive ß-cell failure.

Non-genetic animal models of diabetes offer the possibility to explore effects on glycemic control in the context of different disease etiologies. In a high-fat diet/streptozotocin-induced

diabetic mouse model, insulin secretion is impaired by a single low-dose injection of streptozotocin and peripheral insulin resistance is provoked by feeding a high-fat diet (Luo et al., 1998). In this model, HbA1c levels remained constant over the 4-week study period with multiple dosing of BI 1356. While the improvement of glucose tolerance and the lowering of blood glucose were not greater in the mouse model than in the ZDF model, a highly significant reduction of HbA1c was achieved with BI 1356 after chronic treatment. Furthermore, the reduction was larger than after 4 weeks of treatment in ZDF rats (0.9% vs. 0.3-0.4% in comparison of the 3 mg/kg dose with control). Similarly to the ZDF rats, chronic treatment with BI 1356 in HFD/STZ mice resulted in an increase of basal GLP-1 levels suggesting a similar mechanistic effect of BI 1356 in the two models.

The current data clearly indicate that the absolute degree of glycemic control that can be reached via the DPP-4 mechanism depends on the underlying pathology causing diabetes in a particular animal model. More importantly, BI 1356 improves glycemic control after chronic treatment in various diabetic animal models independent of their disease etiology suggesting that it would be efficacious in treating a broad spectrum of type 2 diabetic patients. Indeed, a long lasting inhibition of DPP-4 and improvement of glucose tolerance, as well as, HbA1c lowering have already been confirmed in humans (Forst et al., 2007). In the clinical study by Forst et al., the maximal mean placebo-corrected change in HbA1c was -0.37% after 4 weeks of treatment of type 2 diabetic patients. This effect was already achieved after a treatment period that covers only one half-life of human glycosylated hemoglobin. The half-lives of glycosylated hemoglobin have been reported to be 30, 16, and 15 days in man, mouse, and rat, respectively (Rendell et al., 1985). In the HFD/STZ mouse model, the decrease in HbA1c after one half-life was about 0.5-0.6%. Extrapolating the efficacy of BI 1356 in the female ZDF rat model to one half-life yields a decrease of about 0.2-0.4%. Thus, it seems as if the achievable degree of glycemic control in these two animal models might roughly translate

into the clinical situation, whereby the mouse model would overestimate and the rat model underestimate the human efficacy to some extent. In general, rodent models with a moderate severity of diabetes appear to have a better predictivity for the efficacy of a DPP-4 inhibitor in humans as a model like the male ZDF rat with a very severe diabetes and a rapid disease progression. It should be emphasized that no animal model fits the human situation in all aspects.

In summary, BI 1356 improves glucose homeostasis after multiple dosing in different animal models of diabetes. The positive effects of BI 1356 on glycemic control are paralleled by a sustained increase in basal GLP-1 levels, which offers the potential for beneficial effects on pancreatic islets that extend beyond lowering hyperglycemia. Additional studies are ongoing to investigate the effects of once daily chronic administration of BI 1356 on ß-cell morphology, preservation and function.

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References

Baggio LL and Drucker DJ (2006) Therapeutic approaches to preserve islet mass in type 2 diabetes. *Annu Rev Med* **57**:267-281.

Balkau B and Eschwège E (1997) Insulin resistance: An independent risk factor for cardiovascular disease? *Diabetes Obes Met* **1**:S23-S31.

Bolen S, Feldman L, Vassy J, Wilson L, Yeh HC, Marinopoulos S, Wiley C, Selvin E, Wilson R, Bass EB, and Brancati FL (2007) Systematic review: Comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. *Ann Intern Med* **147**:386-399.

Drucker DJ (2006) The biology of incretin hormones. *Cell Metab* **3**:153-165.

Eckhardt M, Langkopf E, Mark M, Tadayyon M, Thomas L, Nar H, Pfrengle W, Guth B, Lotz R, Sieger P, Fuchs H, and Himmelsbach F (2007) 8-(3-(R)-Aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective,long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J Med Chem* **50**:6450-6453.

Farilla L, Hongxiang H, Bertolotto C, Kang E, Bulotta A, Mario UDI, and Perfetti R. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in zucker diabetic rats. *Endocrinology* **143**:4397-4408.

Forst T, Uhlig-Laske B, Ring A, Ritzhaupt A, Graefe-Mody U, and Dugi KA (2007) The novel, potent, and selective DPP-4 inhibitor BI 1356 significantly lowers HbA1c after only 4

weeks of treatment in patients with type 2 Diabetes, in *Proceedings of the American Diabetes Association 67th Scientific Sessions*; 2007 June 22-26; Chicago, IL. Abstract 0594-P, American Diabetes Association Chicago, IL.

Franklin I, Gromada J, Gjinovci A, Theander S, and Wollheim CB (2005) β-Cell secretory products activate α-cell ATP-dependent potassium channels to inhibit glucagon release. *Diabetes* **54**:1808-1815.

Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals* 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.

King H, Aubert RE, and Herman WH (1998) Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* **21**:1414-1431.

Luo J, Quan J, Tsai J, Hobensack CK, Sullivan C, Hector R, and Reaven GM (1998) Nongenetic mouse models of non-insulin-dependent diabetes mellitus. *Metabolism* **47**:663-668.

Manson JAE, Colditz GA, Stampfer MJ, Willett WC, Krolewski AS, Rosner B, Arky RA, Speizer FE, and Hennekens CH (1991) A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. *Arch Int Med* **151**:1141-1147.

Meier JJ and Nauck MA (2006) Incretins and the development of type 2 diabetes. *Curr Diab Rep* **6**:194-201.

Mu J, Woods J, Zhou YP, Roy RS, Li Z, Zycband E, Feng Y, Zhu L, Li C. Howard AD, Moller DE, Thornberry NA, and Zhang BB (2006) Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. *Diabetes* **55**:1695-1704.

Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, and Creutzfeldt W (1993) Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **36**:741-744.

Palumbo PJ (2001) Gycemic control, mealtime glucose excursions, and diabetic complications in type 2 diabetes mellitus. *Mayo Clin Proc* **76**:609-618.

Pauly RP, Rosche F, Wermann, M, McIntosh CH, Pederson RA, and Demuth HU (1996) Investigation of glucose-dependent insulinotropic polypeptide-(1-42) and glucagon-like peptide-1-(7-36) degradation in vitro by dipeptidyl peptidase IV using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. A novel kinetic approach. *J Biol Chem* **271**:23222-23229.

Peterson RG (2001) The Zucker diabetic fatty (ZDF) rat, in *Animal models of diabetes: A primer* (Anders AFS and Shafrir E eds) pp 109-128, Harwood Academic Publishers, Amsterdam, The Netherlands.

Pratley RE and Salsali A (2007) Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. *Curr Med Res Opin* **23**:919-931.

Rachman J, Gribble FM, Barrow BA, Levy JC, Buchanan KD, and Turner RC (1996) Normalization of insulin responses to glucose by overnight infusion of glucagon-like peptide 1 (7-36) amide in patients with NIDDM. *Diabetes* **45**:1524-1530.

Rendell M, Stephen PM, Paulsen R, Valentine JL, Rasbold K, Hestorff T, Eastberg S, and Shint DC (1985). An interspecies comparison of normal levels of glycosylated hemoglobin and glycosylated albumin. *Comp Biochem Physiol* 81B: 819-822.

Taylor SI (1999) Deconstructing type 2 diabetes. *Cell* **97**:9-12.

Thomas L, Eckhardt M, Langkopf E, Tadayyon M, Himmelsbach F, and Mark M (2008) (R)-8-(3-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a novel xanthine-based dipeptidyl peptidase 4 inhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors. *J Pharmacol Exp Ther* **325**:175-182.

Todd JF and Bloom SR (2007) Incretins and other peptides in the treatment of diabetes. *Diabet Med* **24**:223-232.

Tourrel C, Bailbé D, Meile M-, Kergoat M, and Portha B (2001) Glucagon-like peptide-1 and exendin-4 stimulate β-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes* **50**:1562-1570.

Villhauer EB, Brinkman JA, Naderi GB, Burkey BF, Dunning BE, Prasad K, Mangold BL, Russell ME, and Hughes TE (2003) 1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(S)-

pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* **46**:2774-2789.

Villhauer EB, Brinkman JA, Naderi GB, Dunning BE, Mangold BL, Mone MD, Russell ME, Weldon SC, and Hughes TE (2002) 1-[2-[(5-Cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(S)-pyrrolidinecarbonitrile: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem* **45**:2362-2365.

Xu G, Stoffers DA, Habener JF, and Bonner-Weir S (1999) Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* **48**:2270-2276.

Footnotes

Parts of this work were previously published as follows:

Thomas L and Mark M (2008) BI 1356 (proposed trade name ONDERO), a novel xanthine-based DPP-4 inhibitor, improves glycaemic control after multiple dosing in various diabetic animal models. *3rd International Conference on Dipeptidyl peptidases and related proteins*; 2008 Apr 23-25; Antwerp, Belgium. Organizing committee De Meester I, Scharpé S, and Haemers A, Antwerp, Belgium.

Thomas L, Tadayyon M, and Mark M (2008) BI 1356 (proposed trade name ONDERO), a novel xanthine-based DPP-4 inhibitor, increases basal GLP-1 and improves glycemic control in diabetic animals, in *Proceedings of the American Diabetes Association 68th Scientific Sessions*; 2008 June 6-10; San Francisco, CA. American Diabetes Association, San Francisco, CA.

The chronic study in male ZDF rats was performed under contract by Rheoscience A/S (Roedovre, Denmark) with Niels Vrang and Mads Tang-Christensen as project managers and Kirsten Lykkegaard as study director.

Legends for Figures

Fig. 1. Inhibition of plasma DPP-4 activity and effects on blood glucose in HFD/STZ mice after multiple once daily oral dosing of BI 1356 at the indicated doses. A: Plasma DPP-4 activity on day 27 of treatment. The measurements were performed 21 h post-dose. B: OGTT in overnight fasted animals on day 17 of treatment. The test was performed 16 h post-dose. C: Reactive glucose AUC for the OGTT shown in B. D: Postprandial glucose at baseline and on day 27 of treatment. Glucose was measured in the non-fasted state 16 h post-dose. Data in all panels are means ± S.E.M. (n = 10/group). Statistical comparison between control and active groups was conducted by one-way ANOVA and by paired Student's *t* test for comparison with baseline values within groups (#, $p < 0.1$; *, $p < 0.05$; ***, $p < 0.001$).

Fig. 2. Time course of HbA1c after multiple once daily oral dosing of BI 1356 at the indicated doses in HFD/STZ mice. Data are means ± S.E.M. (n = 10/group). Statistical comparison between baseline and on-treatment values was conducted by repeated measures one-way ANOVA (**, $p < 0.01$; ***, $p < 0.001$).

Fig. 3. Levels of active GLP-1 (A) and insulin (B) in plasma of HFD/STZ mice on day 28 after multiple once daily oral dosing of BI 1356 at the indicated doses. Blood samples were obtained in the non-fasted state 16 h post-dose. Data are means ± S.E.M. (n = 10/group). Statistical comparison between control and active groups was conducted by one-way ANOVA (#, $p < 0.1$; ***, $p < 0.001$).

Fig. 4. Body weight development (A) and cumulative food intake (B) in male ZDF rats during multiple once daily oral dosing with either 3 mg/kg vildagliptin or BI 1356. Data are means ±

S.E.M. (n = 10-30/group). Statistical comparison between control and active groups at the end of the study was conducted by one-way ANOVA (*, $p < 0.05$).

Fig. 5. OGTT in male ZDF rats (fasted for approximately 8 h) on day 34 after multiple once daily oral dosing of 3 mg/kg vildagliptin or 3 mg/kg BI 1356. Compound administration was 15 min before the glucose challenge and is depicted by an arrow. A: Temporal course of glucose excursion. Mean fasting plasma glucose levels before the glucose challenge varied between 21 and 25 mM but were not significantly different between the groups. B: Reactive glucose AUC. Statistical comparison between control and active groups was conducted by one-way ANOVA (#, $p < 0.1$; *, $p < 0.05$). C: Temporal course of active GLP-1. Statistical comparison between control and active groups was conducted by unpaired Student's *t* test at each time point. BI 1356 was significantly different from the control group at the pre-dose value -15 min ($p < 0.01$) and at all subsequent time points ($p < 0.05$ or $p < 0.01$) except at 15 min. Vildagliptin was not significantly different from the control group at the pre-dose value -15 min, but at all subsequent time points ($p < 0.05$ or $p < 0.01$) except at 45 min. D: Temporal course of insulin. Data in all panels are means ± S.E.M. (n = 10/group).

Fig. 6. Glucose profile in the fed state over 24 h in male ZDF rats on day 21/22 after multiple once daily oral dosing of 3 mg/kg vildagliptin or 3 mg/kg BI 1356 (A). Compound administration is depicted by an arrow and the dark phase by a black rectangle. Total 24 h glucose AUC is shown in B. Data are means ± S.E.M. (n = 10-30/group). Statistical comparison between control and active groups was conducted by one-way ANOVA (*, $p < 0.05$).

Fig. 7. HbA1c after multiple once daily oral dosing of 3 mg/kg vildagliptin or 3 mg/kg BI 1356 in male ZDF rats (A) or of BI 1356 at the indicated doses in female ZDF rats (B). Data

are means ± S.E.M. (n = 10-30/group in A; n = 7/group in B). Statistical comparison between control and active groups was conducted by repeated measures two-way ANOVA (#, $p < 0.1$).

Fig. 8. OGTT in overnight fasted female ZDF rats on day 32 after multiple once daily oral dosing of BI 1356 at the indicated doses. The test was performed 16 h post-dose. A: Temporal course of glucose. B: Reactive glucose AUC. Data in all panels are means ± S.E.M. Statistical comparison between control and active groups was conducted by unpaired Student's *t* test (*, $p < 0.05$).

Figure 1

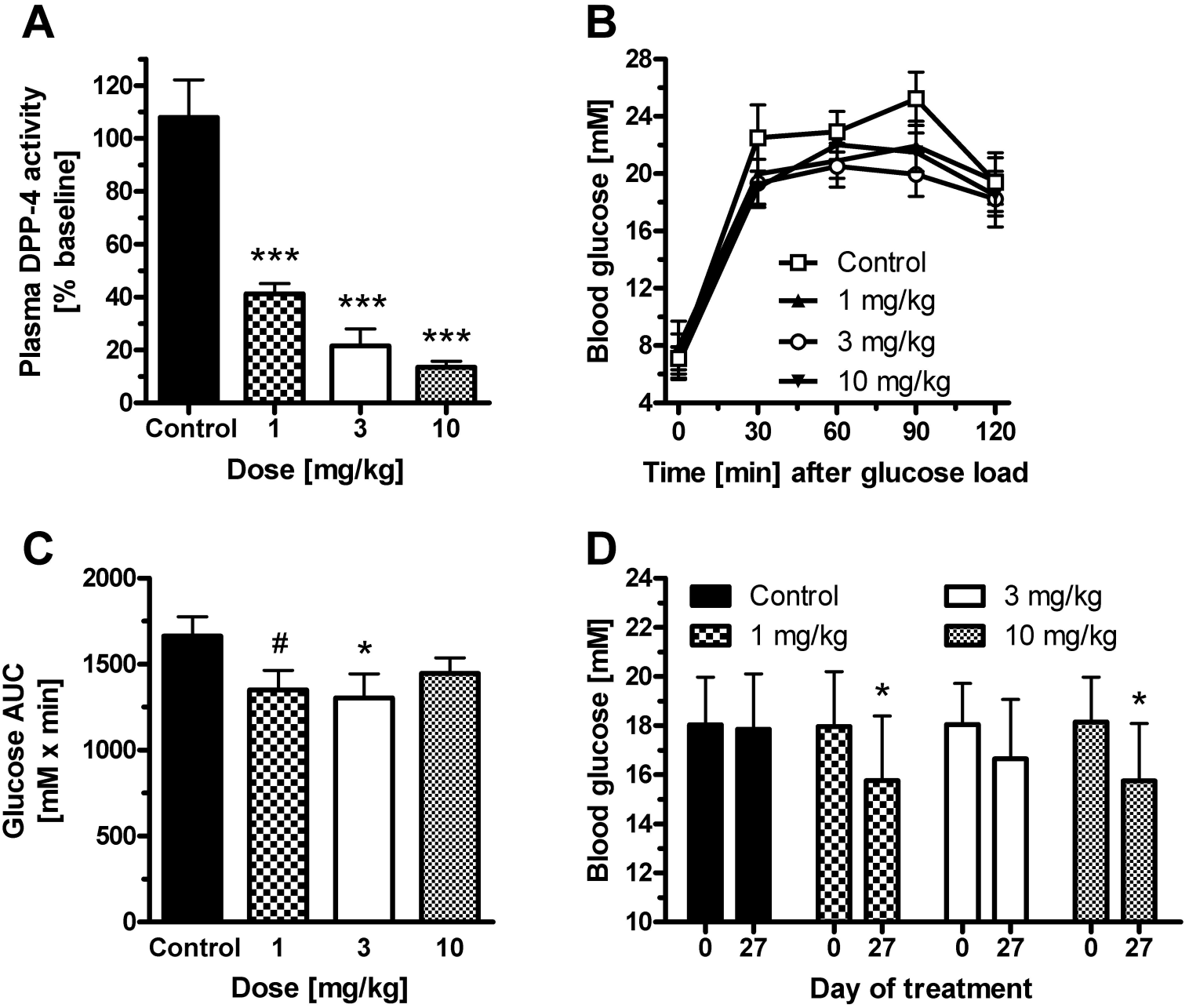
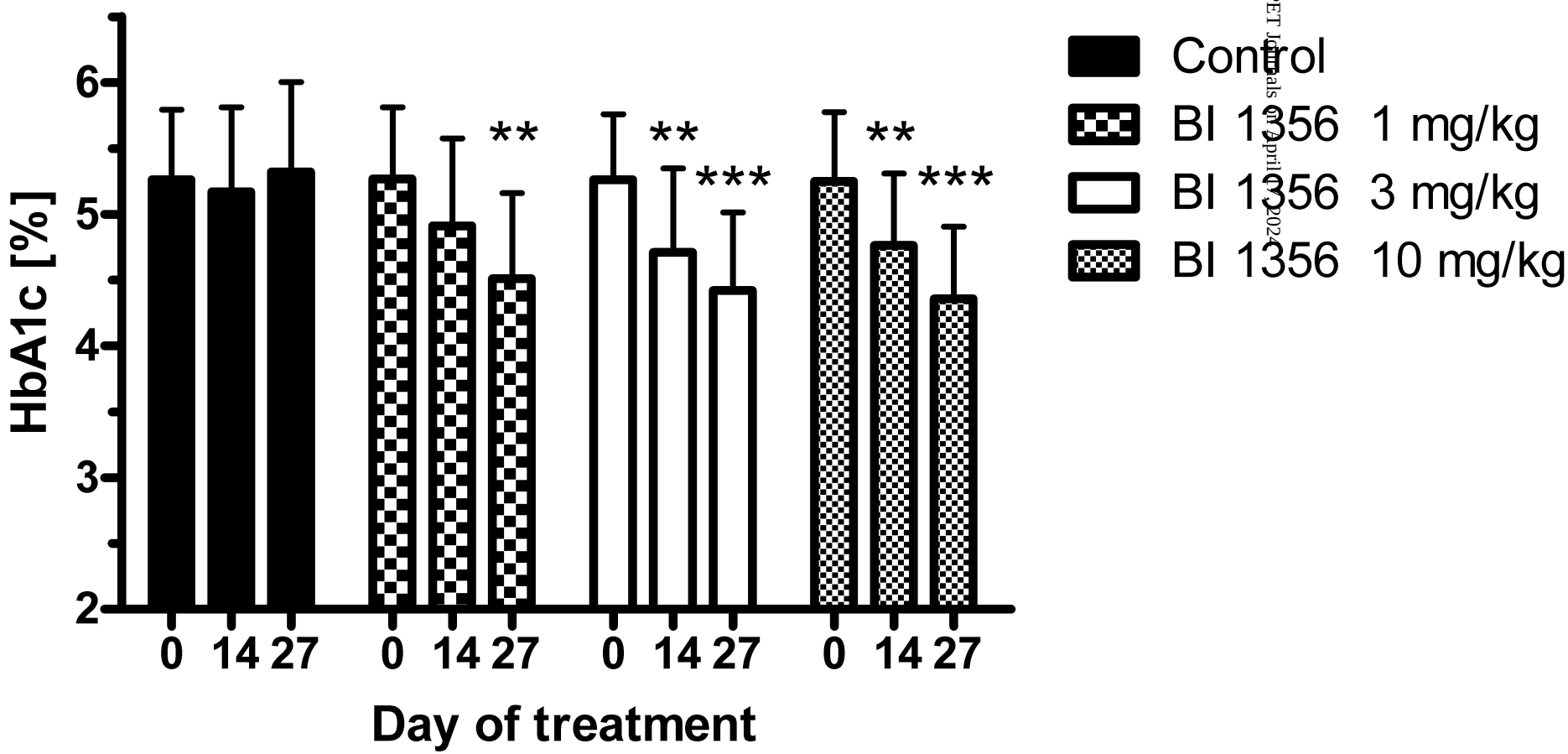


Figure 2



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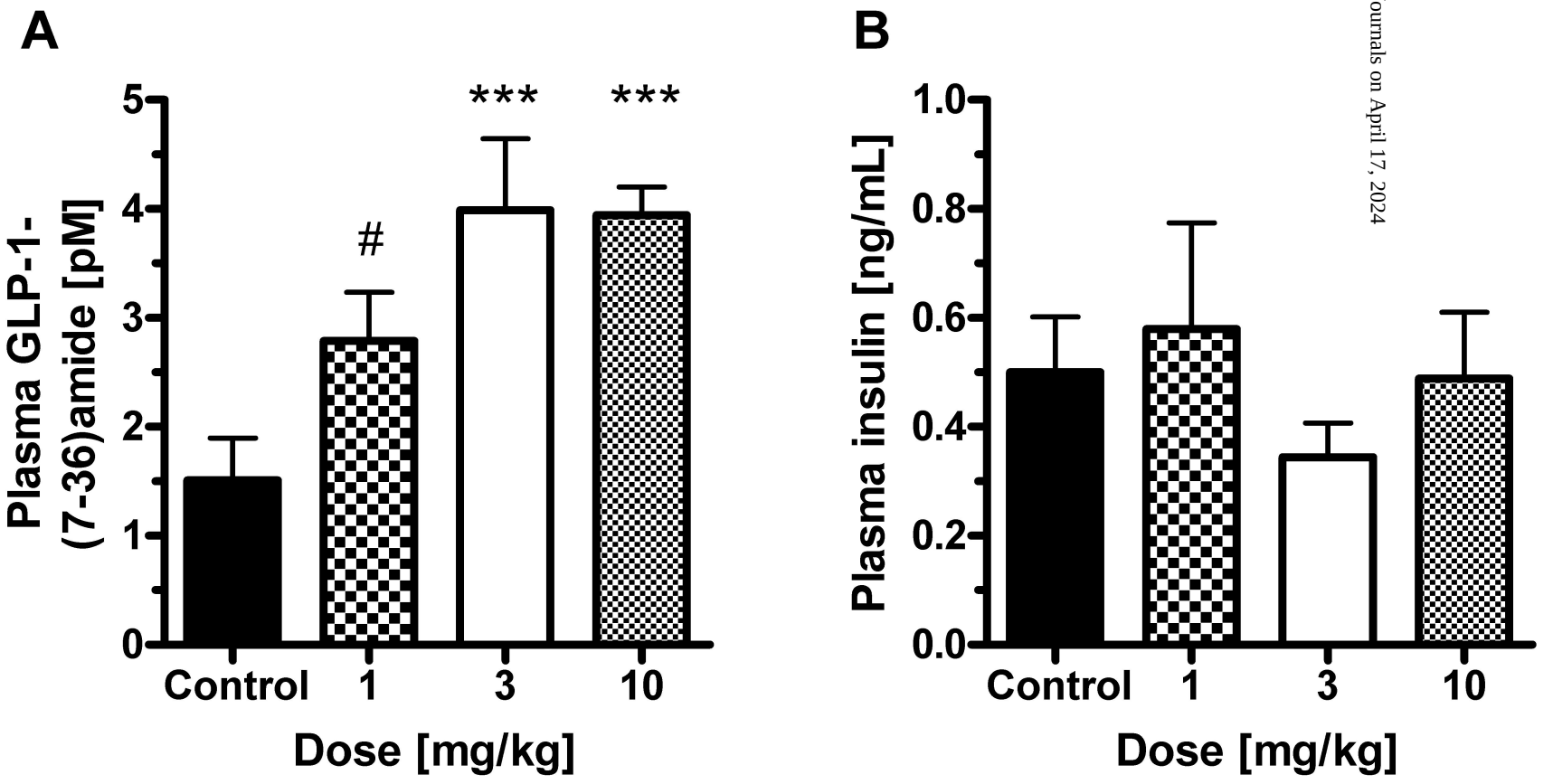

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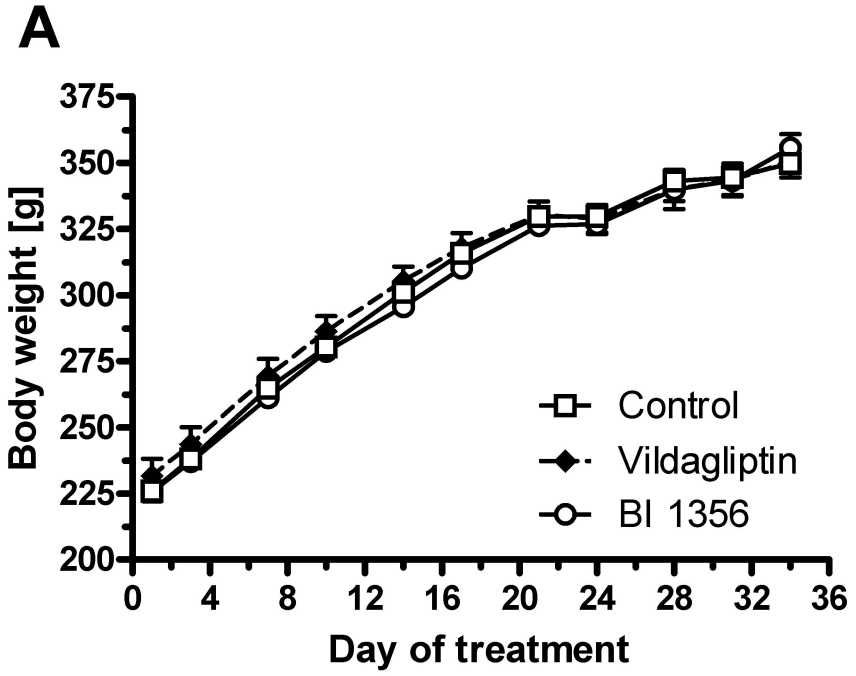

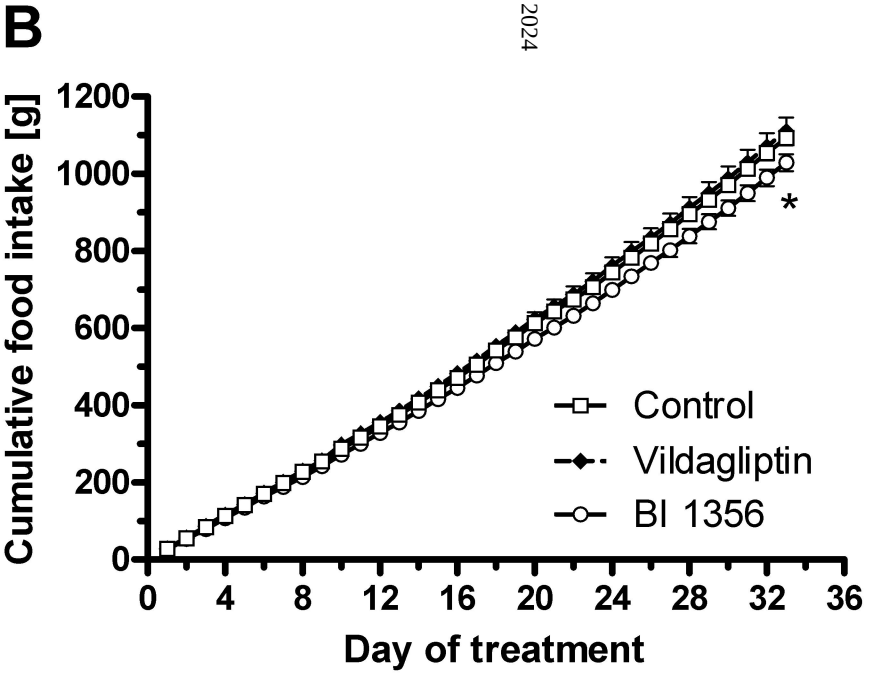

Figure 5

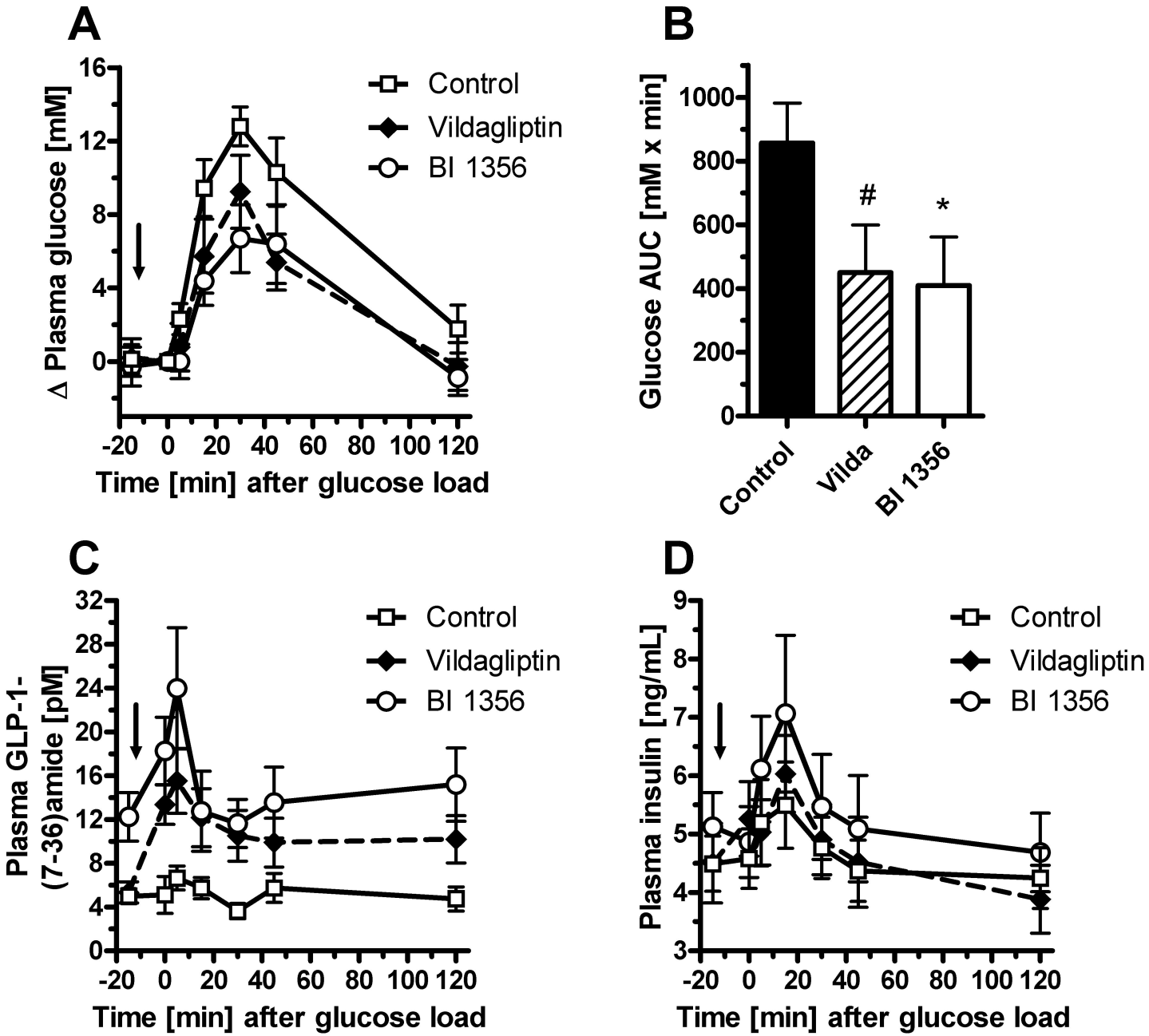
A
Δ Plasma glucose [mM]
Time [min] after glucose load
Control
Vildagliptin
BI 1356
B
Glucose AUC [mM x min]
Control
Vilda
BI 1356
#
*
C
Plasma GLP-1-(7-36)amide [pM]
Time [min] after glucose load
Control
Vildagliptin
BI 1356
D
Plasma insulin [ng/mL]
Time [min] after glucose load
Control
Vildagliptin
BI 1356

Figure 6

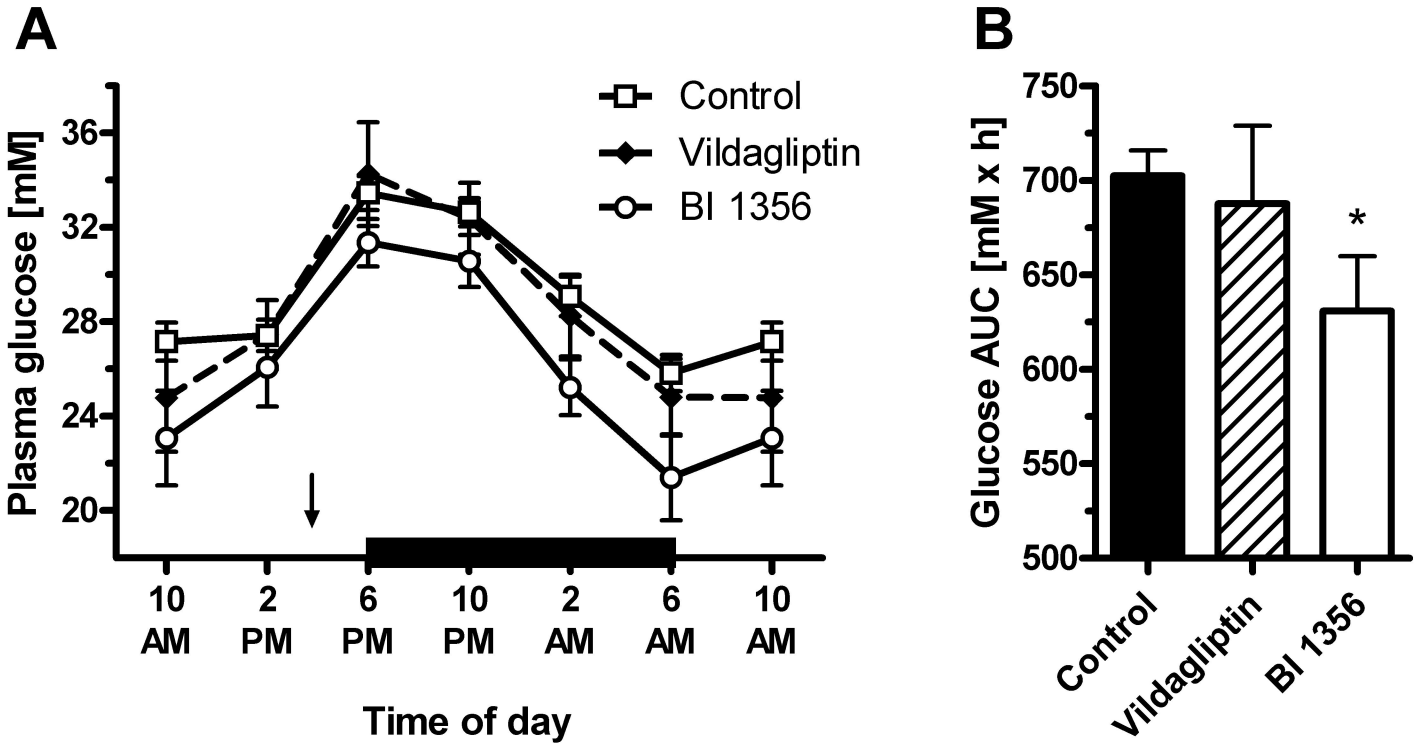
A
Plasma glucose [mM]
36
32
28
24
20
Control
Vildagliptin
BI 1356
10 AM
2 PM
6 PM
10 PM
2 AM
6 AM
10 AM
Time of day
B
Glucose AUC [mM x h]
750
700
650
600
550
500
*
Control
Vildagliptin
BI 1356

Figure 7

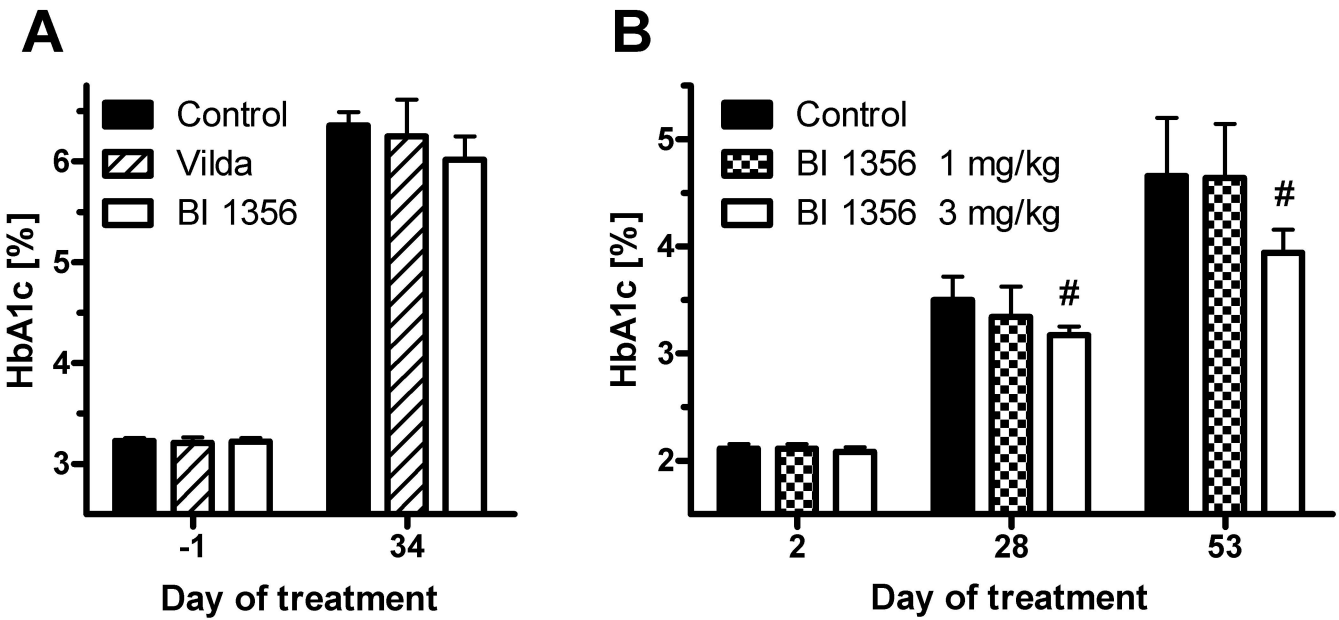
A
Control
Vilda
BI 1356
HbA1c [%]
6
5
4
3
-1
34
Day of treatment
B
Control
BI 1356 1 mg/kg
BI 1356 3 mg/kg
HbA1c [%]
5
4
3
2
#
#
2
28
53
Day of treatment

Figure 8