

JPET #241281

## **Title Page**

### **The Dual Amylin- and Calcitonin Receptor Agonist KBP-042 works as Adjunct to Metformin on Fasting Hyperglycaemia and HbA1c in a rat model of Type 2 Diabetes**

Hjuler ST, Gydesen S, Andreassen KV, Karsdal MA, Henriksen K

STH, SG, KVA, MAK, KH: Nordic Bioscience Biomarkers and Research, Dept. of

Musculoskeletal Diseases

JPET #241281

## Running Title Page

**Running head:** KBP-042 as adjunct therapy to metformin

**Corresponding Author:** Sara Toftegaard Hjuler, Nordic Bioscience, Herlev Hovedgade 207, 2730

Herlev, Denmark, Tel: +45 44 54 77 44, Fax: +45 44 52 52 51, E-mail: [sth@nordicbioscience.com](mailto:sth@nordicbioscience.com).

Number of text pages: 10

Number of tables: 0

Number of figures: 4

Number of references: 32

Abstract: 239 words

Introduction: 475 words

Discussion: 865 words

Section assignment: Drug Discovery and Translational Medicine

### Abbreviations:

ZDF: Zucker Diabetic fatty (rats)

DACRA: Dual Amylin and Calcitonin receptor agonist

HbA1c: Glycated haemoglobin A1c

ADA: American Diabetes Association

EASD: European association for the study of diabetes

OGTT: Oral glucose tolerance test

JPET #241281

IPITT: Intraperitoneal insulin tolerance test

EDTA: Ethylenediaminetetraacetic acid

ELISA: Enzyme-linked immunosorbent assay

SEM: Standard error of the mean

ANOVA: Analysis of Variance

(t)AUC: (total) area under the curve

GLP-1: Glucagon-like peptide 1

## Abstract

KBP-042 is a dual amylin and calcitonin receptor agonist which increases glucose tolerance and insulin action, and reduces body weight in rat models of obesity and pre-diabetes. The objective of the present study was to 1) Evaluate KBP-042 as a treatment of late stage type 2 diabetes in a rat model and 2) Assess the value of adding KBP-042 to the standard of care, metformin, in order to consider KBP-042 as a relevant drug for treating patients with type 2 diabetes. Two studies were included: an intervention and a prevention study. Intervention: Treatment with 5 µg/kg KBP-042 was initiated in 11-weeks old ZDF rats. Glucose tolerance, fasting glycaemia as well as glycated haemoglobin were assessed after 4 weeks. Prevention: Either metformin (400 mg/kg), KBP-042 (5 µg/kg) or a combination of both were administered to ZDF rats for a total of 9 weeks. Glycaemia, glucose tolerance, and insulin tolerance were tested. Furthermore, fasting plasma insulin and glucagon levels were evaluated. Finally, pancreatic content of insulin was assessed as a surrogate marker of beta-cell mass. It was found that KBP-042 was efficient in lowering fasting plasma glucose as well as improving glucose tolerance, both as prevention and intervention of disease progression. Furthermore, KBP-042 was efficient in combination with metformin and had additional effects compared to either therapy alone. In conclusion, KBP-042 is a highly relevant therapeutic candidate against type 2 diabetes, effective both as add-on to metformin and as stand-alone therapy.

## Introduction

Metformin is the standard of care for type 2 diabetes; however, metformin regularly fails to maintain glucose control after progression of the disease, due to lack of efficacy (Pawlyk et al., 2014). Consequently, add-on therapy is required for continued glucose control, and these should preferentially work in combination with metformin, i.e. by targeting disease aspects not regulated by this, such as glucagon hypersecretion and excessive body weight. Alternatively, improving insulin sensitivity could provide the opportunity for synergistic effects with metformin (Hundal et al., 2000; Gong et al., 2012; Pawlyk et al., 2014). At present, however, no treatment has shown the ability to increase insulin sensitivity and glycaemic control while also reducing body weight, neither as stand-alone nor in combination (Kahn et al., 2006; Haas et al., 2014; Scott, 2014; Scheen, 2015). The amylin analogue pramlintide does not have the capacity to improve glycaemic control alone, only as adjunct therapy to insulin. It does so by lowering postprandial hyperglycaemia through suppression of glucagon secretion, slowing of gastric emptying, increasing satiation and facilitating weight loss (Traina and Kane; Ryan et al., 2009; Dunican et al., 2010). Generally, use of pramlintide is limited by its low potency; hence more potent amylin analogues are of substantial interest as their mode of action clearly complements that of metformin (Mack et al., 2010, 2011). Recent studies of an orally delivered novel dual amylin- and calcitonin receptor agonist (DACRA) KBP-042 demonstrated markedly improved efficacy compared to the natural ligands, amylin and calcitonin. Oral KBP-042 improved glucose homeostasis in ZDF rats by reducing fasting plasma glucose, HbA1c levels, ameliorating pancreatic dysfunction, and increasing insulin sensitivity. These effects have not been observed with known amylin analogues (Traina and Kane; Ryan et al., 2009; Dunican et al., 2010). When tested for the previously reported parameters for salmon calcitonin (another dual amylin and calcitonin receptor agonist), KBP-042 showed superior efficacy and is thus promising as a novel treatment for type 2 diabetes (Feigh et al., 2011, 2012; Andreassen

JPET #241281

et al., 2014; Hjuler et al., 2015). All the previous studies dealt with prevention of symptoms (obesity, hyperglycaemia, insulin resistance) rather than initiation of treatment in a model which has progressed into a late stage of disease. Furthermore, the effect of subcutaneous delivery of KBP-042 on fasting plasma glucose and HbA1c have not been assessed. Finally, KBP-042 has only been evaluated as mono therapy, but as most type 2 diabetic patients receive metformin due to its status as 1<sup>st</sup> line treatment as recommended by the ADA and EASD (Thomas and Gregg, 2017), it is relevant to evaluate the additive effects with metformin. In this study, we used ZDF rats to investigate efficacy of the injected KBP-042 in combination with standard of care high dose metformin, to assess the treatment potential in a model that suffers from obesity as well as hyperglycaemia, two hallmarks of human type 2 diabetes.

## Methods

### Animal experiments

All animal procedures were performed in accordance with guidelines from the Animal Welfare Division of the Danish Ministry of Justice under the institutional license issued to Nordic Bioscience (2012-15-2934-00094). Male obese Zucker diabetic fatty rats (*fa/fa*) (ZDF) (Charles River Laboratories, Lyon, France) were obtained at 6 weeks of age and housed pair-wise under controlled temperature on a normal 12-h light-dark cycle with unrestricted access to water and food in a standard Type IV cage. ZDF rats were fed Purina Laboratory Diet #5008 (Brogaarden, Lyngø, Denmark) and allowed 1 week of acclimatization prior to experiments.

Two studies were performed in ZDF rats, and in both studies the treatment groups were matched for body weight, HbA1c and fasting blood glucose levels.

JPET #241281

For subcutaneous delivery the peptide KBP-042 (Andreassen et al., 2014) (Senn Chemicals, Switzerland) was dissolved in sterile saline. All drug administrations were performed in the afternoon.

#### Animal Study 1) 4-week intervention study with KBP-042

11-weeks old ZDF rats (kept on a regular diet to allow full development of diabetes) were assigned Vehicle treatment (n=5) and treatment with KBP-042 (n=5). The vehicle group received s.c. saline and treatment group received 5 µg/kg/day KBP-042 (1 ml/kg) dissolved in saline for 4 weeks. Fasting blood glucose (6 hours) was measured weekly, and HbA1c was measured in the end of each study. Oral glucose tolerance test (OGTT, 1 g/kg) was performed at day 20 of KBP-042 treatment in animals fasted for 12 hours. Animals were administered with vehicle or drug at t = -30, glucose was administered at t = 0. Blood samples were collected just before drug administration and glucose administration, and then following 15, 30, 60, and 120 minutes.

At the end of the 4 weeks of treatment, animals were fasted for 12 hours, blood was sampled for insulin measurements, and animals were euthanized by exsanguinations under isoflurane anaesthesia.

#### Animal Study 2) 9-week combination therapy with KBP-042 and metformin

40 seven-week old ZDF rats were assigned the following groups: Vehicle, s.c. KBP-042 5 µg/kg/day (1 ml/kg), 400 mg/kg/day p.o. metformin (5 ml/kg), and s.c. KBP-042 5 µg/kg/day in combination with 400 mg/kg/day oral metformin. Vehicle received saline s.c. (1 ml/kg) and p.o. (5 ml/kg), the s.c. group received likewise p.o. saline and p.o. group received s.c. saline.

Baseline blood samples were drawn at day -1 after 5 hours fasting for measurements of HbA1c, fasting insulin and glucagon. The animals were administered with drugs for 9 weeks. Fasted (6

JPET #241281

hours) and non-fasted plasma glucose levels were measured on alternating weeks while HbA1c was measured at the end of the study. Intraperitoneal insulin tolerance testing (IPITT) was performed after 6 h fasting (Insulin: 1.0 U/kg, Humulin, Eli Lilly) at week 7 of the study. OGTT was also performed after 8 weeks of treatment, as described above.

After 9 weeks animals were fasted for 5 hours, blood was sampled for insulin and glucagon measurements, and animals were euthanized by exsanguinations under isoflurane anesthesia.

### **Biochemical analysis**

Blood samples were collected from the tail vein of conscious animals in MiniCollect® 1 ml EDTA tubes, and centrifuged at 5000 rpm for 10 min at 4 °C and kept at -20 °C until further analysis.

Blood glucose was monitored by Accu-Check® Avia monitoring system (Roche Diagnostics, Rotkreuz, Switzerland). HbA1c levels by DCA Vantage Analyzer (Siemens, Erlangen, Germany).

Levels of insulin (Mercodia Rat Insulin ELISA, Mercodia AB, Uppsala, Sweden) and glucagon (Glucagon Quantikine ELISA; R&D Systems Europe, Abington, UK) were analysed according to manufacturer`s instructions.

### **Tissue analysis**

Pancreases were excised, homogenized, and extracted in acid-ethanol for subsequent determination of insulin and glucagon content (Leiter et al., 1981). Protein contents of these extracts were estimated by the bicinchoninic acid method (Smith et al., 1985).

### **Statistical analyses**

All data are presented as mean  $\pm$  standard error of the mean (SEM). The statistical analysis of drug effects versus vehicle effects was conducted using one-way ANOVA followed by Tukey's post-hoc



JPET #241281

test. Student's t-test was performed to compare treatment group and vehicle in the intervention study. All analyses were performed using GraphPad Prism software (San Diego, CA, U.S.A). A value of  $P < 0.05$  was considered significant.

## Results

### *Animal Study 1) 4-week intervention study with KBP-042*

ZDF rats were left untreated until 11 weeks of age where fasting blood glucose levels had increased to an average of 28 mM. After development of hyperglycaemia treatment with KBP-042 was initiated. KBP-042 treatment resulted in an immediate reduction in fasting glucose levels, which were sustained throughout the study period (Figure 1A). Furthermore, HbA1c levels at the end of the study were reduced from 9.0 % to 7.2 % in the KBP-treated group (Figure 1B). An OGTT performed at week 8 (Figure 1C&D), demonstrated a significant increase in glucose tolerance in response to KBP-042. Notably, the increased glucose tolerance was achieved with lower plasma insulin levels in the KBP-042 group compared to vehicle (Figure 1E & F). Finally, the intervention with KBP-042 did not lead to significant change in fasting plasma insulin levels at the end of the 4-week study (Figure 1G).

### *Animal Study 2) 9-week combination therapy with KBP-042 and metformin*

The effects of combined treatment with KBP-042 and metformin were investigated in ZDF rats. Treatment with either KBP-042 or metformin led to increases in bodyweight, and the combination led to an even larger weight gain (Figure 2A). Treatment with metformin led to lowering of both fasting and non-fasting blood glucose values in the early stages of the study, which were not maintained at later stages of the study (Figure 2B & C). Meanwhile, KBP-042 induced a marked lowering of both fasting and non-fasting blood glucose levels throughout the entire study (Figure

JPET #241281

2B & C). For the combination treatment, considerable decreases in both glucose parameters were observed, indicating additive effects of the two treatments (Figure 2B & C). Similarly, HbA1c levels in all treatment groups were lowered, and the combination was clearly superior to either treatment alone with a 5 % HbA1c reduction (Figure 2D). Fasting plasma insulin and glucagon were assessed both at baseline and after treatment. Insulin levels in the vehicle group were significantly lowered throughout the study (Figure 2E) corresponding to a loss of beta cells seen in the ZDF rat, leading to drastically increased glucagon levels in the vehicle group. The drastic increase in plasma glucagon was neutralized by KBP-042 treatment and even further reduced with the combination of KBP-042 and metformin (Figure 2F).

In the OGTT, both treatments led to increase in glucose tolerance; however, the kinetics of blood glucose shown in Figure 3A, was distinct between the two treatment types. None of the treatment groups had a large increase in plasma glucose in response to oral glucose load, however, the basal glucose levels of KBP-042 groups (both monotherapy and combination) were lower than metformin and vehicle group. This difference in basal levels is reflected in the AUC. Importantly, combination therapy was superior to either therapy alone (Figure 3B). Both treatments increased insulin action, which was assessed by use of an intraperitoneal insulin tolerance test (IPITT) (Figure 3E). The combination therapy had additive effects to the two treatments, and led to a large reduction in the tAUC for glucose during the IPITT, though also here the difference in blood glucose at  $t = 0$  affects the AUC (Figure 3F).

#### *Pancreatic insulin content was conserved by prevention and not intervention*

The preserving effect on beta cells seen after treatment with a DACRA (Feigh et al., 2012) was estimated by extracting insulin from the pancreases from animal studies 1 and 2. After the intervention study, where animals were left untreated first before initiation of treatment, no

JPET #241281

difference in insulin content in the pancreas was found (Figure 4A). The combination treatment with KBP-042 and metformin administered throughout the study period did however preserve the insulin content (Figure 4B), suggesting that beta cells were preserved in contrast to the intervention study where the insulin content was low in both groups.

## Discussion

The DACRAs are dual amylin and calcitonin receptor agonists, which are agonists that are superior to both amylin and calcitonin on their respective receptors (Christopoulos et al., 1999; Andreassen et al., 2014). As treatment candidates, they separate themselves from amylin as they possess glucoregulatory capacities independent of insulin co-administration (Feigh et al., 2012; Andreassen et al., 2014). Recent studies have highlighted DACRAs as potential treatments for type 2 diabetes, due to their ability to reduce fasting blood glucose, HbA1c, and bodyweight, and to increase insulin action (Feigh et al., 2011, 2012; Andreassen et al., 2014; Hjuler et al., 2016), warranting further analysis of their therapeutic potential in humans and possible combination strategies with existing medications.

The main findings of the current studies were:

- 1) KBP-042, possesses potent glucoregulatory capacities even in severely diabetic animals.
- 2) Combining KBP-042 with metformin leads to additive improvements in glucose control compared to either monotherapy alone.

Administration of KBP-042 provided more robust pharmacodynamic effects than previously published using oral delivery (Andreassen et al., 2014), likely due to a more homogenous exposure and subsequent biological efficacy (Karsdal et al., 2015). Furthermore, KBP-042 has effects on

JPET #241281

fasting plasma glucose and insulin sensitivity that is not seen with amylin agonists such as pramlintide, possibly why pramlintide is only approved in combination with meal-time insulin (Ryan et al., 2009).

When KBP-042 was administered as adjunct to high dose metformin the combination was superior to either drug alone, demonstrating an additive effect. As a large proportion of type 2 diabetic patients are on high dose metformin as first line therapy, the present data suggest that KBP-042 could serve as a novel second line therapy leading to improvements in glucose homeostasis, insulin tolerance and bodyweight. Considering that metformin is known to work primarily by reducing excessive glucose production in the liver (Hundal and Inzucchi, 2003), the additive nature of the combination therapy may be expected, as the modes of action are distinct. The combination study e.g. showed that when levels of fasting insulin were significantly reduced, glucagon levels were increased inappropriately probably due to insufficient paracrine effect of insulin (Lee et al., 2012; Unger and Roth, 2015). This was efficiently ameliorated by treatment with KBP-042, and can possibly be attributed preservation of beta cells (increased paracrine effect), but amylin agonism also has a potent glucagonostatic effect (Gedulin et al., 1997; Akesson et al., 2003).

Another effect mediated by KBP-042 is reduction of gastric emptying (Hjuler et al., 2015). Gastric motility is often increased in type 2 diabetes (Frank et al., 1995; Horowitz et al., 2002), and slowing of the accelerated passing is known to reduce postprandial glucose and important in maintaining blood glucose homeostasis (Phillips et al., 2014) This too is an additive effect to that of metformin. The fasting insulin levels in the intervention study shows that in this model, the insulin producing capacity has already been lost within the period without treatment. This was further corroborated by the unchanged pancreatic content of insulin after the study. However, OGTT clearly demonstrates that KBP-042 treatment, even when introduced in a severely progressed stage, is able to increase glucose tolerance without increasing insulin levels. In fact, insulin levels were lower after KBP-042

JPET #241281

administration. In the preventive setup in Study 2 however, it was indicated that the combination treatment with KBP-042 and metformin is able to preserve beta cells and thereby maintain a sufficient high level of insulin. This finding was also confirmed by the increased insulin content in pancreas after treatment with metformin and KBP-042. Evidence that metformin improves insulin sensitivity also exists, and when considering that KBP-042 acts by increasing insulin action (Hjuler et al., 2016) a reason for potential synergy may reside at this level (Hundal et al., 2000; Gong et al., 2012; Pawlyk et al., 2014). Importantly, the increased insulin action is most likely the key determinant not only for the efficacy of KBP-042, but also for the additive effect observed in the combination therapy group. KBP-042 assists in rebalancing leptin levels, also potentially contributing to the synergy (Andreassen et al., 2014; Hjuler et al., 2015), as well as to a wide range of other metabolic effects (Unger and Roth, 2015).

An increase in bodyweight was observed after KBP-042 treatment in the ZDF rats, which is contradictory to expected reduction in bodyweight. However, this weight gain is also seen with metformin, and it is most likely related to the improved health status of the treated animals, when compared the vehicle group. The data correlate well with previous findings in ZDF rats treated with other types of molecules, such as GLP-1 analogues and metformin, as well as previous DACRA studies, where treatment induces weight gain or rather prevents a disease related weight loss (Feigh et al., 2012; Vrang et al., 2012; Ito et al., 2013; Andreassen et al., 2014). In alignment, data from diet-induced obese rats have clearly demonstrated a weight reducing effect with KBP-042 (Andreassen et al., 2014; Hjuler et al., 2015), underscoring that the lack of weight reduction is a phenomenon seen in ZDF rats only.

In summary, KBP-042 possesses anti-diabetic efficacy, which when combined with metformin leads to a potent treatment of severe hyperglycaemia in a rat model of type 2 diabetes.

JPET #241281

**Authorship Contributions:**

Participated in research design: Hjuler, Henriksen, Karsdal

Conducted experiments: Hjuler, Gydesen, Andreassen

Performed data analysis: Hjuler, Gydesen, Andreassen

Wrote or contributed to the writing of the manuscript: Hjuler, Henriksen

## References

- Akesson, B., Panagiotidis, G., Westermark, P., and Lundquist, I. (2003). Islet amyloid polypeptide inhibits glucagon release and exerts a dual action on insulin release from isolated islets. *Regul. Pept. 111*: 55–60.
- Andreassen, K. V., Feigh, M., Hjuler, S.T., Gydesen, S., Henriksen, J.E., Beck-Nielsen, H., et al. (2014). A novel oral dual amylin and calcitonin receptor agonist (KBP-042) exerts antiobesity and antidiabetic effects in rats. *Am. J. Physiol. Endocrinol. Metab. 307*: E24-33.
- Christopoulos, G., Perry, K.J., Morfis, M., Tilakaratne, N., Gao, Y., Fraser, N.J., et al. (1999). Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Mol. Pharmacol. 56*: 235–42.
- Duncan, K.C., Adams, N.M., and Desilets, A.R. (2010). The role of pramlintide for weight loss. *Ann. Pharmacother. 44*: 538–45.
- Feigh, M., Andreassen, K. V, Neutzsky-Wulff, A. V, Petersen, S.T., Hansen, C., Bay-Jensen, A.C., et al. (2012). Oral salmon calcitonin attenuates hyperglycaemia and preserves pancreatic beta-cell area and function in Zucker diabetic fatty rats. *Br. J. Pharmacol. 167*: 151–63.
- Feigh, M., Henriksen, K., and Andreassen, K. (2011). A novel oral form of salmon calcitonin improves glucose homeostasis and reduces body weight in diet-induced obese rats. *Diabetes. Obes. Metab. 911–920*.
- Frank, J.W., Saslow, S.B., Camilleri, M., Thomforde, G.M., Dinneen, S., and Rizza, R.A. (1995). Mechanism of accelerated gastric emptying of liquids and hyperglycemia in patients with type II diabetes mellitus. *Gastroenterology 109*: 755–765.

JPET #241281

Gedulin, B.R., Rink, T.J., and Young, A.A. (1997). Dose-response for glucagonostatic effect of amylin in rats. *Metabolism*. 46: 67–70.

Gong, L., Goswami, S., Giacomini, K.M., Altman, R.B., and Klein, T.E. (2012). Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet.Genomics* 22: 820–827.

Haas, B., Eckstein, N., Pfeifer, V., Mayer, P., and Hass, M.D.S. (2014). Efficacy, safety and regulatory status of SGLT2 inhibitors: focus on canagliflozin. *Nutr. Diabetes* 4: e143.

Hjuler, S.T., Andreassen, K. V, Gydesen, S., Karsdal, M.A., and Henriksen, K. (2015). KBP-042 improves bodyweight and glucose homeostasis with indices of increased insulin sensitivity irrespective of route of administration. *Eur. J. Pharmacol.* 762: 229–38.

Hjuler, S.T., Gydesen, S., Andreassen, K.V., Pedersen, S.L.K., Hellgren, L.I., Karsdal, M.A., et al. (2016). The dual amylin- and calcitonin-receptor agonist KBP-042 increases insulin sensitivity and induces weight loss in rats with obesity. *Obesity (Silver Spring)*.

Horowitz, M., O'Donovan, D., Jones, K.L., Feinle, C., Rayner, C.K., and Samsom, M. (2002). Gastric emptying in diabetes: clinical significance and treatment. *Diabet.Med.* 19: 177–194.

Hundal, R.S., and Inzucchi, S.E. (2003). Metformin: new understandings, new uses. *Drugs* 63: 1879–1894.

Hundal, R.S., Krssak, M., Dufour, S., Laurent, D., Lebon, V., Chandramouli, V., et al. (2000). Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes* 49: 2063–2069.

Ito, R., Tsujihata, Y., Matsuda-Nagasumi, K., Mori, I., Negoro, N., and Takeuchi, K. (2013). TAK-875, a GPR40/FFAR1 agonist, in combination with metformin prevents progression of diabetes and



JPET #241281

beta-cell dysfunction in Zucker diabetic fatty rats. *Br.J.Pharmacol.* 170: 568–580.

Kahn, S.E., Hull, R.L., and Utzschneider, K.M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840–846.

Karsdal, M.A., Riis, B.J., Mehta, N., Stern, W., Arbit, E., Christiansen, C., et al. (2015). Lessons learned from the clinical development of oral peptides. *Br. J. Clin. Pharmacol.* 79: 720–732.

Lee, Y., Berglund, E.D., Wang, M.Y., Fu, X., Yu, X., Charron, M.J., et al. (2012). Metabolic manifestations of insulin deficiency do not occur without glucagon action.

*Proc.Natl.Acad.Sci.U.S.A* 109: 14972–14976.

Leiter, E.H., Coleman, D.L., Eisenstein, A.B., and Strack, I. (1981). Dietary control of pathogenesis in C57BL/KsJ db/db diabetes mice. *Metabolism* 30: 554–562.

Mack, C.M., Smith, P. a, Athanacio, J.R., Xu, K., Wilson, J.K., Reynolds, J.M., et al. (2011). Glucoregulatory effects and prolonged duration of action of davalintide: a novel amylinomimetic peptide. *Diabetes. Obes. Metab.* 13: 1105–13.

Mack, C.M., Soares, C.J., Wilson, J.K., Athanacio, J.R., Turek, V.F., Trevaskis, J.L., et al. (2010). Davalintide (AC2307), a novel amylin-mimetic peptide: enhanced pharmacological properties over native amylin to reduce food intake and body weight. *Int.J.Obes.(Lond)* 34: 385–395.

Pawlyk, A.C., Giacomini, K.M., McKeon, C., Shuldiner, A.R., and Florez, J.C. (2014). Metformin pharmacogenomics: current status and future directions. *Diabetes* 63: 2590–2599.

Phillips, L.K., Deane, A.M., Jones, K.L., Rayner, C.K., and Horowitz, M. (2014). Gastric emptying and glycaemia in health and diabetes mellitus. *Nat. Rev. Endocrinol.* 11: 112–128.

Ryan, G., Briscoe, T.A., and Jobe, L. (2009). Review of pramlintide as adjunctive therapy in

JPET #241281

treatment of type 1 and type 2 diabetes. *Drug Des. Devel. Ther.* 2: 203–14.

Scheen, A.J. (2015). A review of gliptins for 2014. *Expert Opin. Pharmacother.* 16: 43–62.

Scott, L.J. (2014). Liraglutide: a review of its use in adult patients with type 2 diabetes mellitus. *Drugs* 74: 2161–74.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., et al. (1985). Measurement of protein using bicinchoninic acid. *Anal.Biochem.* 150: 76–85.

Thomas, I., and Gregg, B. (2017). Metformin; a review of its history and future: from lilac to longevity. *Pediatr. Diabetes* 18: 10–16.

Traina, A.N., and Kane, M.P. Primer on pramlintide, an amylin analog. *Diabetes Educ.* 37: 426–31.

Unger, R.H., and Roth, M.G. (2015). A new biology of diabetes revealed by leptin. *Cell Metab.* 21: 15–20.

Vrang, N., Jelsing, J., Simonsen, L., Jensen, A.E., Thorup, I., Soeborg, H., et al. (2012). The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis.

*Am.J.Physiol Endocrinol.Metab* 303: E253–E264.

JPET #241281

## Footnotes

**Conflicts of Interest:** Morten A. Karsdal and Kim Henriksen own stock/stock options in Nordic Bioscience. All other authors report no conflicts of interest.

**Funding:** The Danish Research Foundation (Den Danske Forskningsfond). The Danish Ministry of Science, Technology and Education (VTU).

JPET #241281

## Figure legends

Figure 1: KBP-042 improves the diabetic status of ZDF rats left untreated until 11 weeks old. A) Fasting blood glucose monitored weekly during the 4-week study period. B) HbA1c levels after 4 weeks of treatment with KBP-042 or vehicle C) Blood glucose during OGTT D) Total AUC of blood glucose during OGTT. E) Plasma insulin levels from 0-60 minutes during OGTT F) Total AUC of plasma insulin during OGTT G) Fasting plasma insulin (12 hours fasting) after treatment with KBP-042 or vehicle.

N = 5 rats per group. Statistical analysis between groups in B), D), F), and G) were performed as a Students t-test with the following annotations: \*P<0.05, \*\*P<0.01, vs. Vehicle, n.s. Not significant. Data are expressed as mean  $\pm$  SEM.

Figure 2: KBP-042 in combination with high dose metformin leads to improved metabolic status and glycaemia in ZDF rats. A) Body weight monitored weekly during the 9-week study period. B) Fasting blood glucose monitored bi-weekly during the 9-week study period. C) Non-fasted plasma glucose monitored bi-weekly (alternating weeks to fasting plasma glucose) during the 9-week study period. D) HbA1c levels measured at baseline and after 9 weeks of treatment. E) & F) Fasting plasma insulin and glucagon respectively (5 hours fasting) at baseline and after the 9-week study period. n=10 rats per group, for baseline n=40. Statistical analysis between groups were performed as a One-way ANOVA followed by Tukey's post-hoc test with the following annotations: <sup>‡</sup>P<0.05, <sup>‡‡‡</sup>P<0.001 vs. Baseline \*P<0.05, \*\*\*P<0.001 vs. Vehicle. ††P<0.01, †††P<0.001 vs. KBP-042 mono-treatment. ‡‡P<0.01, ‡‡‡P<0.001 vs. Metformin mono-treatment. Data are expressed as mean  $\pm$  SEM.

JPET #241281

Figure 3: KBP-042 in combination with high dose metformin leads to superior glucose tolerance and insulin action in ZDF rats. A) Kinetics of blood glucose changes during OGTT performed in week 8. B) Total AUC of glucose during OGTT. C) Plasma insulin levels after glucose administration in the OGTT. D) Total AUC of plasma insulin during OGTT. E) Kinetics of blood glucose during the IPITT performed in week 7. F) Total AUC of blood glucose during the IPITT. n=10 rats per group. Statistical analysis between groups were performed as a One-way ANOVA followed by Tukey's post-hoc test with the following annotations: \*\*P<0.01, \*\*\*P<0.001 vs. Vehicle. ††P<0.01, †††P<0.001 vs. KBP-042 mono-treatment. ‡‡P<0.01, ‡‡‡P<0.001 vs. Metformin mono-treatment. Data are expressed as mean ± SEM.

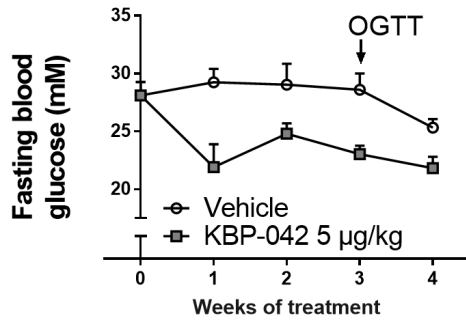
Figure 4: Pancreatic insulin content from extraction after treatment with: A) KBP-042 intervention for 4 weeks (after development of overt diabetes). n=5 per group. Statistical analysis was performed as student's t-test. n.s. = not significant B) Metformin, KBP-042 or a combination of Metformin and KBP-042 for 8 weeks. n=10 per group. Statistical analysis between groups were performed as a One-way ANOVA followed by Tukey's post-hoc test with the following annotations: \*\*\*P<0.001 vs. Vehicle. ††P<0.01 vs. KBP-042 mono-treatment. ‡‡‡P<0.001 vs. Metformin mono-treatment. Data are expressed as mean ± SEM.

JPET #241281

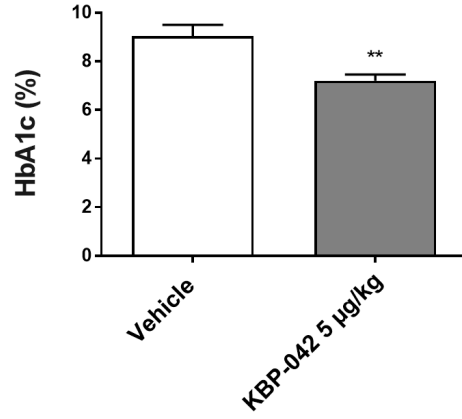
## Figures

# Figure 1

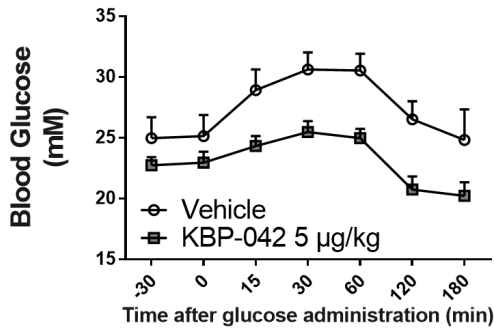
**A**



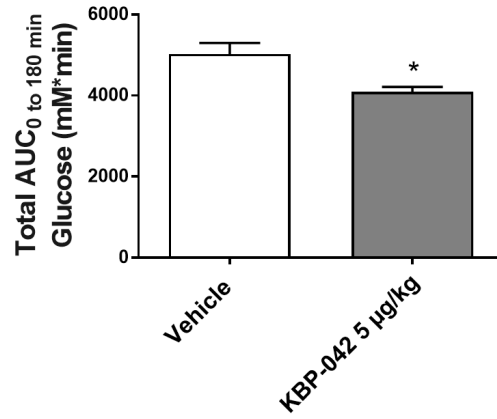
**B**



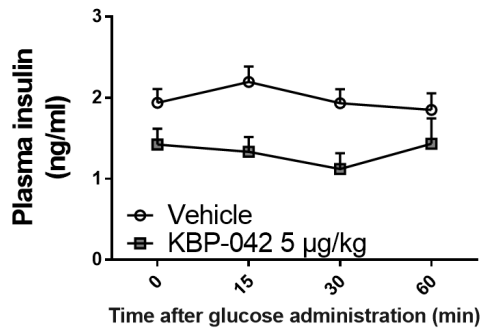
**C**



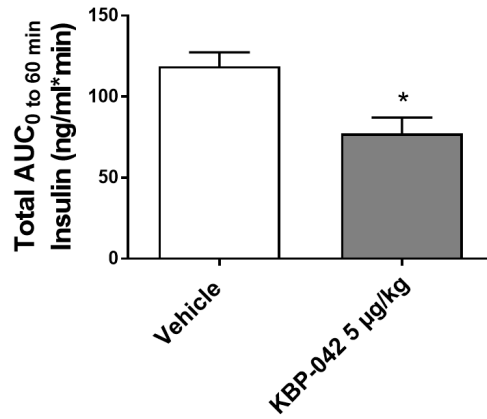
**D**



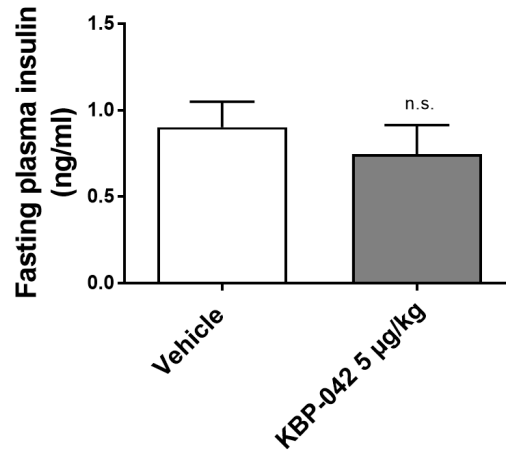
**E**



**F**

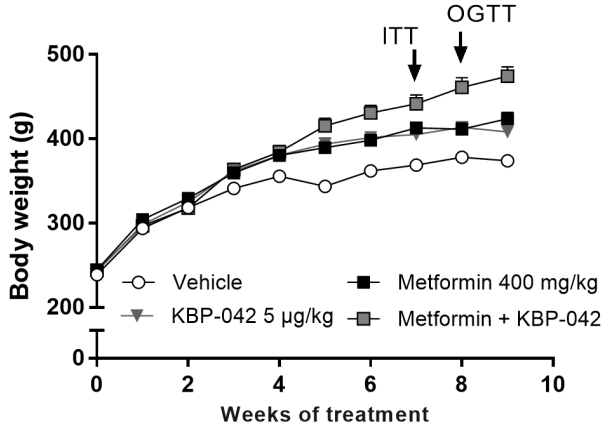


**G**

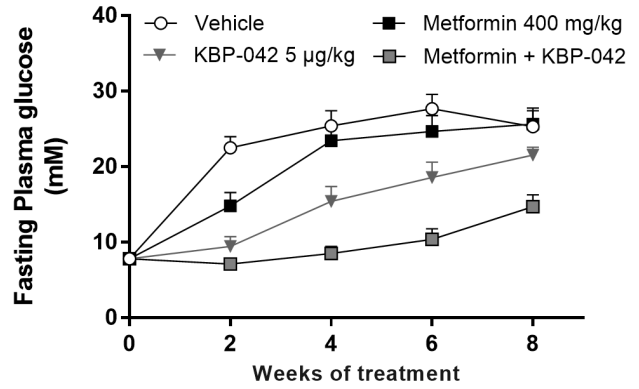


# Figure 2

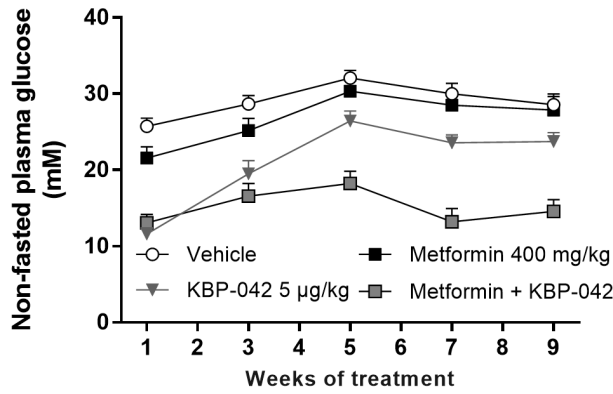
**A**



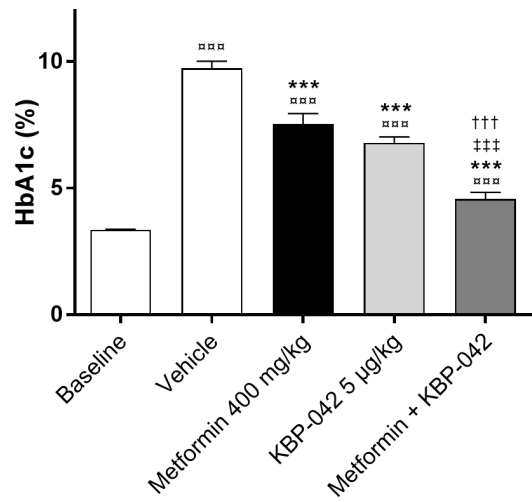
**B**



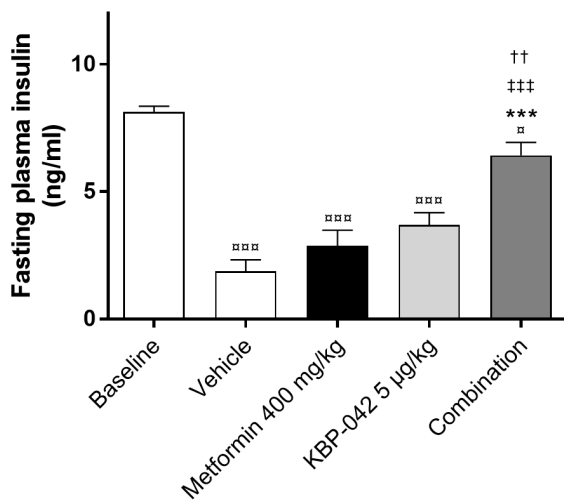
**C**



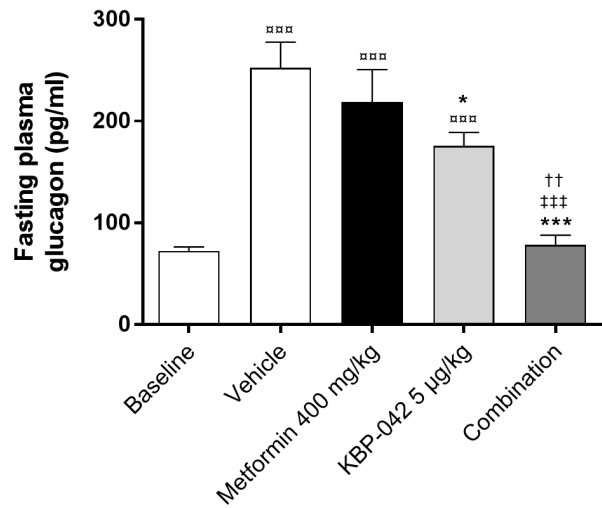
**D**



**E**

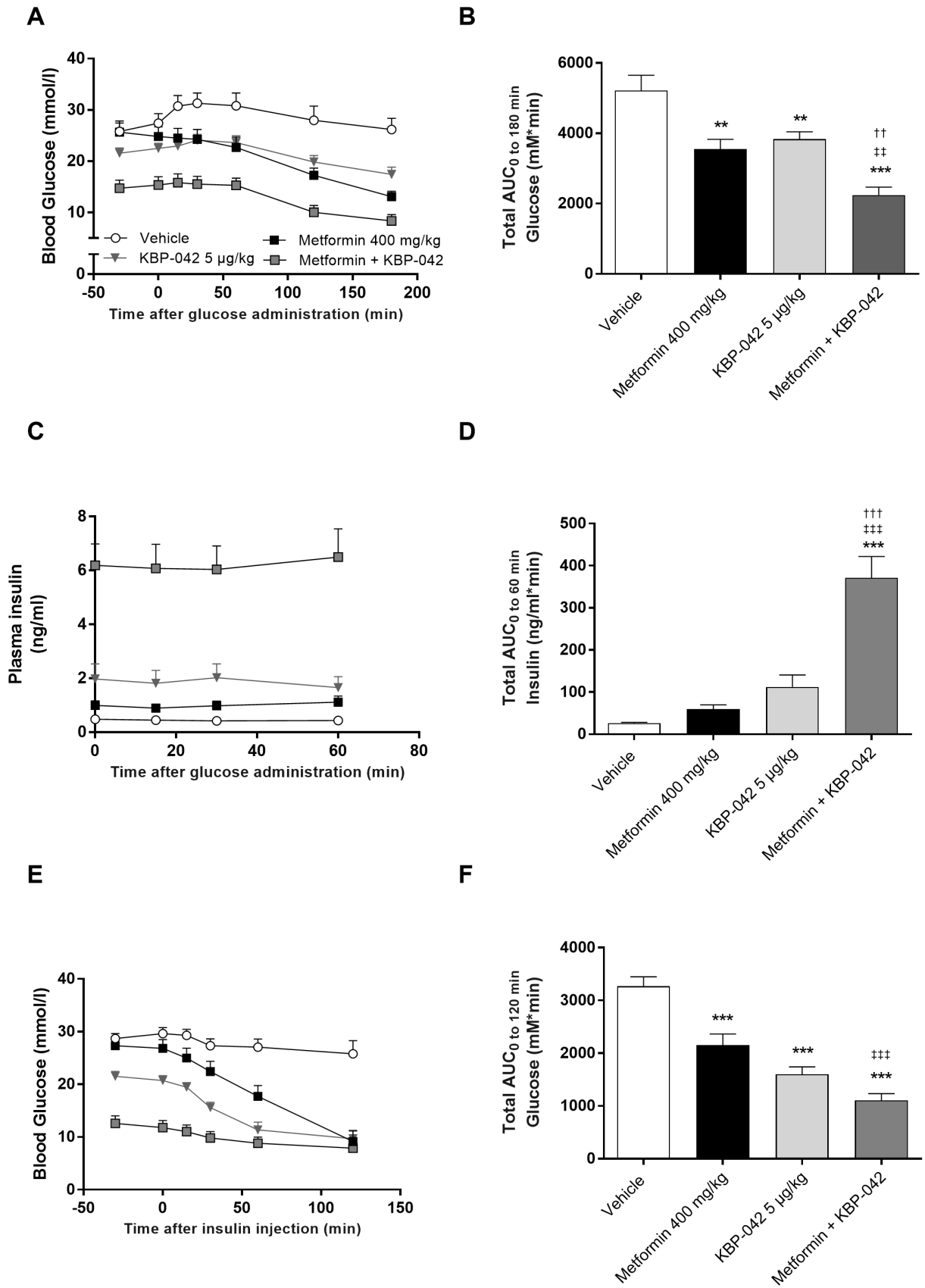


**F**





# Figure 3



# Figure 4

