

The dual amylin and calcitonin receptor agonist KBP-089 and the GLP-1 receptor agonist liraglutide act complementarily on body weight reduction and metabolic profile

Anna Thorsø Larsen

Nordic Bioscience

Sofie Gydesen

Nordic Bioscience

Nina Sonne

Nordic Bioscience

Morten Asser Karsdal

Nordic Bioscience

Kim Henriksen (✉ kh@nordicbio.com)

Nordic Bioscience

Research article

Keywords: weight loss therapy, amylin receptor agonists, GLP-1 receptor agonists, dual use

Posted Date: July 16th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-39438/v1>

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Version of Record: A version of this preprint was published on January 7th, 2021. See the published version at <https://doi.org/10.1186/s12902-020-00678-2>.

Abstract

Background

Weight loss therapy is becoming more and more important, and two classes of molecules, namely amylin receptor and GLP-1 receptor agonists, have shown promise in this regard. Interestingly, these molecules have several overlapping pharmacological effects, such as suppression of gastric emptying, reduction of glucagon secretion and weight loss in common; however, they also have distinct effects on prandial insulin secretion. Hence, a combination of these two mechanisms is of significant interest.

Methods

In this study, we investigated the add-on potential of the dual amylin and calcitonin receptor agonist (DACRA) KBP-089 in combination with the GLP-1 receptor agonist liraglutide as obesity treatment in high-fat diet (HFD) fed rats.

Results

Increasing doses of KBP-089 and liraglutide alone and in combination were studied with respect to their effects on body weight, food intake and glucose metabolism during a 9-week intervention study conducted in HFD rats. Further, the gastric emptying rate during an oral glucose tolerance was assessed. Treatment with KBP-089 and liraglutide dose-dependently lowered body weight 15% (at 2.5 $\mu\text{g}/\text{kg}/\text{day}$) and 7% (at 400 $\mu\text{g}/\text{kg}/\text{day}$) in HFD rats, respectively, while the combination resulted in a 21% body weight reduction, which was mirrored by reduction in fat depot sizes. Gastric emptying and glucose metabolism were improved, primarily by KBP-089, although liraglutide led to a reduction in fasting plasma glucagon.

Conclusion

DACRAs complement GLP-1 on food intake, body weight, and glucose tolerance indicating the potential for an add-on therapy.

Background

Obesity is the result of modern lifestyle and excessive caloric intake, and the number of obese people is increasing. Multiple complications such as insulin resistance, type 2 diabetes, cardiovascular disease, cancer and non-alcoholic fatty liver disease, among others, are frequently associated with obesity (Haslam and James, 2005; Kahn et al., 2006; Guh et al., 2009). A sustained weight loss is key in treatment of obesity; however, treatments beside lifestyle intervention are still few. Bariatric surgery is effective, but is only used in severe obesity due to risk of surgical complications (Pories, 2008), therefore alternative therapies with improved efficacy and low risk of side effects are of great interest. Furthermore, a

significant weight loss is important in treatment of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and other obesity related morbidities (Cusi, 2009; Milić et al., 2014).

Amylin receptor agonists (davalintide and pramlintide) have shown potential for significant body weight reduction and improved appetite control in both preclinical and clinical studies (Aronne et al., 2007; Smith et al., 2008; Mack et al., 2010). Additionally, pramlintide has been approved as adjunct to insulin therapy for treatment of type 1 and type 2 diabetes due to its ability to regulate post-prandial glucose levels, reduce body weight, and HbA1c (Weyer et al., 2001; Ratner et al., 2004; Ryan et al., 2009).

Multiple GLP-1 agonists are approved for treatment of type 2 diabetes and recently high dose liraglutide was also approved for treatment of obesity, as it promotes sustained weight loss via effects on satiety and appetite (Van Can et al., 2014; Crane and McGowan, 2016). Additionally, liraglutide improves postprandial blood glucose concentration, although still with limitations in terms of tolerability challenges, in particular nausea (Astrup et al., 2012; Kanoski et al., 2012; Lean et al., 2014; Davies et al., 2015). There is an increasing focus on combining GLP-1 receptor agonists with additional therapy to obtain greater therapeutic efficacy. GLP-1 and amylin analogues have several overlapping pharmacological effects including marked reductions in food intake, delay of gastric emptying and inhibition of glucagon secretion, although they act through different sites and mechanisms of action (Roth et al., 2012).

Dual Amylin and Calcitonin Receptor Agonists (DACRAs) activate both the amylin receptor and the calcitonin receptor for an extended period of time and elicit superior activity in terms of typical amylin-induced responses (Mack et al., 2010; Andreassen et al., 2014; Gydesen et al., 2016; Hjuler et al., 2016). Previously, combinations of sub-optimal doses of the DACRA KBP-089 and the GLP-1 agonist liraglutide were shown to act complementarily on body weight, food intake and glucose tolerance (Gydesen, Andreassen, et al., 2017), indicating the add-on potential of KBP-089 to liraglutide in obesity treatment. As only sub-optimal doses of KBP-089 and liraglutide have been examined chronically, we here evaluate combination-effects of doses that elicits full response separately (Gydesen, Hjuler, et al., 2017). In this study, we investigated the weight reducing potential of KBP-089 as monotherapy and in combination with the GLP-1 analogue liraglutide in obese high-fat diet fed (HFD) rats.

Methods

Peptide therapy

Synthetic KBP-089 (American Peptide Company, CA, USA) and liraglutide (SynPeptide, Shanghai, China) were dissolved in saline for subcutaneous delivery. The doses chosen for KBP-089 administration in the current *in vivo* investigations were based on previous comparable studies in animal models of obesity using KBP-089 (Gydesen, Andreassen, et al., 2017; Gydesen, Hjuler, et al., 2017) and previous studies with liraglutide (Knudsen, 2010; Gydesen, Andreassen, et al., 2017).

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 (2016-15-0201-00910). 108 male Sprague Dawley (SD) rats (Envigo, Horst, The Netherlands) were obtained at 6 weeks of age and housed pair-wise in standard Type IV cages at the Nordic Bioscience animal facility (21–23 °C, 55–65% relative humidity, 12-h light/dark cycle) with *ad libitum* access to food and water.

From arrival and throughout the study period, the age-matched male SD rats were fed a 60 kcal% fat diet (#58Y1, TestDiet, London, UK). After 10 weeks on high fat diet (HFD), the rats were allocated into treatment groups according to body weight (n = 8–10 rats/treatment group – 8 rats in monotherapy groups and 10 rats in combination therapy groups). The rats received doses of KBP-089 (KBP) (0.625, 1.25 and 2.5 µg/kg sc), liraglutide (L) (200 and 400 µg/kg sc) and the combinations (KBP 0.625 + L 200 µg/kg, KBP 0.625 + L 400 µg/kg, KBP 1.25 + L 200 µg/kg, KBP 1.25 + L 400 µg/kg and KBP 2.5 + L 200 µg/kg, KBP 2.5 + L 400 µg/kg) and vehicle (saline) for 9 weeks. Body weight and food intake were monitored daily in the initial three weeks, then once weekly. Following 4 and 8 weeks of treatment, oral glucose tolerance tests (OGTT) were performed. To assess the treatment effect on gastric emptying, rats received acetaminophen (40 mg/kg) p.o. gavage (4 mL/kg) together with the glucose bolus during OGTT and the appearance of acetaminophen in plasma was measured after 30 min. At study end, animals were euthanized by exsanguination (under isoflurane anaesthesia) followed by dissection. Epididymal, perirenal and subcutaneous inguinal fat depots were surgically removed and weighed.

Glucose tolerance tests

OGTTs were performed at 4 and 8 weeks of treatment in animals fasted for 12 hours. Animals were administered with either vehicle or drug at t=-30 and received glucose p.o. gavage (2 g/kg) at t = 0. EDTA blood samples were collected from the tail vein before drug administration and glucose challenge (0 min) and then the following 15, 30, 60 and 120 min post glucose challenge.

Biochemical analysis

Blood samples were collected in EDTA tubes and centrifuged at 5000 rpm for 10 min at 4 °C and the plasma was kept at -20 °C until further analysis. Blood glucose was monitored by Accu-Check® Avia monitoring system (Roche Diagnostics, Rotkreuz, Switzerland). Plasma levels of insulin (Mercodia Rat Insulin ELISA, Mercodia AB, Uppsala, Sweden), glucagon (Mercodia glucagon ELISA, Mercodia AB, Uppsala, Sweden) and acetaminophen (Acetaminophen Direct ELISA Kit, Immuneanalysis, Pomona, CA) were analyzed according to manufacturer's instruction.

Statistical analysis

The endpoints were: change in bodyweight, food intake, glucose tolerance and insulin levels.

All data are presented as mean ± standard error of the mean (SEM). The statistical analysis of group differences were assessed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparison. All analyses were performed using GraphPad Prism software (GraphPad Prism, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

Results

KBP-089 acts complementary with GLP-1 on food intake and body weight loss

To assess whether KBP-089 acts complimentary with GLP-1, KBP-089 and the GLP-1 analogue liraglutide were administered alone or in combination for 9 weeks in HFD rats. 9 weeks of treatment with KBP-089 (0.625, 1.25 and 2.5 µg/kg) resulted in a dose dependent weight loss (Fig. 1C). Chronic treatment with high concentrations of liraglutide (200 and 400 µg/kg) and KBP-089 (2.5 µg/kg) resulted in a 7% and 15% vehicle-corrected body weight loss, respectively, while the combinations (L 200 µg/kg + KBP 2.5 µg/kg and L 400 µg/kg + KBP 2.5 µg/kg) resulted in a 17% and 21% weight reduction, respectively (Fig. 1A,C). All treatments significantly reduced food intake in the initial phase of the study (Fig. 1D-E), while only high dose KBP-089 and combination therapy reduced food intake during the entire study (Fig. 1F). Based on food intake and body weight change, food efficiency was calculated. Treatment with the two highest KBP-089 doses (1.25 and 2.5 µg/kg) as well as their combinations with liraglutide resulted in a significant reduction in food efficiency compared with vehicle. Additionally, combination treatment was superior to treatment with liraglutide and showed a trend to towards superiority to KBP-089 alone (Fig. 1G).

Treatment with KBP-089 and liraglutide reduces overall adiposity in high fat diet rats

At study end adipose tissues were isolated and weighed. In conjugation with the significant reduction in body weight, the weights of epididymal and perirenal white adipose tissues were significantly reduced after treatment with 2.5 µg/kg of KBP-089 and combinations of KBP-089 and liraglutide, whereas only the combination therapy reduced the weight of inguinal adipose tissue (Fig. 2A-C).

Treatment with KBP-089 and liraglutide improves oral glucose tolerance with reduced insulin levels

OGTTs were performed after 4 and 8 weeks of treatment. After 4 weeks of treatment, KBP-089 (1.25 µg/kg) and the combination of high KBP-089 (2.5 µg/kg) and liraglutide (400 µg/kg) had markedly improved glucose tolerance (Fig. 3A,C). During OGTT after 8 weeks of treatment (Fig. 3B), only the two combination groups (L 200 µg/kg + KBP 2.5 µg/kg and L 400 µg/kg + KBP 2.5 µg/kg) were able to significantly improve oral glucose tolerance considering the iAUC (Fig. 3D). After both short and long-term treatment (4 and 8 weeks) insulin levels were reduced in rats treated with KBP-089 (1.25 and 2.5 µg/kg) and unchanged in rats treated with liraglutide (200 and 400 µg/kg) compared to vehicle, resulting in significantly different iAUC values in KBP-089 and liraglutide treated rats (Fig. 3G,H). All combinations of the two treatments resulted in insulin levels in the same range as the KBP-089 treated rats, being significantly lower compared to vehicle and liraglutide treated rats (Fig. 3G,H).

High dose Liraglutide, KBP-089 and the combination reduced fasting plasma glucagon levels

Fasting plasma glucagon levels and glucagon levels during OGTT were assessed after 8 weeks of treatment. High dose liraglutide (L 400 µg/kg), KBP-089 (1.25 and 2.5 µg/kg) and the combination of slightly reduced fasted plasma glucagon levels compared to vehicle (Fig. 4A). Plasma glucagon levels during OGTT did not differ significantly between treatment (Fig. 4B).

KBP-089 in combination with liraglutide delay gastric emptying rate

Gastric emptying was assessed in the treatment groups receiving the highest doses of KBP-089, high dose liraglutide and the combination of the two. The rate of gastric emptying during OGTT was assessed after 4 and 8 weeks of treatment (Fig. 5A,B). After both 4 and 8 weeks of treatment administration of KBP-089 (2.5 µg/kg) resulted in a significant reduction of gastric emptying rate 30 min after acetaminophen administration, while liraglutide (L 400 µg/kg) had no pronounced effect on gastric emptying (Fig. 5A,B). After 4 weeks of treatment liraglutide reduced gastric emptying by approximately 10% compared to vehicle (Fig. 5A), while liraglutide increased gastric emptying by approximately 18% compared to vehicle after 8 weeks of treatment (Fig. 5B). Additionally, the combination of high dose KBP-089 and liraglutide (L 400 µg/kg + KBP 2.5 µg/kg) significantly delayed gastric emptying, but equally to KBP-089 treatment alone. This effect on gastric emptying rate was unchanged from 4 (Fig. 5A) to 8 (Fig. 5B) weeks of treatment.

Discussion

In this article we present data underlining the potential of combination therapy for obesity with the DACRA KBP-089 and the GLP-1 analog.

In general, combination therapies are showing promise in the treatment of metabolic disorders, such as obesity, NASH and T2DM, including the combination of GLP-1 and amylin therapy (Roth et al., 2012). These agonists have several overlapping effects, although the mechanisms of action might differ (Roth et al., 2012; Jansson and Palsdottir, 2015). A previous study in non-human primates suggested a synergistic reduction in food intake when combining GLP-1 and amylin agonism (Bello et al., 2010). In addition, the combination of sub-optimal doses of the DACRA, KBP-089, and the GLP-1 agonist liraglutide was recently shown to act complementarily (Gydesen, Andreassen, et al., 2017). Therefore, we studied the combination of KBP-089 with liraglutide at their individual maximum efficacious doses.

In this study, we found a significant effect on appetite suppression and body weight loss when combining the two peptides over a period of nine weeks, an effect superior to either monotherapy alone. Importantly, this also manifested in reduction in food efficiency and overall adiposity. Generally, KBP-089 was superior to liraglutide therapy, and the effects on body weight and food intake dose dependently followed KBP-089

concentrations when combining the two therapies. This suggests that KBP-089 is responsible for the majority of the efficacy of the combination therapy. These findings correspond well with earlier observations using lower doses of the two peptides (Gydesen, Andreassen, et al., 2017), and demonstrate an additive effect.

In terms of glucoregulatory actions both amylin receptor agonism (Ratner et al., 2004; Ryan et al., 2009; Mack et al., 2011) and GLP-1R agonism (Vilsbøll et al., 2007; Klein et al., 2014; Van Can et al., 2014) have shown potential. However, the glucose-lowering effect of GLP-1 receptor agonists involves increased post-prandial insulin secretion (Vilsbøll et al., 2008; Ladenheim, 2015). During OGTT, both short and long-term treatment with KBP-089 improved glucose tolerance in accordance with previous studies performed with KBP-089 (Gydesen, Andreassen, et al., 2017; Gydesen, Hjuler, et al., 2017). Interestingly, the effect on blood glucose during OGTT was especially pronounced in combination therapy groups, particularly after eight weeks of treatment, supporting that the peptides act through complimentary pathways, and possibly that the combination leads to increased durability of the glucoregulatory effects compared to stand-alone treatment. Importantly, along with improved glucose clearance, significantly lower plasma insulin levels were observed in KBP-089 (1.25 and 2.5 µg/kg) and combination therapy groups, indicating improved insulin sensitivity. This together with the significant weight loss suggest potential not only as anti-obesity therapy, but also in treatment of obesity related co-morbidities such as type 2 diabetes and NASH (Kahn et al., 2006; Guh et al., 2009; Milić et al., 2014). Surprisingly, liraglutide did not increase plasma insulin as expected for a GLP-1 receptor agonist. Other studies in obese rats found similar lack of liraglutide induced increase in plasma insulin during OGTT (Raun et al., 2007; Gydesen, Andreassen, et al., 2017), suggesting that the lack of effect observed here might be explained by the animal model that is non-diabetic. Plasma glucagon levels were assessed after 8 weeks of treatment. All treatments, except liraglutide (200 µg/kg), tended towards a lowering of fasting plasma glucagon levels compared to vehicle. Though, all treatment groups had nearly constant glucagon levels during OGTT and no significant differences between groups were observed. This suggests that the HFD rat model does not show inappropriate elevated glucagon levels as seen in diabetic conditions and might explain why there is no clear effect of the therapies post glucose challenge.

GLP-1 and amylin analogues are both known to delay gastric emptying (Roth et al., 2012), hence gastric emptying rates were assessed. In accordance with earlier studies using DACRAs (Hjuler et al., 2015, 2016), KBP-089 (2.5 µg/kg) markedly reduced gastric emptying after both short- and long-term treatment. A similar effect was observed in the group receiving high-dose combination therapy. Perhaps surprisingly, liraglutide alone only had minor effect on gastric emptying, even trending towards increasing vehicle-corrected gastric emptying after 8 weeks of treatment. Several clinical studies have shown that chronic treatment with liraglutide delays gastric emptying (Flint et al., 2011; Horowitz et al., 2012; Van Can et al., 2014). However, in a pre-clinical setting the ability of liraglutide to reduce gastric emptying markedly diminished within 14 days of treatment, explaining the lack of effect observed here (Jelsing et al., 2012). The inhibited gastric emptying can positively affect postprandial blood glucose levels by delaying entry of glucose into circulation, a central factor in diabetes treatment. Overall, the weight lowering and glucoregulatory actions of both the mono- and the combination therapies might be limited by the fact

that the HFD rat model does not have indications of insulin resistance and seems to have reached their maximal weight loss. Therefore, further studies in a diabetic model would be of importance.

In conclusion, KBP-089 acts complementary with the GLP-1 analogue, liraglutide, on food consumption, weight loss and glucose tolerance, indicating the potential for an add-on therapy causing additional improvement in metabolic profile.

Abbreviations

ANOVA

Analysis of Variance

DACRA

Dual Amylin and Calcitonin Receptor Agonists

ELISA

Enzyme-linked immunosorbent assay

GLP-1

Glucagon-like peptide 1

HFD

High Fat Diet

(i)AUC

(incremental) Area Under the Curve

OGTT

Oral Glucose Tolerance Test

SEM

Standard error of the mean

Declarations

All studies were approved by the Danish Animal Inspectorate (approval number: 2016-15-0201-00910).

The data is available for sharing upon request.

Funding by the Danish Research Foundation (Den Danske Forskningsfond) was granted to ATL and SGY (Grant numbers N/A).

Author contributions:

Participated in the research design: SGY, KH, MK

Conducted experiments: ATL, SGY

Performed data analysis: ATL, SGY

All authors have read and approved the final manuscript.

Competing interests: MK and KH own stocks in Nordic Bioscience. MK and KH hold patents on KBPs. All authors are employees of Nordic Bioscience.

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Figures

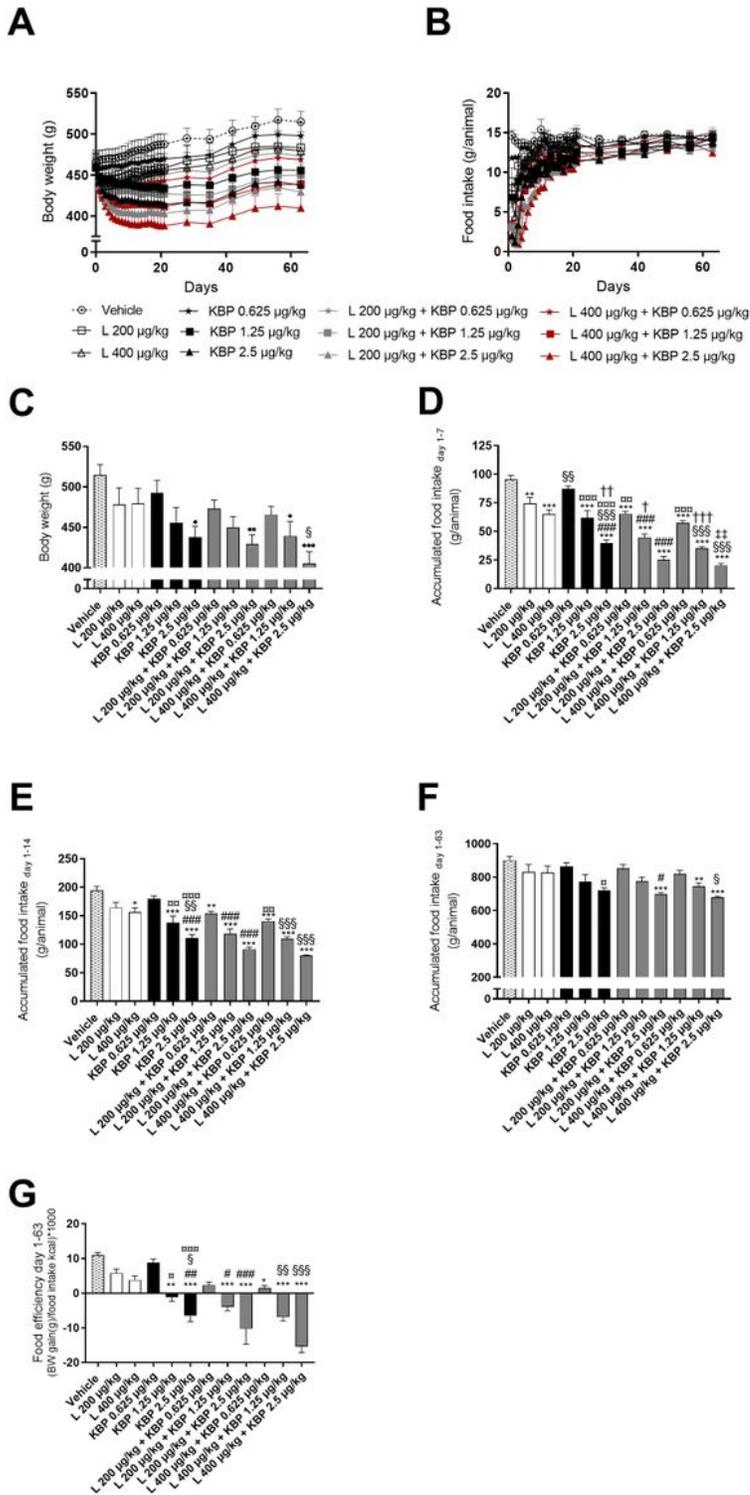


Figure 1

Body weight (A) and food intake (B) during the study. End-point body weights (C). Accumulated food intake for the initial 7 (D) and 14 (E) days and for the entire duration of the study (F). Calculated food efficiency (G). N=8-10 rats per group. Statistical analysis between groups in C-G were performed as a one-way ANOVA followed by Tukey's post-hoc test with the following annotations: *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle, #P < 0.05, ##P < 0.01 vs. liraglutide (200 µg/kg), §P < 0.05, §§P < 0.01 vs. liraglutide

(400 µg/kg), α P < 0.05, $\alpha\alpha$ P < 0.01, $\alpha\alpha\alpha$ P < 0.001 vs. KBP-089 (0.625 µg/kg), † P < 0.05, †† P < 0.01, ††† P < 0.001 vs. KBP-089 (1.25 µg/kg) and ‡P<0.01 vs. KBP-089 (2.5 µg/kg). All data are means \pm SEM.

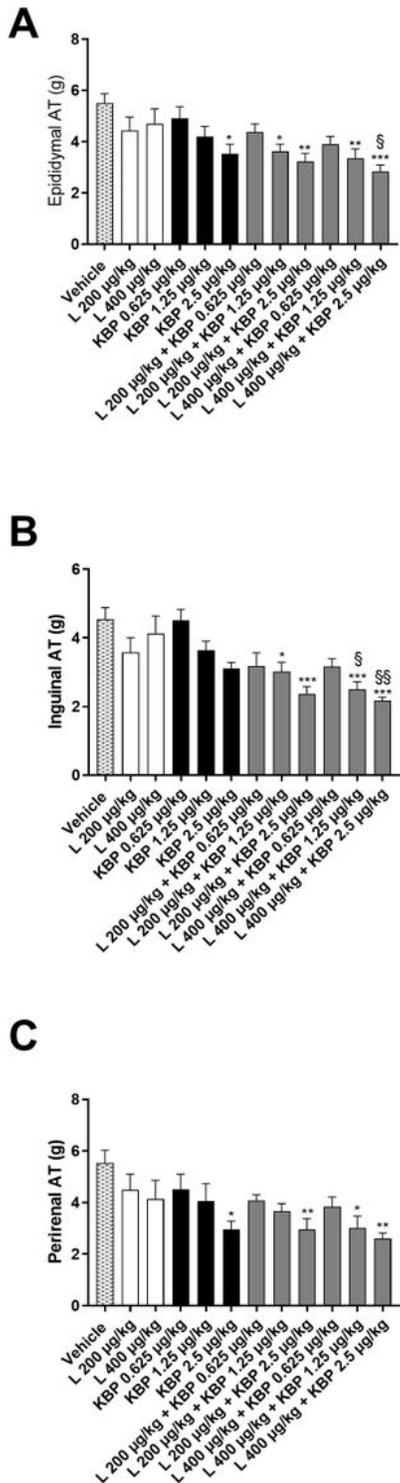


Figure 2

Weights of epididymal (A), inguinal (B) and perirenal (C) white adipose tissue (AT) at study end (n=8-10 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 < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle, §P < 0.05, §§P < 0.01 vs. liraglutide (400 µg/kg). All data are means ± SEM.

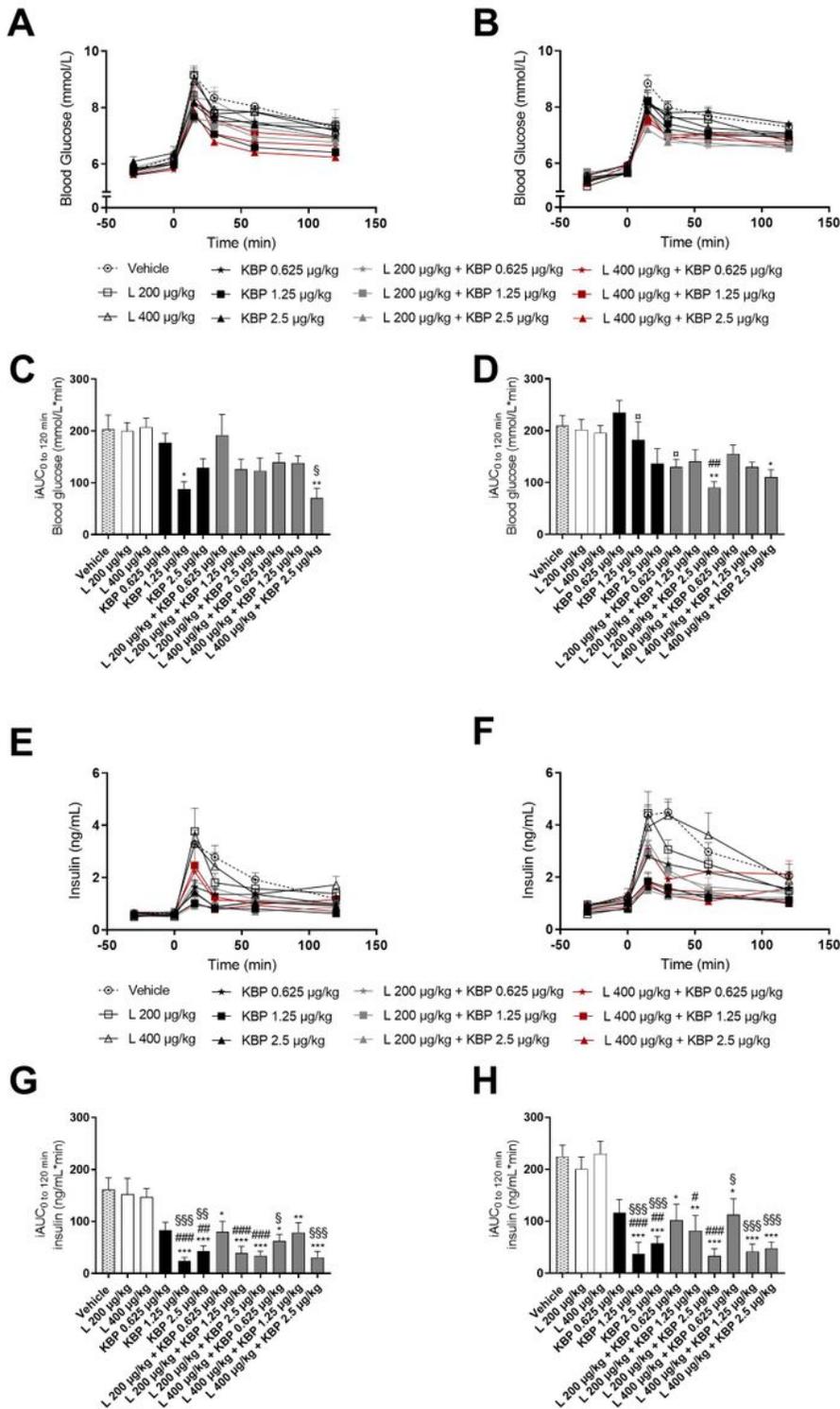


Figure 3

Plasma glucose (A and B) and insulin (E and F) during oral glucose tolerance test (OGTT) after 4 and 8 weeks, respectively. The incremental area under the curve (iAUC) shown for glucose (C and D) and insulin (G and H) during OGTT after 4 and 8 weeks, respectively. n=8-10 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 < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle, #P < 0.05, ##P < 0.01 vs. liraglutide (200 µg/kg), §P < 0.05, §§P < 0.01 vs. liraglutide (400 µg/kg), α P < 0.05 vs. KBP-089 (0.625 µg/kg). All data are means ± SEM.

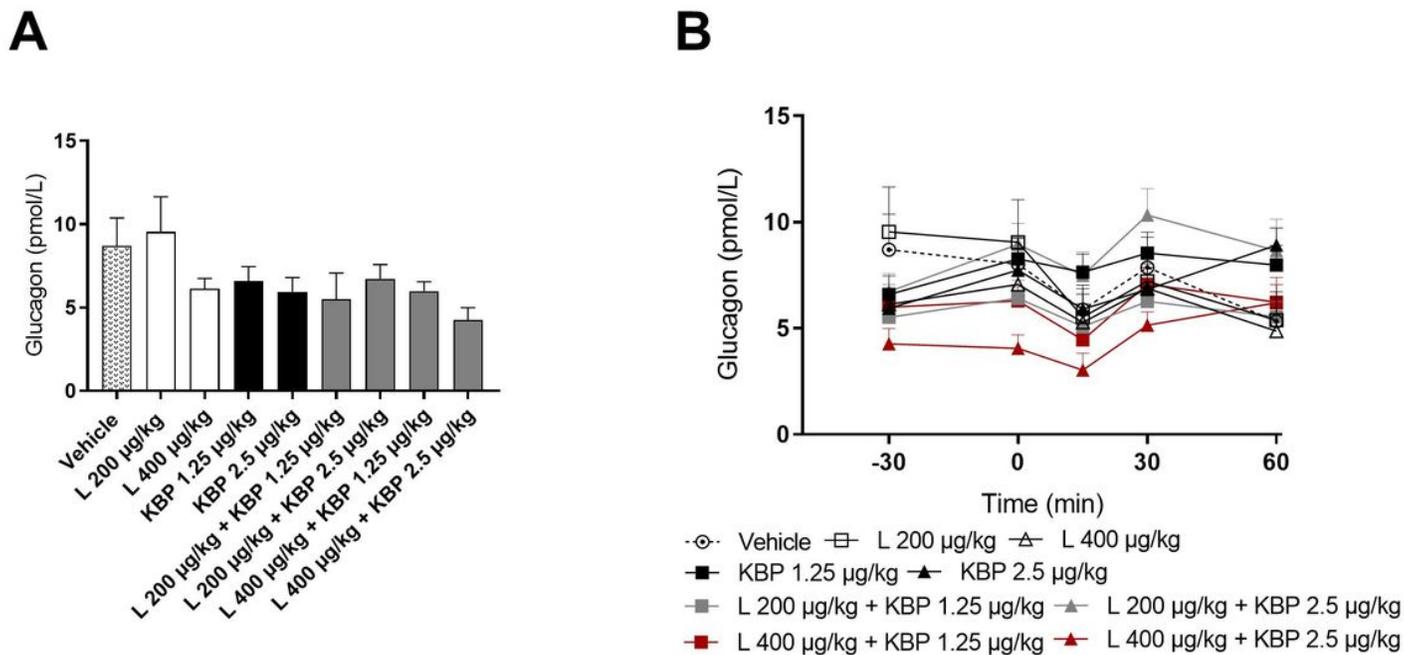
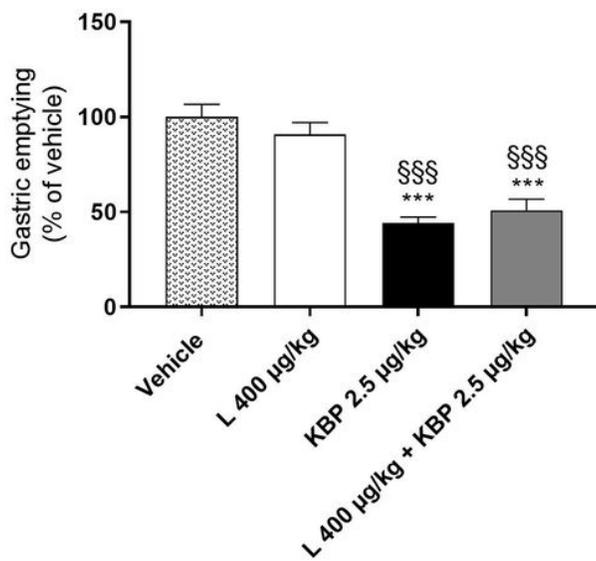
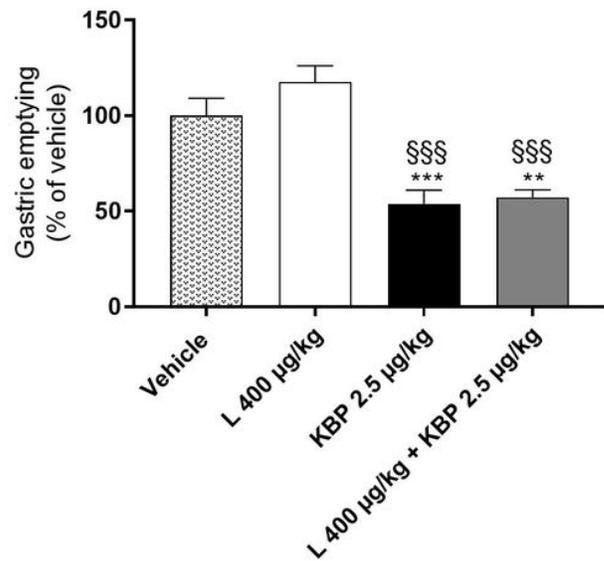


Figure 4

Fasting plasma glucagon levels (A) and plasma glucagon levels during OGTT after 8 weeks of treatment (B). n=8-10 rats per group. Statistical analysis between groups were performed as a one-way ANOVA followed by Tukey's post-hoc test. All data are means ± SEM.

A**B****Figure 5**

Gastric emptying after 4 (A) and 8 (B) weeks of treatment. Gastric emptying was estimated by the appearance of acetaminophen in plasma 30 min post dosing and calculated as % change relative vehicle. 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 and §§§P < 0.001 vs. liraglutide (400 µg/kg). All data are means ± SEM.

Supplementary Files

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- [NC3RsARRIVEGuidelinesChecklistfillablecompleted.pdf](#)