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RESEARCH ARTICLE

Translational Physiology

The enduring metabolic improvement of combining dual amylin and calcitonin receptor agonist and semaglutide treatments in a rat model of obesity and diabetes

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Abstract

Long-acting dual amylin and calcitonin receptor agonists (DACRAs) are novel candidates for the treatment of type 2 diabetes and obesity due to their beneficial effects on body weight, glucose control, and insulin action. However, how the metabolic benefits are maintained after long-lasting treatment is unknown. This study investigates the long-term anti-obesity and anti-diabetic treatment efficacy of the DACRA KBP-336 alone and combined with the GLP-1 analog semaglutide. Zucker diabetic Sprague Dawley (ZDSD) rats with obesity and diabetes received KBP-336 (4.5 nmol/kg Q3D), semaglutide (50 nmol/kg Q3D), or the combination for 7 mo, and the treatment impact on body weight, food intake, glucose control, and insulin action was evaluated. Furthermore, serum levels of the cardiac fibrosis biomarker endotrophin were evaluated. KBP-336, semaglutide, and the combination lowered body weight significantly compared with the vehicle, with the combination inducing a larger and more sustained weight loss than either monotherapy. All treatments resulted in reduced fasting blood glucose levels and HbA1c levels and improved glucose tolerance compared with vehicle-treated rats. Furthermore, all treatments protected against lost insulin secretory capacity and improved insulin action. Serum levels of endotrophin were significantly lowered by KBP-336 compared with vehicle. This study shows the benefit of combining KBP-336 and semaglutide to obtain significant and sustained weight loss, as well as improved glucose control. Furthermore, KBP-336-driven reductions in circulating endotrophin indicate a clear reduction in the risk of complications. Altogether, KBP-336 is a promising candidate for the treatment of obesity and type 2 diabetes both alone and in combination with GLP-1 analogs.

NEW & NOTEWORTHY These studies describe the benefit of combining dual amylin and calcitonin receptor agonists (DACRA) with semaglutide for long-term treatment of obesity and type 2 diabetes. Combination treatment induced sustained weight loss and improved glucose control. A DACRA-driven reduction in a serological biomarker of cardiac fibrosis indicated a reduced risk of complications. These results highlight DACRAs as a promising candidate for combination treatment of obesity and type 2 diabetes and related long-term complications.

DACRA; GLP-1; obesity; type 2 diabetes; weight loss

INTRODUCTION

Obesity is constantly increasing affecting a considerable proportion of the population and is associated with an increased risk of several long-term complications including type 2 diabetes and cardiovascular disease (1, 2). Significant weight loss is crucial to reduce the risk of these obesity-related comorbidities (3). A substantial weight loss of at least 15% has a disease-modifying effect in people with type 2 diabetes (4, 5), highlighting the importance of weight management not only in the treatment of obesity itself but also as a primary treatment of obesity-related comorbidities. Major advancements in anti-obesity therapy are occurring and additional weight management drugs have been approved within the past years. However, new treatments, including combination therapy using different classes of compounds, are still being investigated aiming for improved efficacy both on weight reduction and on comorbidities.

The GLP-1 analog semaglutide and the GLP-1-GIP dual receptor agonist tirzepatide elicit substantial weight loss and improve glucose control, and are approved by the US Food and Drug Administration (FDA) for both chronic weight

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management and treatment of type 2 diabetes (6–17). They are presently the most efficacious drugs for type 2 diabetes and obesity; however, they still do not match the weight loss elicited by bariatric surgery (18).

Among the leading potential combination partners for the incretins, the long-acting amylin analogs are found (19–23). One is the amylin analog cagrilintide, which has shown promising metabolic effects in combination with semaglutide and currently is in clinical development as CagriSema for the treatment of obesity and type 2 diabetes (19, 20, 24). The group of dual amylin and calcitonin receptor agonists (DACRAs) termed KBPs (Key Bioscience Peptides) are highly potent agonists of both amylin and calcitonin receptors (25, 26) developed for the treatment of obesity and type 2 diabetes. Several preclinical studies have shown beneficial effects on both weight loss, glucose control, and insulin sensitivity (25–27). Importantly, the mode of action of DACRAs complements the effects of incretins (28–30), underscoring the potential of these as combination possibilities. However, to what extent the combination efficacy is sustainable and to what extent it manifests in reductions of the complications are unknown parameters.

In this study, we assess the durability of the combination of the long-acting DACRA KBP-336 with the GLP-1 analog semaglutide in an obese and type 2 diabetic rat model. Importantly, we also investigate the impact on comorbidities, through assessment of the circulating levels of endotrophin, which is a well-characterized biomarker of heart and kidney failure both clinically (31–38) and preclinically (39).

MATERIALS AND METHODS

Peptides

Synthetic KBP-336 (Bachem, Switzerland) was dissolved in acetate buffer (10 mM acetate buffer + 50 mg/mL mannitol, pH 4), while semaglutide (Bachem, Switzerland) was dissolved in saline (0.9%) for subcutaneous (s.c.) delivery. The dose chosen was based on previous preclinical studies (26, 28, 29). The DACRA KBP-336 is one of three long-acting KBPs (KBP-066A, KBP-088A, and KBP-336) previously tested in vivo (25–29, 39–41). All peptides active the amylin and calcitonin receptor with similar potency, and any minor potency differences can be compensated in vivo by adjusting the dose. The peptide sequences are similar with only a few amino acid differences, and in vivo studies show that they elicit the same effects (25–29, 39–41).

Animal Studies

All animal procedures were performed following guidelines from the Animal Welfare Division of the Danish Ministry of Justice under the institutional license issued to Nordic Bioscience (2021-15-0201–00886). Rats were housed pairwise in standard type IV cages (Scanbur A/S, Denmark) under a controlled temperature (21–23°C, 55%–65% relative humidity) and normal 12-h light/dark cycle with ad libitum access to food and water.

Male Zucker Diabetic Sprague Dawley rats (ZDSD, Charles River Laboratories, Wilmington, MA) were obtained at the age of 5–6 wk and fed a 60 kcal% fat high-fat diet (HFD, No. D12492, Research Diets) from arrival and throughout the study. The rats were left untreated for 10 wk to induce obesity and hyperglycemia before they were allocated into treatment groups according to body weight (562.9 ± 42.4 SD) and fasting blood glucose levels (8.3 ± 0.6 SD). Rats received subcutaneous injections of vehicle (acetate-mannitol buffer, $n = 17$), KBP-336 (4.5 nmol/kg, $n = 20$), semaglutide (50 nmol/kg, $n = 20$), or a combination of KBP-336 and semaglutide (4.5 nmol/kg + 50 nmol/kg, $n = 16$) every third day for 7 mo. Food intake and body weight were monitored daily for the initial 40 days of the study, then every third day on dosing days. Fasting (6 h) blood glucose levels were measured every other week throughout the study, while HbA1c levels were measured at baseline, mid-study, and the study end. Blood samples were collected approximately every other month. An oral glucose tolerance test (OGTT) was performed in overnight fasted rats at the study end. The rats were euthanized by exsanguination under isoflurane anesthesia. Inguinal, epididymal, and perirenal adipose tissue depots were surgically removed and weighed, and pancreases were surgically removed and stored for analysis of insulin content.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed in overnight (11 h) fasted rats 24 h postdosing. A glucose bolus (1 g/kg, Sigma Aldrich, Søborg, Denmark) was administered orally. EDTA blood samples were collected, and blood glucose levels were measured before the glucose challenge and the following 15, 30, 60, and 120 min.

Gastric Emptying

The gastric emptying rate was assessed during the OGTT. Rats received acetaminophen (40 mg/kg) by oral gavage together with the glucose bolus, and the appearance of acetaminophen in plasma was measured 30 min postadministration.

Tissue Processing

Pancreases were homogenized and extracted in acid-ethanol (1.5% HCl in 70% EtOH) for measurement of insulin content. Insulin contents (Mercodia High Range Rat Insulin ELISA, Mercodia AB, Uppsala, Sweden) were analyzed according to the manufactures' instructions. Protein contents were estimated using Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Hercules, CA).

Biochemical Analysis

Blood samples for serum were collected in Eppendorf tubes and centrifuged twice for 10 min before the serum was kept at −20°C until further analysis. Blood samples for plasma were collected in EDTA tubes and centrifuged for 10 min at 4°C and the plasma was kept at −20°C until further analysis. Blood glucose was monitored by the Accu-Check Avia monitoring system (Roche Diagnostics, Switzerland). HbA1c levels were measured by a DCA Vantage Analyzer (Siemens, Munich, Germany). Plasma levels of insulin (Mercodia Rat Insulin ELISA, Mercodia AB) were analyzed according to the manufacturers' instructions. The biomarker rPRO-C6 (endotrophin), a matrikine released from the C-terminal C5 domain of collagen type VI a3 chain, was measured in serum samples using competitive ELISA

(Nordic Bioscience, Herlev, Denmark) as described previously (39). The gastric emptying rate was determined by measuring acetaminophen in the plasma according to the manufacturers' instructions (Acetaminophen Forensic ELISA kit, Neogen Toxicology, Lansing, MI).

Statistics and Software

The statistical analyses of group differences were conducted using one-way ANOVA followed by Holm-Sidak's post hoc test for multiple comparisons. Statistical analyses of nonparametric data were conducted using Kruskal–Wallis test followed by Dunn's post hoc test for multiple comparisons. Normality of data distribution was determined by Kolmogorov–Smirnov normality test. All analyses were performed using GraphPad Prism 10 software (San Diego, CA). A value of $P < 0.05$ was considered statistically significant. All data are presented as means ± SE.

RESULTS

The Combination of KBP-336 and Semaglutide Induces Sustained Weight Loss

Obese and diabetic ZDSD rats were treated with KBP-336 (4.5 nmol/kg), semaglutide (50 nmol/kg), or the combination of the two for 7 mo to evaluate the long-term treatment efficacy. In the initial 3 mo of the treatment period, all treatments resulted in a significant weight loss compared with

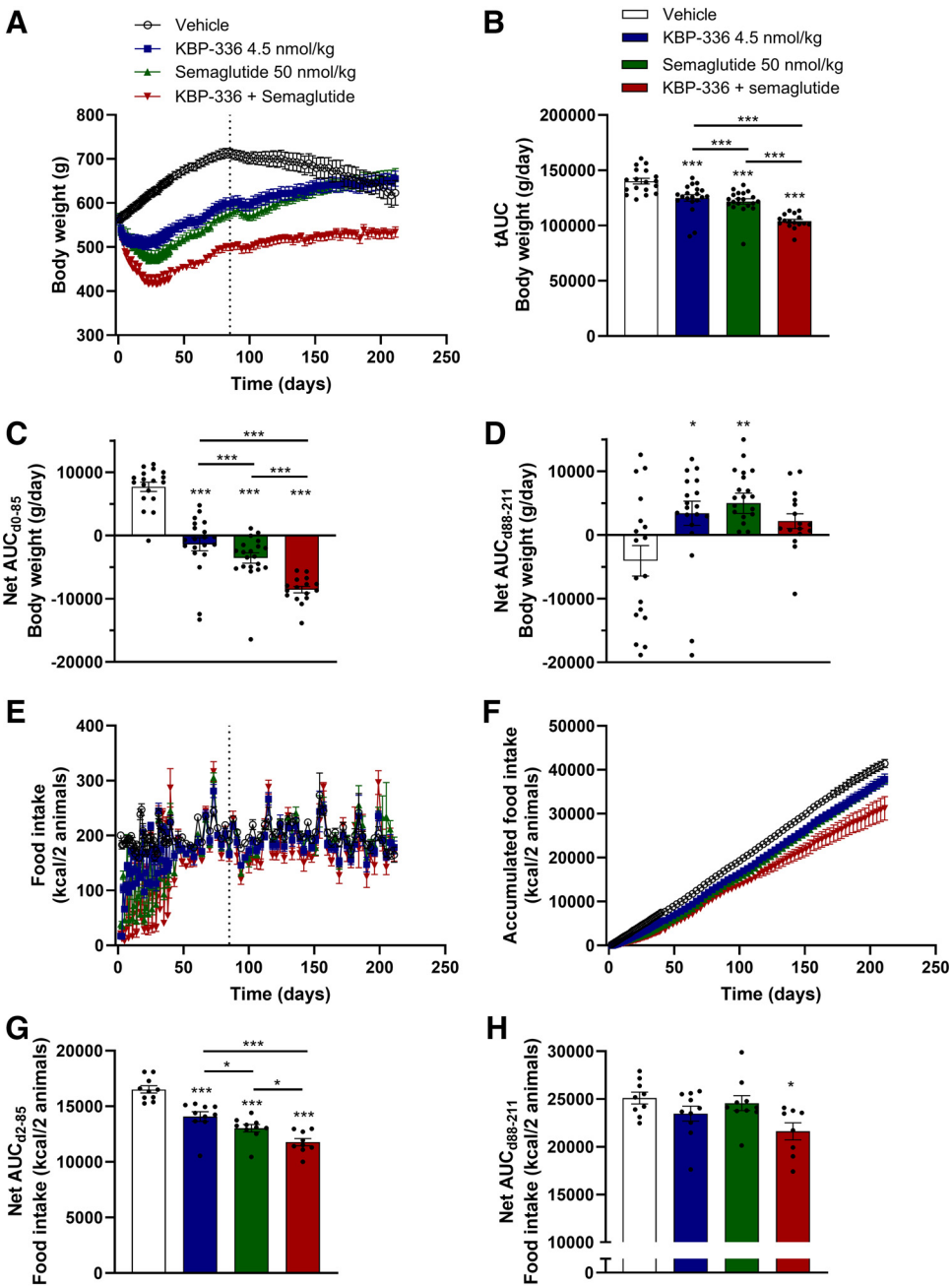

Figure 1. Treatment effect on body weight and food intake. Body weight during the study (*A*) and the total area under the curve (tAUC) (*B*). Net AUC from *day 0* to *day 85* (*C*) and from *day 88* to *day 211* (*D*). Food intake during the study (*E*) and the calculated accumulated food intake per two rats (*F*). Net AUC of food intake from *day 0* to *day 85* (*G*) and from *day 88* to *day 211* (*H*). The dotted lines in *A* and *E* indicate *day 85*. $n = 17$ (vehicle), $n = 20$ (KBP-336), $n = 20$ (semaglutide), and $n = 16$ (KBP-336 + semaglutide). Statistical differences between groups were evaluated with one-way ANOVA followed by Holm–Sidak's multiple comparisons test (*B*, *C*, *D*, *G*, and *H*). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. vehicle unless otherwise indicated in the figure. All data are presented as means ± SE. ANOVA, analysis of variance.

the vehicle-treated rats that continued to gain weight (Fig. 1, *A* and *C*). Notably, combination therapy resulted in a significantly larger weight loss than either monotherapy, while semaglutide treatment induced a slightly larger weight loss compared with KBP-336 (Fig. 1, *B* and *C*). In all groups, the weight loss was obtained by a reduction in food intake especially in the initial phase of the treatment period (Fig. 1, *E–H*). In line with the weight loss effect, combination therapy resulted in a larger reduction in food intake compared with monotherapies (Fig. 1, *F–H*). From *day 85* onward, the vehicle-treated rats started to lose weight, while rats receiving monotherapy gained weight resulting in similar body weight at the study end (Fig. 1, *A* and *D*). Combination therapy, in contrast, resulted in sustained weight management and the rats weighed significantly less than the vehicle rats at the study end (Fig. 1, *A* and *D*). The treatment effect on body weight was reflected in the adiposity at the study end (Fig. 2). In line with the sustained weight loss, combination therapy resulted in markedly lower visceral adiposity compared with both vehicle and monotherapy (Fig. 2, *B* and *C*). KBP-336 and semaglutide-treated rats had significantly more inguinal adipose tissue compared with the vehicle-treated rats, while no differences in epididymal and perirenal adipose depots were observed (Fig. 2*B*).

KBP-336 Induces Long-Term Sustained Glucose Control Alone and in Combination with Semaglutide

All treatments resulted in stable fasting blood glucose levels throughout the treatment period, which is in significant contrast to the vehicle-treated rats that showed a drastic progression of hyperglycemia during the study (Fig. 3, *A* and *B*). At treatment initiation, the rats were hyperinsulinemic (average fasting insulin 5.1 ± 1.3 SD ng/mL). Evaluation of fasting insulin levels showed that all treatments resulted in significantly lower circulating insulin levels compared to with vehicle until *day 100* of the treatment period (Fig. 3, *C* and *D*), which is in line with the significant difference in glucose control (Fig. 3, *A* and *B*). As the disease progresses, the vehicle-treated rats lose their insulin secretory capacity resulting in declining levels of circulating insulin, which is in clear contrast to the stable levels in the treatment groups (Fig. 3*C*). This benefit of the treatments was also evident in the HbA1c levels. All treatments resulted in significantly lower HbA1c levels compared with the vehicle-treated rats whose HbA1c levels increased markedly during the study (Fig. 3, *E* and *F*). Of note, KBP-336 and combination-treated rats even improved HbA1c levels compared with the baseline (Fig. 3*E*). Pancreatic insulin content was determined at the study end to further evaluate the treatment effect on the pancreas. All treatments resulted in preserved pancreatic insulin content, albeit only KBP-336 and the combination therapy elicited significantly higher pancreatic insulin levels than the vehicle (Fig. 3*G*). Evaluation of pancreatic insulin content (Fig. 3*G*) in conjugation with fasting insulin levels (Fig. 3*C*) shows that all treatments increase insulin sensitivity compared with the vehicle and strongly indicate preservation of β-cell function.

Combination Therapy Induces Additional Benefit on Glucose Tolerance

To evaluate the treatment effect on glucose tolerance, an oral glucose tolerance test was performed at the study end (Fig. 4). All treatments resulted in significantly improved glucose tolerance compared with the vehicle (Fig. 4, *A–D*), with an additional benefit of combination therapy (Fig. 4, *A* and *E*). Plasma insulin levels were evaluated during the test to shed light on the mechanism behind the treatment effects on glucose levels (Fig. 4, *B, D,* and *F*). Both monotherapies resulted in significantly higher plasma insulin levels compared with the vehicle when evaluated by iAUC (Fig. 4*D*), while only semaglutide resulted in significantly higher levels than the vehicle when evaluated by tAUC (Fig. 4*F*). The group receiving combination therapy obtained the significantly improved glucose tolerance with plasma insulin levels similar to the vehicle (Fig. 4), indicating an improved insulin action. All treatments significantly slowed the gastric emptying rate compared with the vehicle but with no differences between the treatments (Fig. 4*G*).

KBP-336 Reduces Circulating Levels of the Fibrosis Marker Endotrophin

Circulating endotrophin is predictive for cardiovascular and renal complications as a function of type 2 diabetes and deteriorated insulin sensitivity (31, 33, 39). Therefore, we assessed serum levels of endotrophin, which increased during the study in all groups (Fig. 5*A*), indicating an overall increase

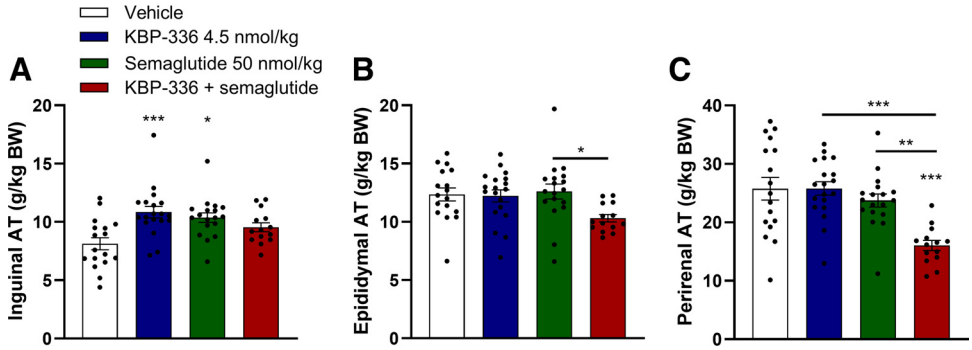

Figure 2. Adiposity at the study end. Weights of inguinal (*A*), epididymal (*B*), and perirenal (*C*) adipose tissue (AT) normalized to body weight (BW). $n = 17$ (vehicle), $n = 19$ (KBP-336), $n = 18$ (semaglutide), and $n = 14$ (KBP-336 + semaglutide) due to limited material. Statistical differences between groups were evaluated with one-way ANOVA followed by Holm–Sidak's multiple comparisons test (*B* and *C*) or Kruskal–Wallis test followed by Dunn's multiple comparisons test (*A*). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. vehicle unless otherwise indicated in the figure. All data are presented as means ± SE. ANOVA, analysis of variance.

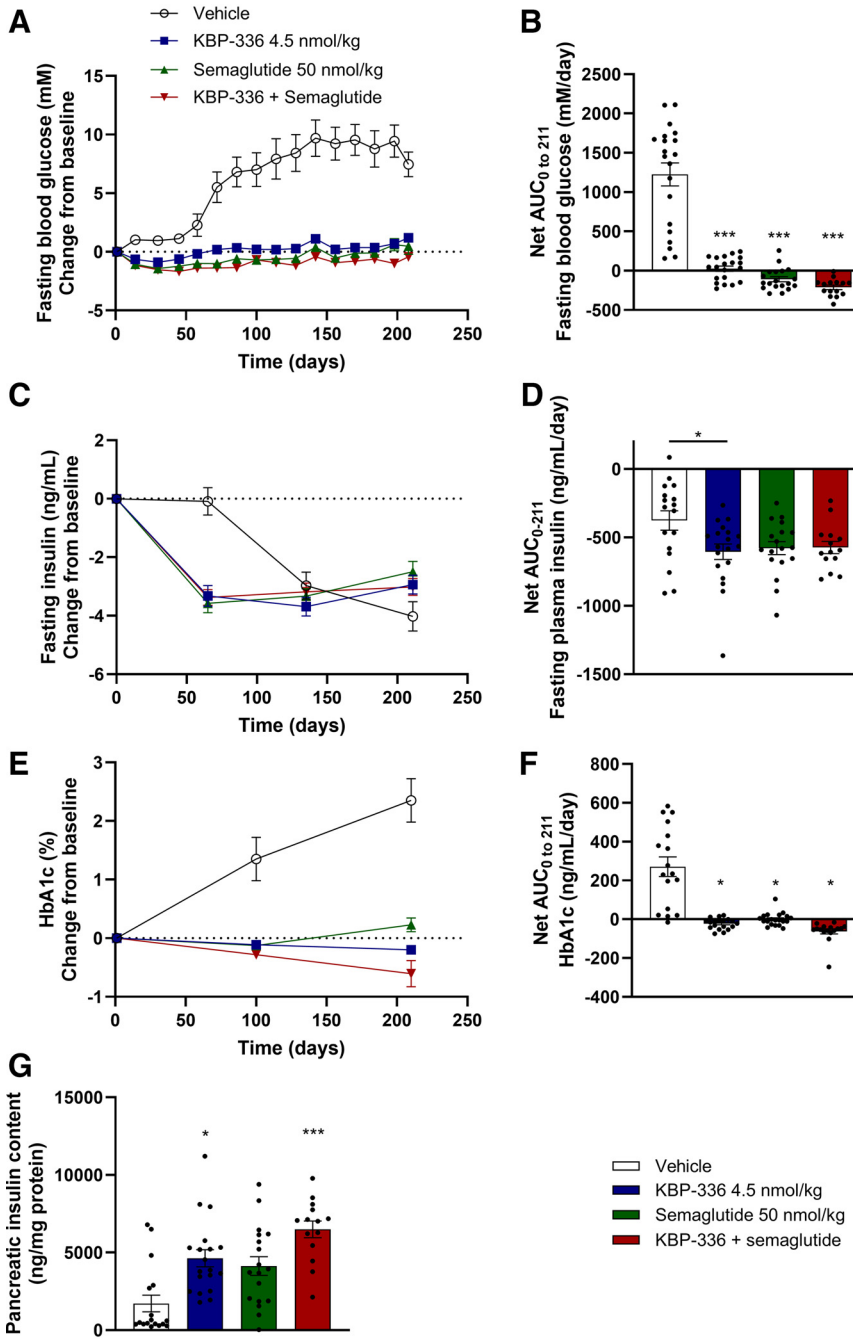

Figure 3. Treatment effect on glucose control. Fasting blood glucose shown as change from baseline (*A*) and the calculated net area under the curve (AUC) (*B*). Fasting plasma insulin levels during the study shown as change from baseline (*C*) and the calculated net AUC (*D*). HbA1c levels during the study shown as change from baseline (*E*) and the calculated net AUC (*F*). Pancreatic insulin content normalized to protein (G). $n = 17$ (vehicle), $n = 20$ (KBP-336), $n = 20$ (semaglutide), and $n = 16$ (KBP-336 + semaglutide), and $n = 19$ (KBP-336), $n = 18$ (semaglutide), and $n = 14$ (KBP-336 + semaglutide) for plasma insulin and pancreatic insulin content due to limited material. Statistical differences between groups were evaluated with one-way ANOVA followed by Holm-Sidak's multiple comparisons test (*B*, *D*, and *F*) or Kruskal–Wallis test followed by Dunn's multiple comparisons test (*G*). $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. vehicle unless otherwise indicated in the figure. All data are presented as means ± SE. ANOVA, analysis of variance.

in the endotrophin levels over time across the groups. However, the increase was most pronounced in the vehicle and semaglutide-treated rats, while KBP-336 treatment resulted in stable endotrophin levels until *day 135*, which were significantly lower than the vehicle group (Fig. 5, *A* and *B*). Interestingly, semaglutide did not lower endotrophin levels, while the combination group showed a trend toward it, particularly in the latter half of the study (Fig. 5, *A* and *B*).

DISCUSSION

In obesity and type 2 diabetes, the treatment gap between pharmacotherapies and bariatric surgeries is narrowing; however, to finally bridge it, combination therapies are needed. Here, the novel long-acting DACRA, KBP-336 is very relevant, and therefore we sought to investigate the durability of the metabolic benefit of the long-acting DACRA KBP-336 in combination with the long-acting GLP-1 analog semaglutide. We found highly promising outcomes in the combination group, as weight, fasting, and prandial glucose control all were improved beyond the effect of either monotherapy, data which clearly underline the potential of the combination of an incretin and an amylin/DACRA-based therapy.

All treatments resulted in an initial weight loss with the combination therapy inducing significantly larger weight

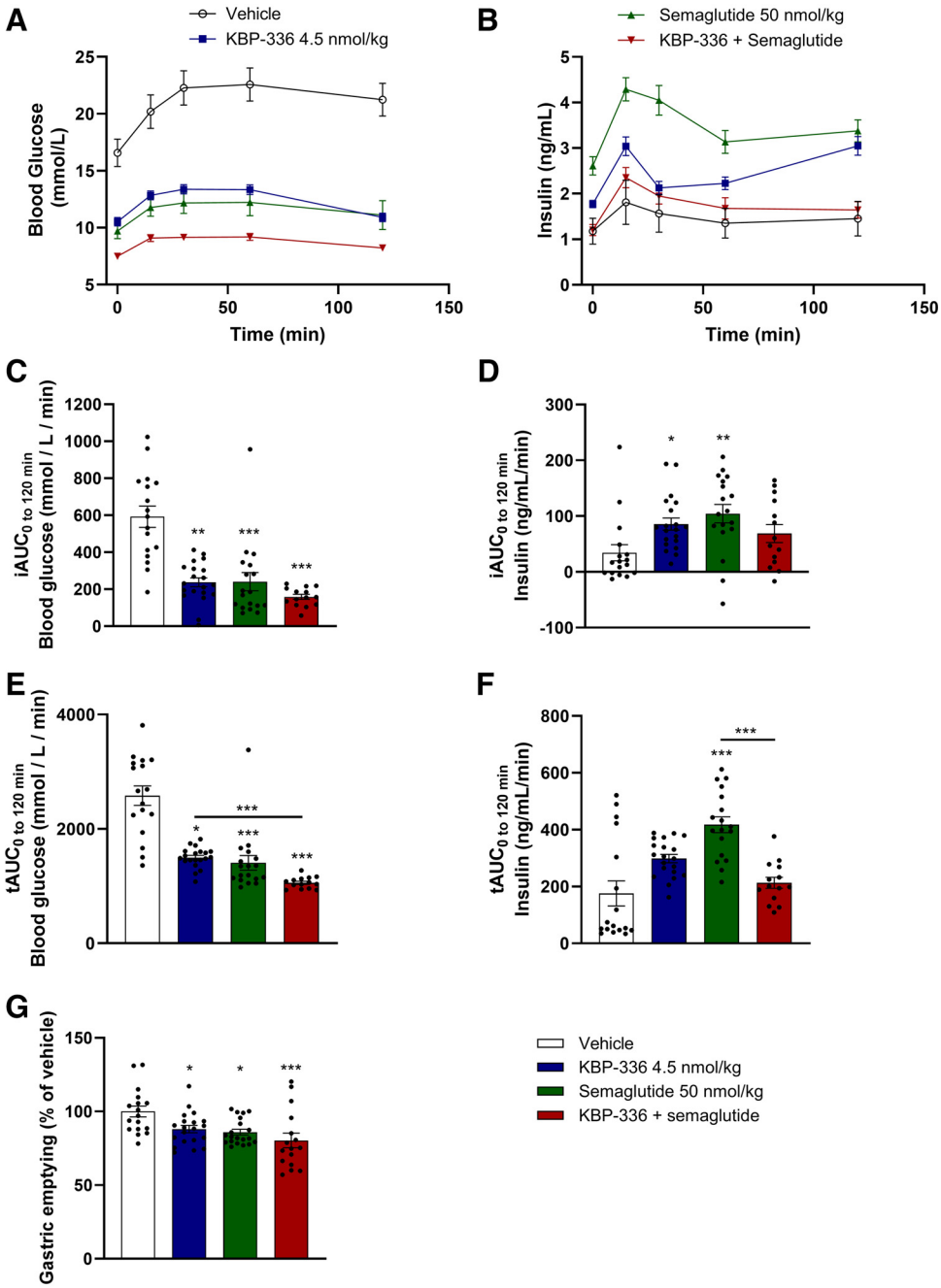

Figure 4. Oral glucose control at the study end. Blood glucose levels (*A*) and plasma insulin levels (*B*) during the test. Calculated incremental area under the curve (AUC) and total AUC for blood glucose (*C* and *E*) and for plasma insulin (*D* and *F*). The gastric emptying rate shown as percentage of the vehicle (*G*). $n = 17$ (vehicle), $n = 20$ (KBP-336), $n = 18$ (semaglutide), and $n = 14$ (KBP-336 + semaglutide) due to limited sample material. Statistical differences between groups were evaluated with Kruskal–Wallis test followed by Dunn's multiple comparisons test (*C*–*F*) or one-way ANOVA followed by Holm–Sidak's multiple comparisons test (*G*). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. vehicle unless otherwise indicated in the figure. All data are presented as means ± SE. ANOVA, analysis of variance.

loss and a clear benefit in terms of sustained weight management. Notably, the vehicle-treated rats started to lose weight as the hyperglycemia became more severe, resulting in a model reflecting severe diabetes during the second half of the treatment period. During this second phase of the study, the monotherapies lost their effects on body weight, which is comparable to observations in diabetic ZDF rats treated with KBP (25, 26, 29). The beneficial effect of combination therapy is in line with previous shorter preclinical studies combining KBP and semaglutide (28, 29) and supported by other preclinical studies highlighting the benefit of combining a DACRA and a GLP-1 analog for weight management (21, 42, 43). The sustained weight loss obtained by combination therapy was reflected in the visceral adiposity at the study end. Interestingly, the increased weight in the monotherapy groups was mainly reflected in the metabolic favorable subcutaneous adipose tissue (44), suggesting that the rats receiving monotherapy have a beneficial metabolic profile compared with the vehicle-treated rats despite the weight gain during the last phase of the treatment period. Visceral adipose tissue is, in contrast to subcutaneous adipose tissue, associated with insulin resistance and consequently type 2 diabetes (44, 45), which corresponds to the observed differences in insulin action between vehicle and active therapy and underscores that the treatments improve insulin sensitivity. Loss of lean mass, and thereby negative impact on energy expenditure, is a known concern during long-term weight loss, as the overall loss of lean mass as a function of

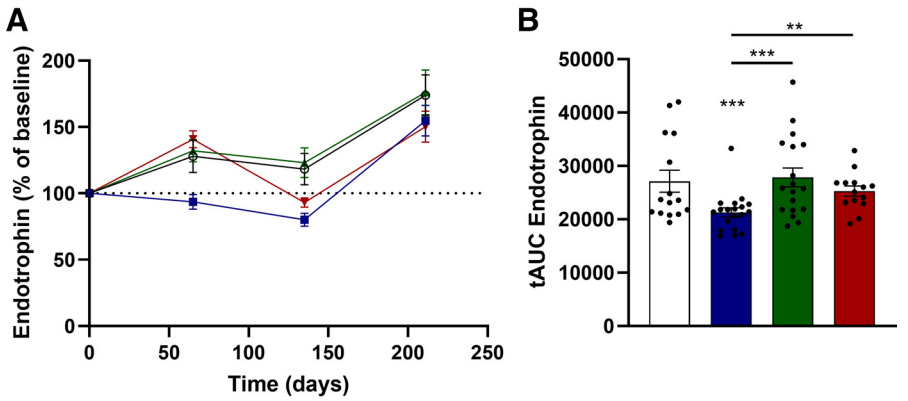

Figure 5. Serum levels of the extracellular remodeling biomarker endotrophin. Serum levels of endotrophin, measured by rPRO-C6, during the study shown as change from baseline (*A*) and the calculated total area under the curve (tAUC) (*B*). $n = 15$ (vehicle), $n = 19$ (KBP-336), $n = 18$ (semaglutide), and $n = 14$ (KBP-336 + semaglutide) due to limited sample material. Statistical differences between groups were evaluated with one-way ANOVA followed by Holm–Sidak's multiple comparisons test. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. vehicle unless otherwise indicated in the figure. All data are presented as means ± SE. ANOVA, analysis of variance.

weight loss accounts for up to 10–40% of the total weight loss (46–48). Clinical data show that semaglutide-induced weight loss leads to loss of lean mass; however, the impact on fat mass is markedly higher relative to the total body weight resulting in an improvement of the overall body composition (49–51). Furthermore, preclinical data have shown that KBP treatment increases lean body mass as a percentage of the total body mass (52). However, it is unknown whether the treatments affected lean mass in the present study.

The overall improvement in metabolic profile induced by all treatments was especially evident when evaluating the glucose control. In line with previous preclinical studies (28, 29), both monotherapies and the combination therapy resulted in stable and significant improvement of glucose control and tolerance. Although the significant weight loss obtained with combination therapy contributes to the additional effect on overall glucose control, the glucoregulatory benefit of both KBP-336 and semaglutide was sustained throughout the treatment period despite the weight regain, suggesting that the treatments have beneficial effects on glucose control independent of weight, as previously described with KBP treatment (25–27, 29). Interestingly, combination therapy resulted in significantly improved postprandial glucose tolerance with insulin levels similar to the vehicle, indicating a clear improvement in insulin sensitivity, as previously documented in preclinical KBP studies. Importantly, this effect was independent of weight loss (27). In addition, clinical studies using GLP-1 analogs show an improvement in insulin sensitivity, at least partly, obtained through weight loss (53, 54). A noteworthy observation was that the combination therapy resulted in a higher pancreatic insulin content compared with the monotherapies. Although this difference was not statistically significant, it indicates an additional benefit of combination therapy in long-term treatment. The beneficial effect on glucose control together with the preserved pancreatic insulin content could indicate preservation of beta-cell mass as suggested in previous preclinical studies using a DACRA (55) or a GLP-1 analog (56, 57). Overall, the present study highlights the benefits of combining a DACRA and a GLP-1 analog in the treatment of both obesity and type 2 diabetes as shown in clinical studies combining the DACRA cagrilintide and semaglutide (19, 20, 24). Notably, previous preclinical studies show that the DACRA KBP-336 has comparable or even superior anti-obesity and anti-diabetic treatment efficacies when compared with the DACRA cagrilintide (26). If this translates into a clinical setting, it indicates that KBP-336 could be a promising candidate for combination therapy with semaglutide or other incretin-based compounds treating obesity and type 2 diabetes.

Comorbidities of obesity and type 2 diabetes are the main reasons for increased morbidity and mortality, and long-term therapy should reduce the risk of these (58). As the rats in the present study were fed HFD, the diet likely has an impact on circulating levels of triglycerides, free fatty acids, and cholesterol as previously shown in ZDSD rats (59). As changes in these parameters, as well as inflammatory biomarkers, would mainly be driven by weight loss, the similar body weights at the study end complicate the interpretation of these parameters. Circulating levels of endotrophin, a collagen type VIa3 derived fragment, are known to predict a series of complications of the metabolic syndrome (60), and importantly, the mechanism behind this includes a strong relation to changes in insulin resistance (61). Our assessment of circulating endotrophin showed a beneficial response to KBP-336 and the combination group, while semaglutide did not reduce endotrophin levels, despite the improvements in the metabolic parameters. We speculate that the direct effect of KBP-336 on insulin sensitivity (27) underlies the suppression of endotrophin in this study, and as semaglutide does not directly improve insulin sensitivity, this explains the lack of change in this study. We speculate that the reductions elicited by KBP-336 and the combination groups are indicative of improvements in cardiovascular and renal complications, as these parameters are strongly predicted by endotrophin in patients with obesity and type 2 diabetic (33, 35–38), as well as in preclinical studies (39). Finally, the lack of reduction of endotrophin in the semaglutide group is most likely due to the specific rat model. In the AWARD-7 study, which compared insulin glargine to the GLP-1 analog dulaglutide in people with type 2 and chronic kidney disease, it was found that circulating endotrophin increased during the trial period in the insulin group while remaining stable in the dulaglutide group (62). In addition, it is well-established that semaglutide reduces overall cardiovascular and renal risks in both obesity and type 2 diabetes populations (63, 64).

To summarize, combination therapy using KBP-336 and semaglutide resulted in a significant and sustained weight loss superior to either monotherapy. All treatments resulted

in long-lasting improvement in glucose control and tolerance as well as insulin action, while KBP-336 reduced circulating levels of endotrophin, indicating an improvement of the overall disease stage as well as late complications. Altogether, KBP-336 is a promising candidate for the treatment of obesity and type 2 diabetes both alone and in combination with GLP-1 analogs.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The graphical abstract was created with BioRender.com.

GRANTS

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DISCLOSURES

M.A.K. and K.H. own stock in Nordic Bioscience A/S. All authors are employed by Nordic Bioscience A/S. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

A.T.L., K.E.M., and K.H. conceived and designed research; A.T.L. and K.E.M. performed experiments; A.T.L., K.E.M., and S.A.M. analyzed data; A.T.L., K.E.M., and K.H. interpreted results of experiments; A.T.L. and K.E.M. prepared figures; A.T.L. drafted manuscript; A.T.L., K.E.M., M.A.K., and K.H. edited and revised manuscript; A.T.L., K.E.M., S.A.M., M.A.K., and K.H. approved final version of manuscript.

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