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Dose Frequency Optimization of the Dual Amylin and Calcitonin Receptor Agonist KBP-088: Long-Lasting Improvement in Food Preference and Body Weight Loss[S]

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ABSTRACT

Dual amylin and calcitonin receptor agonists (DACRAs) are novel candidates for treatment of type 2 diabetes and obesity because of their beneficial effects on body weight, blood glucose, insulin sensitivity, and food preference, at least short-term. DACRAs activate the receptors for a prolonged time period, resulting in metabolic effects superior to those of amylin. Because of the prolonged receptor activation, different dosing intervals and, hence, less frequent receptor activation might change the efficacy of DACRA treatment in terms of weight loss and food preference. In this study, we compared daily dosing to dosing every other day with the aim of understanding the optimal balance between efficacy and tolerability. Obese and lean male Sprague-Dawley rats were treated with the DACRA KBP-088, applying two different dosing intervals (1.5 nmol/kg once daily and 3 nmol/kg every other day) to assess the effect on body weight, food intake, glucose tolerance, and food preference when given the choice between chow (13% fat) and a high-fat diet (60% fat). Treatment with KBP-088 induced significant weight loss, reduction in adiposity, improvement in glucose control, and altered food preference toward food that is less calorie-dense. KBP-088 dosed every other day (3 nmol/kg) was superior to KBP-088 once daily (1.5 nmol/kg) in terms of weight loss and improvement of food preference. The beneficial effects were evident in both lean and obese rats. Hence, dosing KBP-088 every other day positively affects overall efficacy on metabolic parameters regardless of the lean/obese state, suggesting that less-frequent dosing with KBP-088 could be feasible.

SIGNIFICANCE STATEMENT

Here, we show that food preference can be altered chronically toward choices that are less calorie-dense by pharmacological treatment. Further, pharmacological dosing regimens affect the efficacy differently, as dosing every other day improved body weight loss and alterations in food preference compared with daily dosing. This suggest that alterations of the dosing regimens could be feasible in the treatment of obesity.

Introduction

Potent dual amylin and calcitonin receptor agonists (DACRAs) are novel candidates for the treatment of type 2 diabetes and obesity because of their beneficial effects on body weight, blood glucose, and insulin sensitivity (Gydesen et al., 2016, 2017b; Hjuler et al., 2016, 2017). DACRAs activate not only the amylin receptor but also the calcitonin receptor (Andreassen et al., 2014a). Notably, compared with the natural ligands, DACRAs activate the receptors for a prolonged time period (Andreassen et al., 2014b; Gydesen et al., 2016), resulting in metabolic effects superior to those of amylin (Larsen et al., 2019), known effects of which include weight loss and reduced food intake (Mollet et al., 2004; Isaksson et al., 2005; Mack et al., 2010). The prolonged receptor activation suggests that different dosing intervals and, hence, less-frequent receptor activation might improve the efficacy of the DACRAs. A previous DACRA study investigating tolerability by dose escalation showed that each escalation step induced a transient suppression of food intake while reducing the magnitude over time. In addition, cumulative food intake was lower in the groups that were not dose-escalated, suggesting that the peptide is better tolerated when dose-escalated if using food intake as a surrogate measurement of tolerability (Gydesen et al., 2017a). The use of dose escalation to improve tolerability is also observed with pramlintide (Pullman et al., 2006; Aronne et al., 2007; Traina and Kane, 2011). Whether the balance between tolerability and efficacy of DACRAs can be optimized is not known.

One way to improve efficacy of weight loss therapy is to manipulate the voluntary food intake toward choices that are less calorie-dense. Hence, affecting food preference pharmacologically is of high interest. Amylin agonism has been shown to promote the consumption of a low-fat diet relative to a more calorie-dense diet, which adds an interesting aspect to the known weight-reducing effect (Eiden et al., 2002; Mack et al., 2007, 2010).

A previous 1-week study with the DACRA KBP-089 showed that treatment improved food preference toward a higher

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ABBREVIATIONS: CI, confidence interval; DACRA, dual amylin and calcitonin receptor agonist; HFD, high-fat diet; KBP, KeyBioscencePeptide; OGTT, oral glucose tolerance test.

accumulated chow consumption compared with vehicle when given the choice between chow and milk chocolate. When dissecting these data, it was evident that DACRA-treated rats tended to have a higher consumption of chow relative to chocolate during the first 3 days of treatment, when the food suppressive effect is most pronounced (unpublished data). This pattern is also observed in some (Mack et al., 2007) but not all (Mack et al., 2010) studies with amylin agonism when rats are given the choice between a low-fat chow diet and a high-fat diet (HFD) (Mack et al., 2007). These observations led to the hypothesis that a dosing regimen other than daily dosing may even further improve food preference. As DACRAs demonstrate superior metabolic effects compared with amylin, this may also apply to the food preference effect potentially even long-term. Today, most food preference studies using amylin or amylin agonists use HFD-fed rats because of the treatment-induced weight loss. However, obese individuals have altered plasma concentration of appetite regulatory hormones (Tschöp et al., 2001; Batterham et al., 2003; Steinert et al., 2017); thus, whether DACRA treatment improves food preference in a similar fashion in both obese and lean rats long-term is presently unknown.

In this study, we compared daily dosing to dosing every other day to characterize the tolerability and efficacy on weight, adiposity, and food intake of these dosing regimens. The long-term food preference effect of these regimens was investigated in obese as well as lean rats to investigate how the receptor activation frequency affects the preference for calorie-dense food.

Methods

Peptide Therapy. Synthetic KBP-088 (SynPeptide, Shanghai, China) was dissolved in saline (0.9%) for subcutaneous delivery. The dose range chosen for KBP-088 administration was based on previous studies using KBP-088 (Gydesen et al., 2016; Larsen et al., 2019) and other DACRAs (Hjuler et al., 2015, 2016, 2017; Gydesen et al., 2017a).

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). Animals were housed pairwise in enriched standard type IV cages under controlled temperatures (21–23°C, 55%–65% relative humidity) and a normal 12-hour light/dark cycle with ad libitum access to food and water.

Chronic Study. Twenty-four male Sprague-Dawley rats (Envigo, Venray, The Netherlands) were obtained at 5–6 weeks of age and were fed a 60 kcal% fat diet (#D12492, 5.24 kcal/g; ResearchDiets, New Brunswick, NJ) from arrival and throughout the study period. After 12 weeks on HFD, rats were allocated into treatment groups according to body weight (466 g b.wt. ± 33 S.D., $n = 8$ rats per treatment group). Rats were treated for 9 weeks by subcutaneous injections: vehicle (saline) or the DACRA KBP-088 in two different dosing intervals corresponding to a daily dose of 1.5 nmol/kg. KBP-088 was dosed either once daily (1.5 nmol/kg) or every other day (3 nmol/kg). Body weight and food intake were monitored daily throughout the study. After 3 and 8 weeks of treatment, oral glucose tolerance tests (OGTTs, 2 g/kg) were performed 24 hours postinjection of treatment. At the end of the study, rats were euthanized by exsanguination followed by dissection. Epididymal, perirenal, and subcutaneous inguinal fat depots were surgically removed and weighed.

Food Preference Studies. The effect of KBP-088 on diet preference was assessed in both lean and obese rats offered ad libitum access to normal chow (#5002, 3.11 kcal/g; LabDiet, St. Louis, MO) and 60 kcal% HFD (#D12492, 5.24 kcal/g; ResearchDiets). There were four groups in both studies: control (one diet choice, saline once daily), vehicle (two diet choices, saline once daily), KBP-088 once daily (two diet choices, 1.5 nmol/kg once daily), and KBP-088 every other day (two diet choices, 3 nmol/kg every other day).

In the obesity study, 48 male Sprague-Dawley rats (Envigo) were obtained at 5–6 weeks of age and were fed a 60 kcal% HFD (#D12492; ResearchDiets) for 11 weeks before the start of the experiment (455 g b.wt. ± 29 S.D.). The rats were acclimatized to both diets for 7 days before 6 weeks of treatment was initiated ($n = 12$ rats per treatment group), except the control group (saline), which only received HFD. Food intake and body weight were monitored daily throughout the study. An OGTT (2 g/kg) was performed after 5 weeks of treatment. At the end of the study, the rats were euthanized by exsanguination followed by dissection. Epididymal, perirenal, and subcutaneous inguinal fat depots were surgically removed and weighed.

In the lean study, 48 male Sprague-Dawley rats (Envigo) were obtained at 12 weeks of age and were fed normal chow (#5002; LabDiet) 5 weeks before the start of the experiment (369 g b.wt. ± 15 S.D.). The rats were acclimatized to both diets (chow and HFD) for 2 weeks before 5 weeks of treatment was initiated ($n = 12$ rats per treatment group). The control group (saline) had access to chow but not HFD. Food intake and body weight were monitored daily throughout the study. An OGTT (2 g/kg) was performed at the end of the study, and animals were euthanized by exsanguination followed by dissection. Epididymal, perirenal, and subcutaneous inguinal fat depots were surgically removed and weighed.

Oral Glucose Tolerance Test. OGTTs were performed in overnight-fasted rats. A glucose (2 g/kg) bolus (Sigma-Aldrich, Copenhagen, Denmark) was administered by oral gavage (4 ml/kg) at time 0. EDTA blood samples were collected from the tail vein before glucose challenge (0 minutes) and 15, 30, 60, and 120 minutes post–glucose challenge. Blood glucose was monitored at time 0, 15, 30, 60, 120, and 180 minutes post–glucose challenge.

Biochemical Analysis. Blood samples were collected in EDTA tubes and centrifuged at 5000 rpm for 10 minutes at 4°C, and 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) were analyzed according to the manufacturer's instructions.

Statistical Analysis. All analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA). Gaussian distribution was evaluated with the Shapiro-Wilk test, with the exception of food intake, to which D'Agostino-Pearson normality test was applied because of a low n (two rats per cage). Group differences were assessed using one-way ANOVA followed by Tukey's post hoc test for multiple comparison of parametric data. For nonparametric data, Kruskal-Wallis test with Dunn's post hoc test was applied. All data are represented with 95% confidence intervals (CI), except body weight data at randomization, which are described with S.D. A value of $P < 0.05$ was considered statistically significant.

Results

Dosing Every Other Day Improves Body Weight Loss Compared with Daily Dosing. Treatment with KBP-088 once daily (1.5 nmol/kg) and every other day (3 nmol/kg) for 9 weeks resulted in reduced food intake, albeit by two different patterns: KBP-088 once daily transiently reduced food intake, which normalized within the first 10 days of treatment, whereas KBP-088 every other day resulted in fluctuating food intake throughout the study (Fig. 1B). The fluctuations reflected the dosing intervals as a marked suppression of food intake after dosing that normalized on nondosing days (Fig. 1B). Despite the different food intake patterns, KBP-088 once daily and every other day had similar accumulated food intake, although KBP-088 treatment every other day

tended to be lower than once daily (Fig. 1D). In line with the food intake, KBP-088 every other day resulted in fluctuations in body weight throughout the study (Fig. 1A). Interestingly, KBP-088 every other day resulted in significantly more weight loss than KBP-088 once daily at the end of the study, although both treatments caused significant weight loss compared with vehicle (Fig. 1C). At the end of the study, adipose tissues were isolated and weighed. Both treatment groups significantly reduced the inguinal, epididymal, and perirenal adipose tissue depots compared with vehicle. In conjugation with the

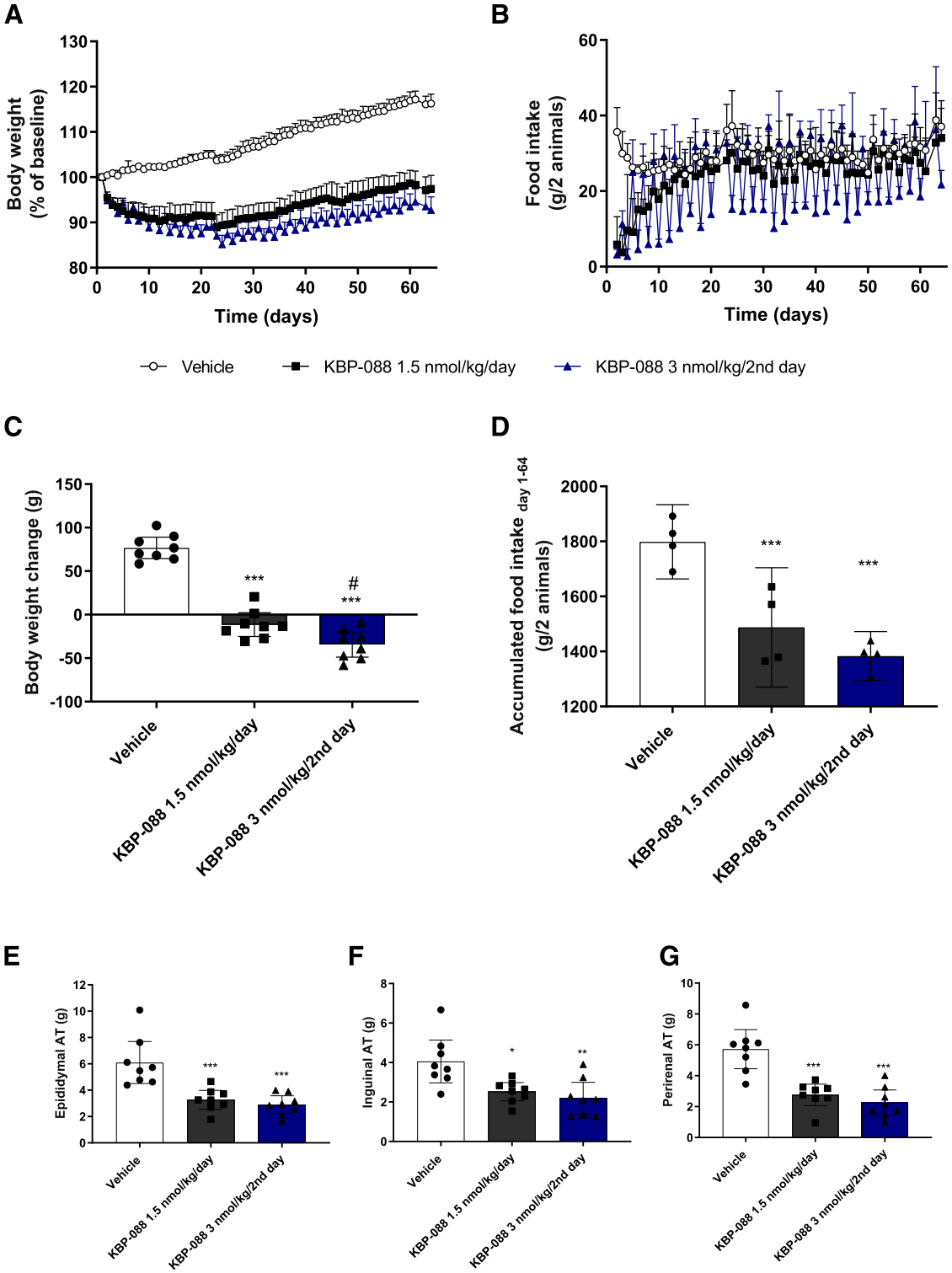

Fig. 1. Body weight as a percentage of initial body weight (A) and food intake (B) during the study. Body weight change at study end (C) and accumulated food intake during the entire study (D). Weights of epididymal (E), inguinal (F), and perirenal (G) adipose tissue (AT) depots at study end. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle and $^{\#}P < 0.05$ compared with 1.5 nmol/kg KBP-088. $n = 8$ rats per treatment group. Data are shown as means with 95% CI.

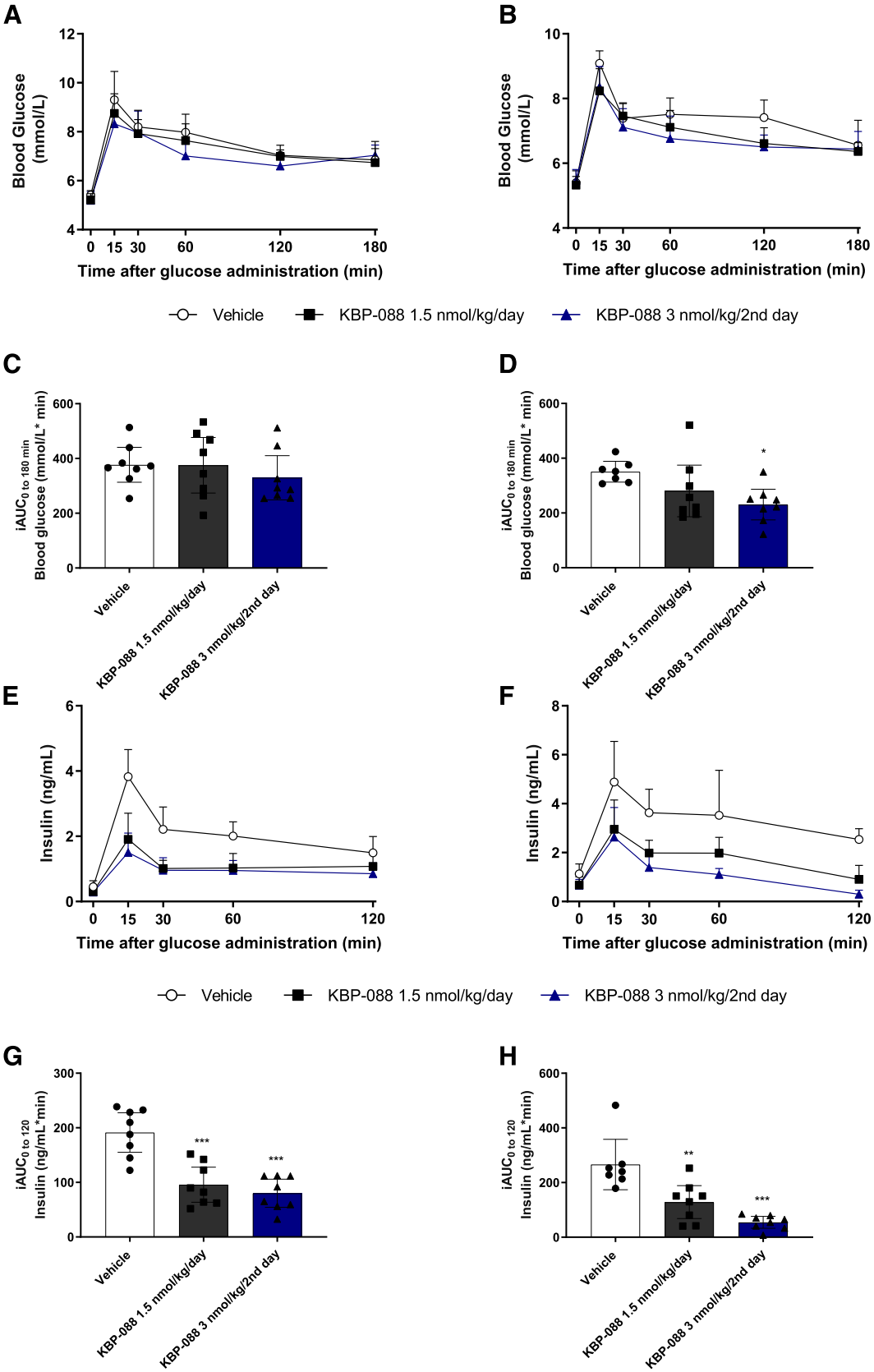

Fig. 2. Plasma glucose (A and B) and insulin (E and F) during OGTT after 3 and 8 weeks of treatment, respectively. The incremental area under the curve (iAUC) shown for glucose (C and D) and insulin (G and H) during OGTT after 3 and 8 weeks of treatment, respectively. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle. $n = 8$ rats per treatment group. Data are shown as means with 95% CI.

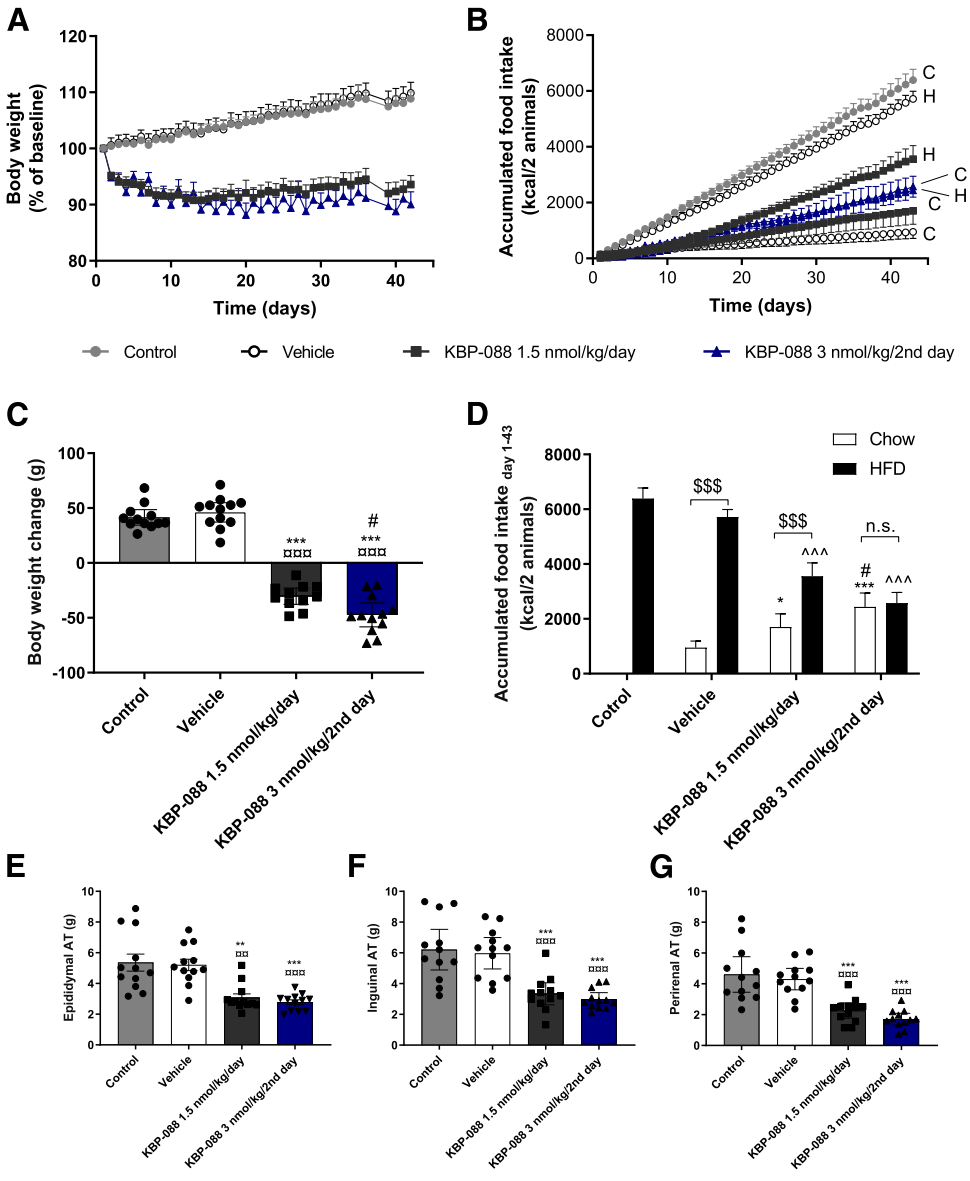

Fig. 3. HFD-fed rats. Body weight as a percentage of initial body weight (A) and accumulated food intake (B) during the study: "H" refers to kcal obtained from HFD, and "C" refers to kcal obtained from chow diet. Body weight change (C) and accumulated food intake at study end (D). Weights of epididymal (E), inguinal (F), and perirenal (G) adipose tissue (AT) depots at study end. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Epididymal AT data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle chow. $^{\wedge\wedge\wedge}P < 0.001$ compared with vehicle HFD. $^{\square\square}P < 0.01$; $^{\square\square\square}P < 0.001$ compared with control. $^{\#}P < 0.05$ compared with 1.5 nmol/kg KBP-088 and \$\$\$ compared with chow. n = 12 rats per treatment group. Data are shown as means with 95% CI. n.s. - not significant.

body weight loss at the end of the study, the group dosed with KBP-088 every other day tended to lose more adipose tissue, albeit the difference between the treatment groups was small and nonsignificant (Fig. 1, E–G).

The food preference studies in lean and obese rats, which used the same once-daily and every-other-day dosing regimen, strongly supported the findings described above, although these studies lasted for 5 and 6 weeks, respectively (Fig. 3, A, C, and E–G; Fig. 5, A, C, and E–G for the obese and lean study, respectively).

Dosing with KBP-088 Every Other Day Further Improves Oral Glucose Tolerance Compared with Daily Dosing. In the 9-week study, OGTTs were performed after 3 and 8 weeks of treatment, 24 hours postdosing of the rats. After 3 weeks of treatment, KBP-088 once daily (1.5 nmol/kg) and every other day (3 nmol/kg) significantly reduced insulin levels, whereas blood glucose was comparable to vehicle levels (Fig. 2, A, C, E, and G). After 8 weeks of treatment, the effect was more pronounced. Blood glucose was significantly lowered with KBP-088 every other day but not once-daily treatment, although both treatments significantly reduced insulin levels (Fig. 2, B, D, F, and H).

The OGTTs performed in the two food preference studies in obese and lean rats corroborated the significant improvement in glucose tolerance, which was mainly mediated by a reduction in insulin levels (Figs. 4 and 6).

KBP-088 Induces a Long-Lasting Improvement in Food Preference. The aim of the food preference studies was to determine whether KBP-088 induces long-lasting changes in food preference and whether KBP-088 dosing intervals (1.5 nmol/kg once daily and 3 nmol/kg every other day) influenced food preference differently. Furthermore, we investigated this in both HFD-fed obese and chow-fed lean rats to shed light on potential differences due to body weight.

In both obese and lean rats, the accumulated chow intake was significantly higher in the KBP-088–treated groups compared with vehicle, whereas HFD intake was significantly lower (Fig. 3, B and D; Fig. 5, B and D). This suggested an altered food preference toward the choice that was less calorie-dense, which lasted throughout the study. Interestingly,

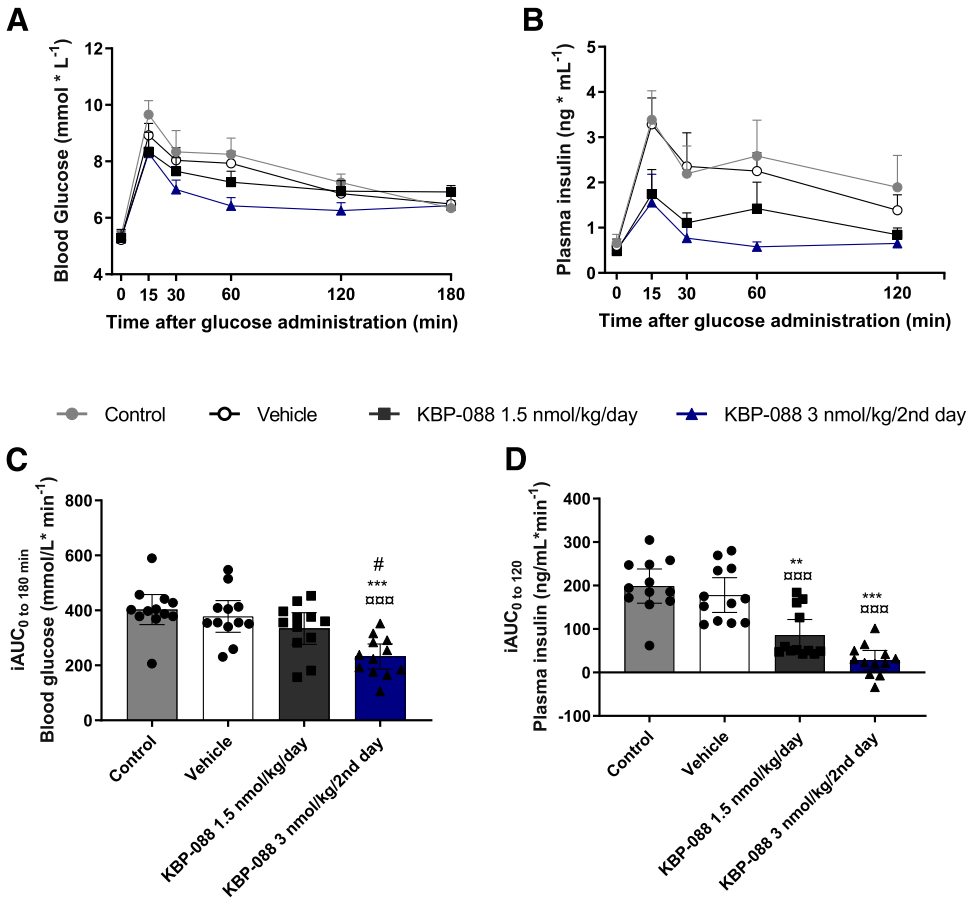

Fig. 4. HFD-fed rats. Plasma glucose (A) and insulin (B) during OGTT after 5 weeks of treatment. The incremental area under the curve (iAUC) shown for glucose (C) and insulin (D) during OGTT. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle. $^{¤¤¤}P < 0.001$ compared with control. $^{\#}P < 0.05$ compared with 1.5 nmol/kg KBP-088. $n = 12$ rats per treatment group. Data are shown as means with 95% CI.

KBP-088 every other day resulted in a significantly higher chow intake compared with KBP-088 once daily, although the total caloric intake did not differ significantly between the two treatment groups (Fig. 3D; Fig. 5D). This applied to both the obese and lean rats. In addition, treatment with KBP-088 every other day resulted in equal consumption of HFD and chow, whereas vehicle and KBP-088 once-daily groups had a significantly higher caloric intake of HFD compared with chow (Fig. 3D; Fig. 5D). Again, this observation was valid in both the obese and lean rats.

Discussion

In this article, we demonstrate that dosing KBP-088 every other day positively affects efficacy and improves food preference to an even greater extent than dosing once daily.

All three studies showed that treatment with KBP-088 once daily (1.5 nmol/kg) and every other day (3 nmol/kg) resulted in an initial reduction in food intake. Whereas KBP-088 once daily resulted in a transient reduction in food intake, KBP-088 every other day resulted in fluctuating food intake throughout the study, reflecting the dosing intervals. Fluctuations in food intake and body weight have previously been observed with the DACRA KBP-089 when dosed every other day (Gydesen et al., 2017a). The continuous fluctuations in food intake suggest that a longer rest period between receptor reactivation results in a greater response upon redosing. Marked reductions in food intake might be a sign of nausea and, hence, poor tolerability of the treatment. Lack of tolerability, which can lead to side effects, is experienced in some studies using amylin analogs. One of the most common side effects of the amylin analog pramlintide is transient nausea, which is experienced by both diabetic and obese individuals Aronne et al., 2007. Increased nausea was presumably not a problem in the present studies, as the applied doses of KBP-088 did not induce pica behavior when measured by kaolin consumption (Supplementary Data). Similar results have been observed with another DACRA, KBP-042, in which only a very high dose (50 μg/kg ≈ 15 nmol/kg) induced pica behavior (Hjuler et al., 2016). In addition, studies have shown that an amylin-induced reduction in food intake is not related to pica behavior or conditioned taste aversion (Lutz et al., 1995; Rushing et al., 2002; Mack et al., 2007). These observations suggest that significant reductions in food intake are not related to nausea or conditioned taste aversion in rats, although clinical data may contradict this.

The present data show that dosing every other day positively affects the KBP-088–induced body weight loss, albeit adipose depots did not differ significantly between rats dosed once daily and every other day. This may indicate that dosing every other day results in loss of lean mass in contrast to once daily (Gydesen et al., 2017b), although some observations contradict this. First, all three studies in obese and lean rats demonstrated the same trend: adipose tissues of rats treated every other day were smaller than that of rats treated once daily, with one exception favoring dosing every other day. We acknowledge that the difference is very small and statistically nonsignificant; however, the fact that the observation is

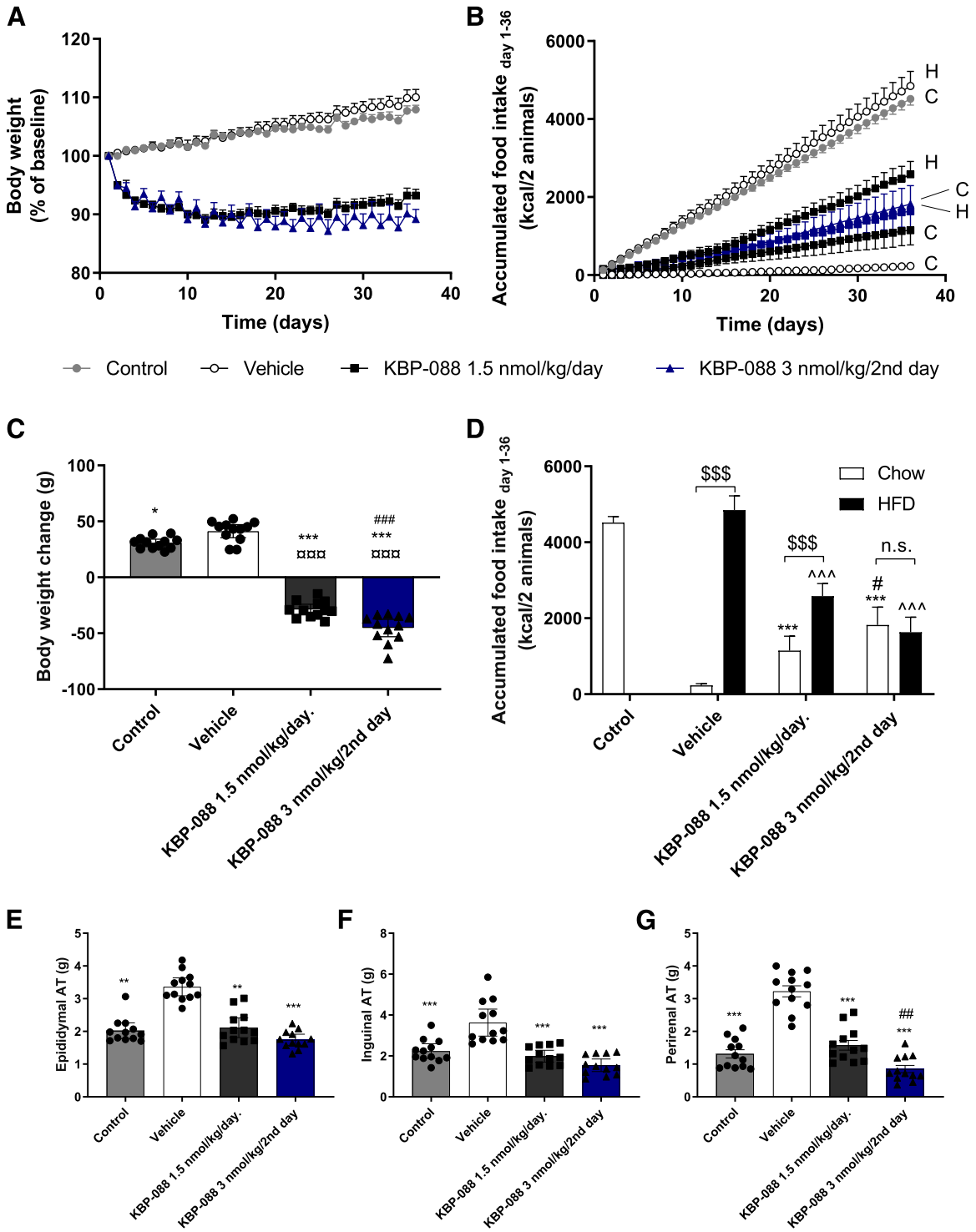

Fig. 5. Lean rats. Body weight as percentage of initial body weight (A) and accumulated food intake (B) during the study: "H" refers to kcal obtained from HFD, and "C" refers to kcal obtained from chow diet. Body weight change (C) and accumulated food intake at study end (D). Weights of epididymal (E), inguinal (F), and perirenal (G) adipose tissue (AT) depots at study end. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Epididymal AT data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with vehicle chow. $^{\wedge\wedge\wedge}P < 0.0001$ compared with vehicle HFD. $^{¤¤¤}P < 0.001$ compared with control. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ compared with 1.5 nmol/kg KBP-088, and $$$ compared with chow. n = 12 rats per treatment group. Data are shown as means with 95% CI. n.s. - not significant.

evident in all three studies strengthens its validity. Second, the oral glucose tolerance tests showed that KBP-088 every other day improved glucose tolerance even further than KBP-088 once daily. Given that insulin sensitivity is strongly correlated to body weight and lean mass (Tuomilehto et al., 2001; McAuley et al., 2002; Fukushima et al., 2016; Kim and Park, 2018), this suggests that dosing every other day does not compromise lean mass. Previous studies with DACRAs have shown that higher doses (up to 500 μg/kg ≈ 150 nmol/kg) do not improve insulin sensitivity in obese nondiabetic rats

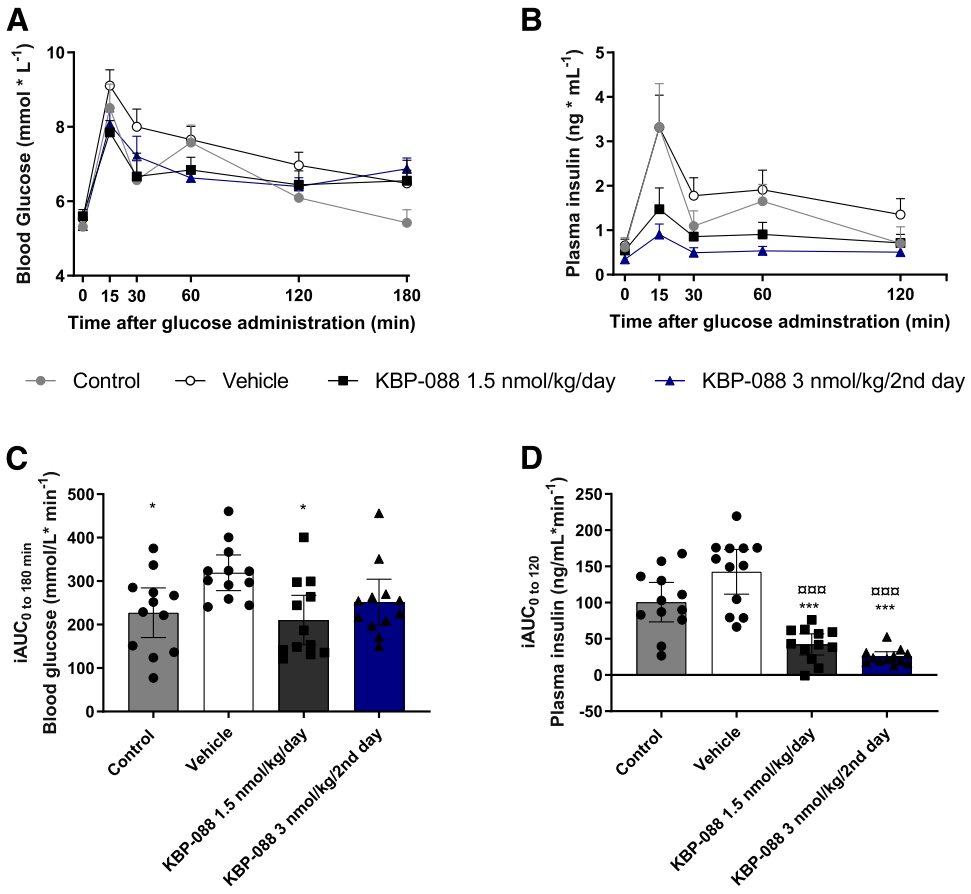

Fig. 6. Lean rats. Plasma glucose (A) and insulin (B) during OGTT performed at study end. The incremental area under the curve (iAUC) shown for glucose (C) and insulin (D) during OGTT. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. $^{*}P < 0.05$; $^{***}P < 0.001$ compared with vehicle. $^{¤¤¤}P < 0.001$ compared with control. n = 12 rats per treatment group. Data are shown as means with 95% CI.

(Hjuler et al., 2016; Gydesen et al., 2017a; Sonne et al., 2020). This indicates that weight loss rather than dose concentration drives the improved insulin sensitivity observed with the every-other-day dosing regimen and that the weight loss is not at the expense of lean mass. Again, this underlines the impact of the longer rest period between receptor reactivation applied with dosing every other day compared with once daily. All OGTTs were performed with 24-hour predosing, and hence, the observed difference between KBP-088 every other day and once daily is unlikely to be due to acute effects.

One way to achieve weight loss is to manipulate the voluntary food consumption toward choices that are less calorie-dense. Amylin agonism is known to affect the feeding pattern in this manner, along with improvement in appetite control (Aronne et al., 2007; Mack et al., 2007; Smith et al., 2007). The DACRA KBP-089 has been shown to improve food preference in rats in a 1-week study offering milk chocolate and chow ad libitum (Gydesen et al., 2017b). However, the long-term effects on food preference were not investigated in that study, and further, taste preference (the sweetness of chocolate) rather than calorie density might have been the focus. Consequently, we attempted to switch the focus to the extent possible to calorie density (HFD vs. chow) rather than taste preference (chocolate vs. chow). In the present study we show that KBP-088 induces a sustained improvement in food preference in terms of a higher intake of food that is less calorie-dense in both lean and obese rats given ad libitum access to chow and HFD. This underlines that the improved food preference is independent of the lean/obese state and, thereby, potential differences in appetite regulatory hormones (Tschöp et al., 2001; Batterham et al., 2003; Steinert et al., 2017). Interestingly, KBP-088 every other day further increased intake of less-calorie-dense food at the expense of high-calorie-dense food compared with dosing once daily, indicating that dose frequency plays an important role in terms of food preference. With the improvement follows a change in dietary macronutrient consumption (chow vs. HFD) between rats dosed every other day and once daily, which may theoretically cause the observed differences in weight loss and insulin sensitivity. However, studies collectively demonstrate that different dietary macronutrient compositions have no effect on body weight and insulin sensitivity regardless of the diet being eucaloric (with a fixed and identical caloric intake between groups) (Lewis et al., 2013; Berryman et al., 2018; Lundsgaard et al., 2019), hypocaloric (de Luis et al., 2012; Wang et al., 2013), or hypercaloric (Mauler et al., 2009; Draznin et al., 2012; Iggman et al., 2014). Based on these findings, it seems unlikely that dietary macronutrient composition is involved in the differences observed between rats dosed every other day and once daily.

Alhough the mechanism facilitating the improved food preference is unknown, we speculate that KBP-088 acts centrally, as amylin agonism is known to affect dopamine release in the hypothalamus (Brunetti et al., 2002), which affects the feeding patterns (Szczypka et al., 1999, 2000). This is supported by the observation that the DACRA salmon calcitonin reduces fat intake and to some extent sucrose intake possibly via the ventral tegmental area and/or the area

postrema (Mietlicki-Baase et al., 2017; Whiting et al., 2017) and has been suggested to be involved in blocking the reward mechanism normally induced by alcohol (Kalafateli et al., 2019). Hence, it is likely that KBP-088 influences food preference via central mechanisms. More studies are required before this question may be answered.

The secretion of satiety hormones may change due to the lean/obese state, as is the case with leptin (Jenkins et al., 2012; Singh et al., 2016; Hausmann et al., 2019; Unamuno et al., 2019). The beneficial interplay between amylin agonism and leptin is well described (Roth et al., 2008; Trevaskis et al., 2008, 2010; Turek et al., 2010; Seth et al., 2011; Moon et al., 2012; Duffy et al., 2018) and supports that KBP treatment lowers endogenous leptin, suggesting attenuation of leptin resistance (Andreassen et al., 2014a; Hjuler et al., 2015, 2016). Other hormones, such as cortisol, which is secreted during stress and nausea but also induces hyperphagia, are likewise altered in response to DACRA treatment. Both in healthy individuals (Žofková et al., 1987; Laurian et al., 1988; Trainer et al., 1991) and patients who had undergone hip surgery (Gabopoulou et al., 2002), cortisol increased shortly after administration of the DACRA salmon calcitonin. However, chronic treatment seems to have the opposite effect in a preclinical setting with diabetic rats (Adeyemi and Olayaki, 2018). Consequently, cortisol levels may increase with acute treatment but diminish over time as tolerability improves and food intake normalizes. Based on the known DACRA-induced food suppressive effect (Wielinga et al., 2007; Feigh et al., 2011; Andreassen et al., 2014a; Braegger et al., 2014), the increase in cortisol is likely a stress response rather than an orexigenic response. Although KBP treatment does not induce pica behavior in rats (Supplementary Data and Hjuler et al., 2016), we may in this context use food suppression as a surrogate for nausea in humans and, most importantly, as an indication of increased cortisol. Dosing KBP-088 once daily would then likely result in a transient increase in cortisol, which would return to vehicle levels as food intake normalized or possibly go lower with reference to Adeyemi and Olayaki (2018). On the contrary, dosing every other day, which maintains a fluctuating food intake, would theoretically translate to similarly fluctuating cortisol levels. Unfortunately, we did not sample our studies to investigate this matter.

In conclusion, chronic KBP-088 treatment improves food preference in terms of a higher intake of food that is less calorie-dense in both lean and obese rats. Importantly, these positive effects on food preference were maintained throughout the study period. In addition, we show that dosing KBP-088 every other day further improves food preference and positively affects the efficacy on metabolic parameters overall, suggesting that less-frequent dosing with KBP-088 could be feasible. How continual fluctuating food intake and body weight affect tolerability is still unclear.

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Authorship Contributions

Participated in research design: Larsen, Sonne, Karsdal, Henriksen.

Conducted experiments: Larsen, Sonne.

Performed data analysis: Larsen, Sonne, Andreassen.

Wrote or contributed to the writing of the manuscript: Larsen, Sonne, Henriksen.

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