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The Dual Amylin- and Calcitonin-Receptor Agonist KBP-042 Increases Insulin Sensitivity and Induces Weight Loss in Rats with Obesity

Sara Toftegaard Hjuler[1], Sofie Gydesen[1], Kim Vietz Andreassen[1], Steffen Lund Kjær Pedersen[1], Lars I. Hellgren[2], Morten Asser Karsdal[1], and Kim Henriksen[1]

Objective: In this study, KBP-042, a dual amylin- and calcitonin-receptor agonist, was investigated as a treatment of obesity and insulin resistance in five different doses (0.625 µg/kg–10 µg/kg) compared with saline-treated and pair-fed controls.

Methods: Rats with obesity received daily s.c. administrations for 56 days, and glucose tolerance was assessed after one acute injection, 3 weeks of treatment, and again after 7 weeks of treatment. To assess the effect on insulin sensitivity, rats received 5 µg/kg KBP-042 for 21 days before hyperinsuline-mic–euglycemic clamp.

Results: KBP-042 induced a sustained weight loss of up to 20% without any significant weight reduction in the pair-fed groups. Decreases in adipose tissues and lipid deposition in the liver were observed, while plasma adiponectin was increased and plasma leptin levels were decreased. Acute administration of KBP-042 led to impaired glucose tolerance and increased plasma lactate, while this diabetogenic effect was reversed by chronic treatment. Finally, assessment of insulin sensitivity using the hyperinsulinemic–euglycemic clamp showed that KBP-042 increased the glucose infusion rate.

Conclusions: The study indicates that KBP-042 combines two highly relevant features, namely weight loss and insulin sensitivity, and is thus an excellent candidate for chronic treatment of obesity and insulin resistance.

Introduction

Obesity is one of the greatest public health challenges of the 21st century (1). Obesity can lead to insulin resistance and type 2 diabetes (2), which are associated with a range of metabolic dysfunctions (3,4). Weight loss, improved glycemic control, and increased insulin action to reduce strain on the β cells are key points for improving disease status. This can be achieved by different interventions (exercise, diet, medication, surgery) which all cause improvements in metabolic profiles and increase of insulin sensitivity and β-cell function (5,6). However, as lifestyle changes often result in only minor weight reductions followed by a rapid regain of weight (7), there is a need for treatments targeting multiple factors of the obesity-related diseases. These include insulin resistance and β-cell failure to avoid development of type 2 diabetes, as well as diabetic complications.

Activation of amylin receptors has already been linked with reduction of food intake (8), increased responsiveness to leptin (9-11), weight loss (12,13), and indications of increased energy expenditure (11,13-16). However, amylin is a short-lasting agonist *in vivo*, and there is a need for improved ligands. KBP-042 is a dual amylin- and calcitonin-receptor agonist with highly potent antiobesity and antidiabetic effects (17), although a long-term chronic treatment has not yet been tested.

In this study, KBP-042 was tested in a long-term treatment of prediabetic rats with obesity, in order to evaluate KBP-042's potential as a chronic treatment of obesity. We further examined whether the beneficial effects on glucose homeostasis were maintained throughout the study, and finally we investigated whether treatment with KBP-042 could increase insulin sensitivity and reduce hepatic steatosis.

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Methods

Peptide therapy

Recombinant KBP-042 peptide (Unigene Laboratories, Boonton, NJ) was dissolved in saline for subcutaneous (s.c.) delivery. The doses for KBP-042 administration in the studies were based on previous studies in animal models of obesity and type 2 diabetes and ranged from 10 μg/kg to 0.625 μg/kg (∼2.87–0.18 nmol/kg/day) (17,18).

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). All male Sprague Dawley rats were obtained at 6 weeks of age and housed under controlled temperature (20°C ± 2) on a normal 12-h light–dark cycle with *ad libitum* access to water and food. Normal diet control rats (ND) were fed rodent chow (5002, LabDiet, St. Louis, MO) and high-fat diet (HFD) rats a 60% fat kcal diet (#D12495, Research Diets Inc., NJ). After 10 weeks of high-fat feeding rats were assigned into groups ($n = 10$) and controlled for equal mean body weight.

Acute study. Food was removed in the afternoon (4 p.m.). After 16 to 18 h of fasting an oral glucose tolerance test (OGTT) was performed. Rats received a single dose of saline (vehicle) or peptide (10 μg/kg, 5 μg/kg, 2.5 μg/kg, 1.25 μg/kg, 0.625 μg/kg). After 30 min, a glucose bolus (2 g/kg, Sigma-Aldrich, Copenhagen, Denmark) was administered by oral gavage. Blood glucose was monitored by Accu-Check® Avia monitoring system (Roche Diagnostics, Rotkreuz, Switzerland) and EDTA-plasma was obtained from the lateral tail vein at $t = 0, 15, 30, 60,$ and 120 min.

Pica test. Fasted animals were administered s.c. with 5, 10, or 50 μg/kg KBP-042 or vehicle (saline). After dosing, animals had free access to normal chow or kaolin pellets (5TBP, Test diet, MO) and food and kaolin intake was monitored after 4 and 24 h.

Chronic study. Each rat was dosed once daily with either saline (vehicle, pair-fed 5 μg/kg, pair-fed 10 μg/kg) or KBP-042 (10 μg/kg, 5 μg/kg, 2.5 μg/kg, 1.25 μg/kg, 0.625 μg/kg) in the afternoon for 8 weeks. The two pair-fed groups were food restricted to match the daily food intake of their corresponding treatment groups (5 μg/kg or 10 μg/kg). Pair-fed animals received an average of the daily intake of their treated paired group every day in the afternoon. Food intake and body weight were monitored daily for the first 6 days, then weekly. OGTT, performed as in the acute study and intravenous glucose tolerance tests (IVGTT) were performed after 3 and 7 weeks of treatment. IVGTT was performed in the morning after 18 h of fasting. Each rat received a single dose of either saline (vehicle, pair-fed 5 μg/kg, pair-fed 10 μg/kg) or peptide (10 μg/kg, 5 μg/kg, 2.5 μg/kg, 1.25 μg/kg, 0.625 μg/kg), after 30 min glucose (0.5 g/kg, Sigma-Aldrich, Copenhagen, Denmark) was administered in the lateral tail vein and blood glucose was monitored and EDTA-plasma was obtained at $t = 0, 5, 15, 30, 60,$ and 120 min, as described above. To assess effect on gastric emptying, overnight-fasted rats received s.c. KBP-042 injection, were administered 40 mg/kg acetaminophen by oral gavage (4 mL/kg) after 30 min and the appearance of acetaminophen in plasma was monitored (19). Blood was collected 30 min after administration from the tail vein and acetaminophen levels were measured in EDTA-plasma (Acetaminophen Direct ELISA Kit, Immuneanalysis, Pomona, CA). Gastric emptying was calculated as % change relative to ND rats.

After 8 weeks, EDTA-Aprotinin plasma samples were collected for hormonal analyses after 3 h fasting. Animals were euthanized under isoflurane inhalation followed by exsanguination. Excised tissue was snap-frozen in liquid nitrogen and stored at −80°C, and plasma was stored at −20°C samples until further analysis.

Hyperinsulinemic–euglycemic clamp

Insulin-mediated whole body glucose uptake was estimated in rats fed either HFD or ND (as described above). The HFD rats were stratified into HFD vehicle or HFD-KBP-042 groups ($n = 5$–7). ND vehicle and HFD vehicle rats received saline injections while HFD-KBP-042 received 5 μg/kg of KBP-042 s.c. for 21 days. After the treatment period, animals were subjected to a hyperinsulinemic–euglycemic clamp experiment explained in details in the Supporting Information.

Plasma analysis

Plasma levels of lactate (L-lactate colorimetric assay, Abcam, Cambridge, UK), insulin (Mercodia Rat Insulin ELISA, Mercodia AB, Uppsala, Sweden), leptin (Rat Leptin ELISA, Millipore Corporation, Billerica, MA), glucose-dependent insulinotropic peptide (GIP) (Rat/Mouse GIP (Total) ELISA, Merck Millipore, Billerica, MA), and adiponectin (Rat Adiponectin ELISA, Millipore Corporation, Billerica, MA) were analyzed according to manufacturer's instruction.

Tissue analysis

Lipids were extracted from liver samples with addition of internal standards and triacylglycerol (TAG) was isolated from the total lipid extract using aminopropyl solid-phase extraction cartridges, *trans*-methylated, and quantified using Gas Chromatography–Flame Ionization Detector as previously described (20).

Statistical analysis

Data were statistically analyzed by one-way ANOVA multiple comparison followed by Tukey's test. In Supporting Information Table S1, ND controls were compared with HFD vehicle using Student's *t*-test. Values of $P < 0.05$ were considered to be significant.

Results

KBP-042 mediated substantial and sustained reductions in body weight

The baseline characteristics of HFD rats and lean controls confirmed the obese and prediabetic status of the HFD rats (Supporting Information Table S1).

After treatment with KBP-042 for 8 weeks, a dose-dependent and sustained reduction of body weight was observed. A large weight loss was observed in the initial phase of the study (Figure 1A, B) in the three highest treatment groups (2.5 μg/kg, 5 μg/kg, and 10 μg/kg), as well as the two corresponding pair-fed groups (pair-fed 5 μg/kg and pair-fed 10 μg/kg). This corresponds well with the large reduction in food intake in the first 6 days of treatment (Figure 1D). Due to the drastic reduction in food intake, pica behavior was tested as a surrogate for nausea in rats. The two highest doses, 5 and 10 μg/kg KBP-042 did not give rise to kaolin intake whereas a high dose of KBP-042 not used in this study (50 μg/kg) provoked pica behavior
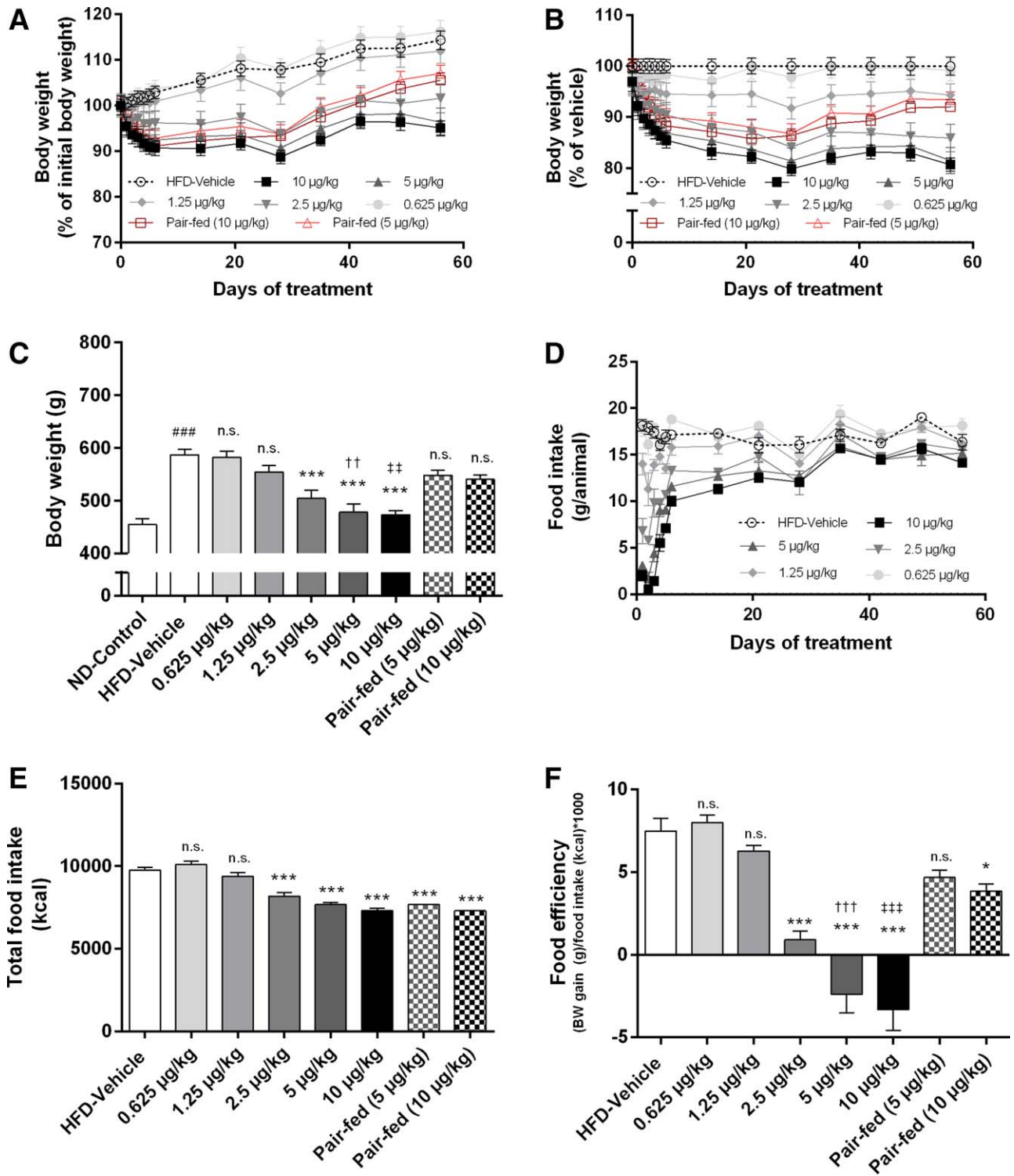
Figure 1 (**A**) Body weight progression in % of initial body weight during the study from randomization at day 0 to last day of treatment, day 56. (**B**) Vehicle-corrected body weights. (**C**) End point body weights. (**D**) Food intake of all treatment groups during the entire study. Food intake was monitored every day for the first 6 days followed by weekly monitoring. Pair-fed groups were fed the same as the average for their corresponding treatment group (5 µg/kg or 10 µg/kg). (**E**) Accumulated food intake for the entire duration of the study expressed in kcal/2 animals. (**F**) Calculated food efficiency. n = 10 for all groups except vehicle (n = 12). Statistical analysis between groups for panels C, E, and F performed as a one-way ANOVA followed by Tukey's post hoc test with the following annotations: ###$P < 0.001$ vs. normal diet control (ND). *$P < 0.05$, ***$P < 0.001$ vs. high-fat diet (HFD) vehicle. ††$P < 0.01$, †††$P < 0.001$ vs. pair-fed 5 µg/kg. ‡‡$P < 0.01$, ‡‡‡$P < 0.001$ vs. pair-fed 10 µg/kg. Data are expressed as mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(Supporting Information Figure S1). After the transient reduction in feeding, food intake increased during the study. The pair-fed groups gained weight again after feeding increased; inversely, treatment with KBP-042 sustained the initial weight reduction throughout the 56 days, with significant reductions in the 2.5 µg/kg, 5 µg/kg, and 10 µg/kg groups compared with the HFD vehicle (Figure 1C). The accumulated food intake corresponds well with the weight change for the three highest treatment groups (2.5 µg/kg, 5 µg/kg, and 10 µg/kg) (Figure 1E), although the pair-fed groups which received the same amount of food as their corresponding treatment group did not lose significant weight. Accordingly, treatment with 2.5, 5, and 10 µg/kg KBP-042 resulted in drastic and significant reduction in food efficiency compared with pair-fed (Figure 1F), suggesting increased energy expenditure.

KBP-042 reduced adipose tissue and ectopic lipid accumulation

After treatment three different adipose tissues were isolated and as seen in Figure 2A–C, the weights of isolated epididymal and perirenal adipose tissues were significantly reduced after treatment with 10 µg/kg of KBP-042. The perirenal adipose tissue in the 2.5, 5, and 10 µg/kg groups was reduced significantly while inguinal fat was not. The same reduction was not seen in the pair-fed controls.

Lipid accumulation in liver was assessed as hepatic TAG concentration (Figure 2D). As expected the HFD vehicle group had dramatically higher TAG levels compared with ND group. This accumulation was significantly reduced after treatment with KBP-042 (10 µg/kg), while the corresponding pair-fed control group did not show a significant reduction in liver TAG. In order to assess the treatment effect on fatty acid metabolism in selective ways (e.g. metabolism of saturated vs. monounsaturated vs. polyunsaturated), the fatty acid composition of hepatic TAG was analyzed. The results showed that there was no difference in the relative distribution, i.e., the treatment caused a general reduction in TAG without effecting the metabolism of specific fatty acid types (Supporting Information Table S2).

Finally, adiponectin and leptin levels were measured after 56 days of treatment (Figure 2E, F). Adiponectin was significantly increased in response to treatment with all doses of KBP-042 except 0.625 µg/kg. For plasma leptin a statistically significant reduction was seen when comparing 10 µg/kg KBP-042 with the corresponding pair-fed control.

In summary, fat depots, lipid, and adipokine data support a strongly improved metabolic status as a function of treatment with KBP-042.

Chronic treatment with KBP-042 improved glucose tolerance with reduced insulin levels

OGTT was performed after the first injection, as well as after 3 and 7 weeks of treatment.

The acute OGTT showed a slightly impaired glucose tolerance for the 10 µg/kg group compared with HFD vehicle (Figure 3A, D). A hyperglycemic effect was observed 30 min after s.c. administration of KBP-042 at $t = 0$ compared with vehicle (5.9 mM) for 5 µg/kg (6.8 mM, $P = 0.033$) and for 10 µg/kg (7.4 mM, $P < 0.001$) groups. The total area under the curve (tAUC) was significantly increased after injection of 10 µg/kg KBP-042 (Figure 3D). However, the insulin levels during the first 60 min after glucose administration were reduced in animals dosed with KBP-042 (Figure 3G, J).

After 3 weeks of treatment with KBP-042 or saline, the three highest doses of KBP-042 resulted in a significantly lowered tAUC (Figure 3B, E). Insulin levels were lowered by KBP-042 except in the 0.625 µg/kg group (Figure 3H, K). Pair-fed 10 µg/kg group also had a reduced insulin response (Figure 3K).

During OGTT after week 7 (Figure 3C) the two highest dose groups had improved glucose tolerance when tAUC was considered (Figure 3F). The two highest dose groups showed increased glucose tolerance, while drastically reduced insulin levels were observed within the first 60 min after glucose administration (Figure 3I, L). Pair-feeding did not change glucose handling compared with HFD vehicle.

After administration of KBP-042, plasma lactate was dose-dependently increased in treatment of naive animals (Supporting Information Figure S2A) and resulted in a 1.5 mM increase in plasma lactate 30 min after s.c. administration of 10 µg/kg KBP-042. Interestingly, the KBP-042-provoked lactate response was completely blunted by chronic treatment (Supporting Information Figure S2B, C).

KBP-042 reduced gastrointestinal mobility and plasma levels of the gut hormone GIP

The rate of gastric emptying during OGTT was assessed in response to acute dosing with KBP-042, after treatment for 3 weeks, or after 7 weeks (Figure 4A, C, E, respectively). Acute s.c. administration of KBP-042 resulted in a significant reduction of gastric emptying 30 min after acetaminophen administration for the three highest treatment groups (2.5 µg/kg, 5 µg/kg, and 10 µg/kg) (Figure 4A). In animals treated for 3 weeks with KBP-042, gastric emptying was reduced for all treatment groups. The two pair-fed groups displayed a slower rate of gastric emptying due to food restriction; however, they still have significantly higher rates of gastric emptying compared with 5 µg/kg and 10 µg/kg groups of KBP-042 (Figure 4C).

After 7 weeks of treatment the reduced gastric emptying was still significant at most doses compared with HFD vehicle. The pair-fed groups were no longer different from the HFD controls (Figure 4E).

GIP levels in plasma were quantified 0 to 30 min after acute glucose administration, and after 3 and 7 weeks of treatment (Figure 4B, D, F). After acute administration of KBP-042, GIP levels were significantly lower in the groups treated with 2.5 to 10 µg/kg KBP-042 (Figure 4B). After treatment for 3 weeks, all groups displayed a drastic reduction in plasma GIP. The two pair-fed groups demonstrated significantly lowered GIP levels compared with HFD vehicle probably due to food restriction. They were still significantly higher than their corresponding treatment controls (5 µg/kg and 10 µg/kg) (Figure 4D). After 7 weeks of treatment, plasma GIP levels were reduced; however, the changes were only significant in the three highest treatment groups. The reductions in pair-fed groups were no longer present after 7 weeks of treatment (Figure 4F).

KBP-042 maintained peripheral glucose tolerance with lower insulin levels irrespective of altered gastric emptying

To circumvent the gastrointestinal tract and assess peripheral glucose tolerance, IVGTTs were performed after 3 and 7 weeks of treatment (Figure 5). In both tests, all KBP-042 groups showed a trend towards lower blood glucose compared with vehicle and pair-
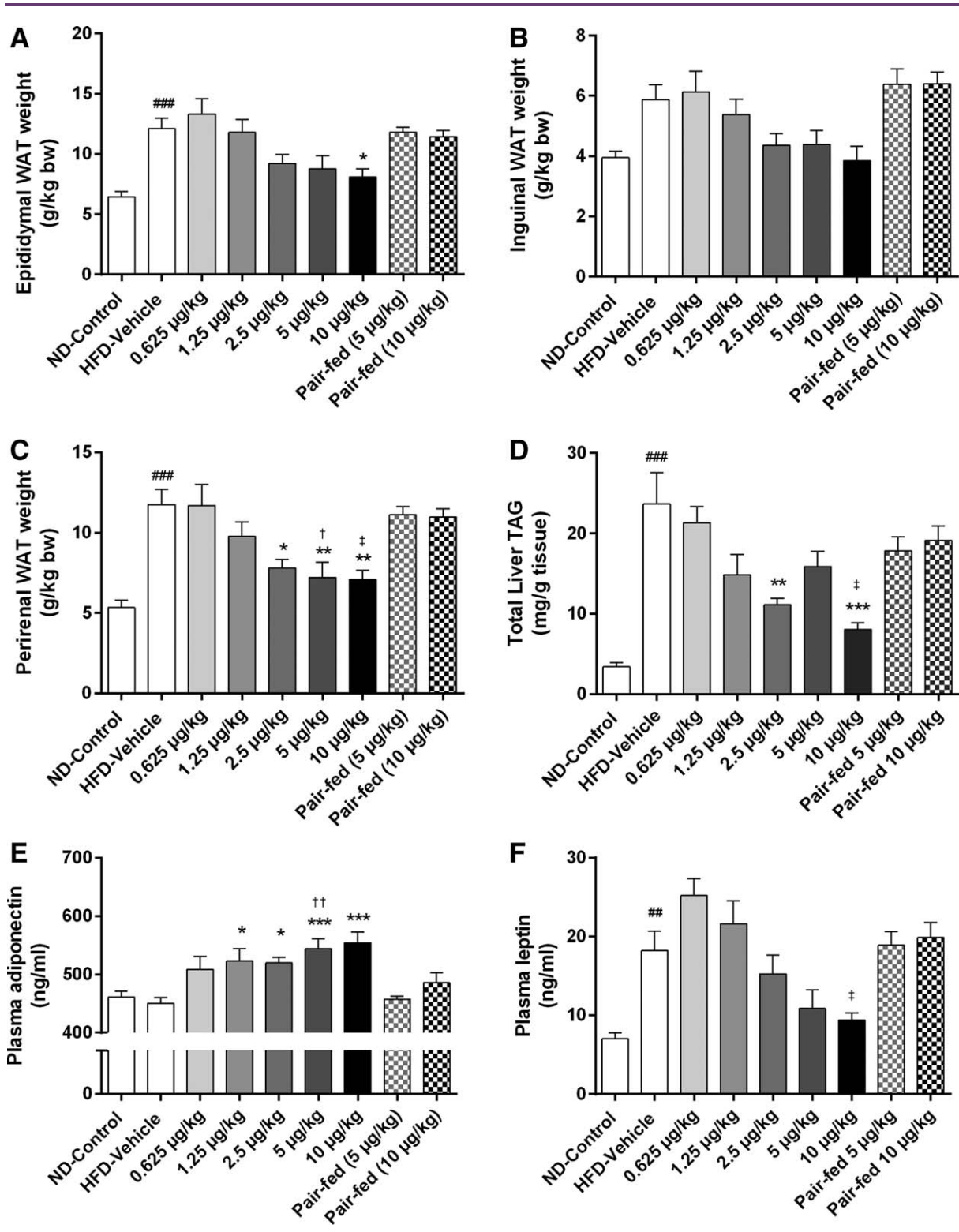
Figure 2 (**A–C**) Weight of isolated epididymal, inguinal, and perirenal white adipose tissue (WAT), respectively, after 56 days of treatment. (**D**) Total tri-acylglyceride content extracted from liver tissue after treatment with KBP-042 or saline for 56 days. (**E,F**) Plasma adiponectin and leptin levels, respectively, after 56 days of treatment. n = 10 for all groups except vehicle (n = 12). Statistical analysis between groups performed as a one-way ANOVA followed by Tukey's post hoc test with the following annotations: ##$P < 0.01$, ###$P < 0.001$ vs. normal diet control (ND). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. high-fat diet (HFD) vehicle. †$P < 0.05$, ††$P < 0.01$ vs. pair-fed 5 µg/kg. ‡$P < 0.05$ vs. pair-fed 10 µg/kg. Data are expressed as mean ± SEM.

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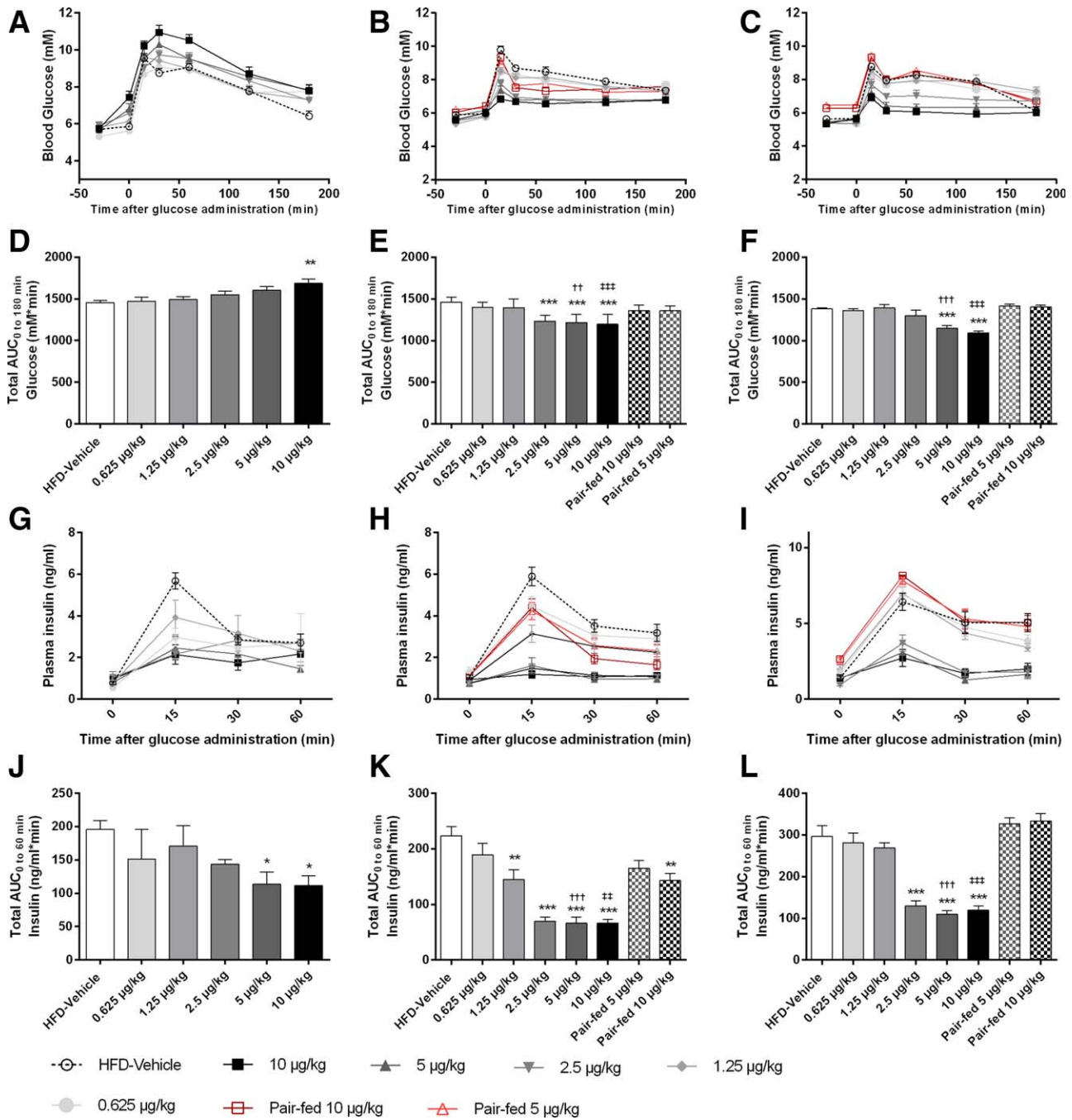OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Figure 3 Blood glucose and insulin levels during oral glucose tolerance test (OGTT) performed in animals treated with KBP-042 or vehicle once (left), for 3 weeks (middle), or 7 weeks (right). Animals were challenged with an oral glucose bolus (2 g/kg) at time = 0 and dosed with either KBP-042 or saline at t = −30. (**A–C**) Blood glucose levels during acute OGTT, OGTT after 3 weeks, and OGTT after 7 weeks, respectively. (**D–F**) Area under the curve (AUC) for acute OGTT, OGTT after 3 weeks, and OGTT after 7 weeks, respectively. (**G–I**) Insulin levels during acute OGTT, OGTT after 3 weeks, and OGTT after 7 weeks, respectively. (**J-L**) Insulin levels during acute OGTT, OGTT after 3 weeks, and OGTT after 7 weeks, respectively, expressed as AUC. n = 10 for all groups except vehicle (n = 12). Statistical analysis between groups 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. high-fat diet (HFD) vehicle. $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ vs. pair-fed 5 µg/kg. $\ddagger\ddagger P < 0.01$, $\ddagger\ddagger\ddagger P < 0.001$ vs. pair-fed 10 µg/kg. Data are expressed as mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fed controls 5 and 10 min after glucose administration (Figure 5A, B). This manifested in a lowered $\text{tAUC}_{0-120 \text{ min}}$ for the 2.5 µg/kg KBP-042 group only in the first test after 3 weeks and not after 7 weeks of treatment. No effect was observed for pair-fed groups.

Interestingly, when insulin levels were quantified, the tAUC for insulin was significantly reduced in KBP-042 1.25-10 µg/kg groups after 3 weeks of treatment (Figure 5G). After 7 weeks of treatment, groups treated with 2.5 µg/kg and 5 µg/kg KBP-042 had
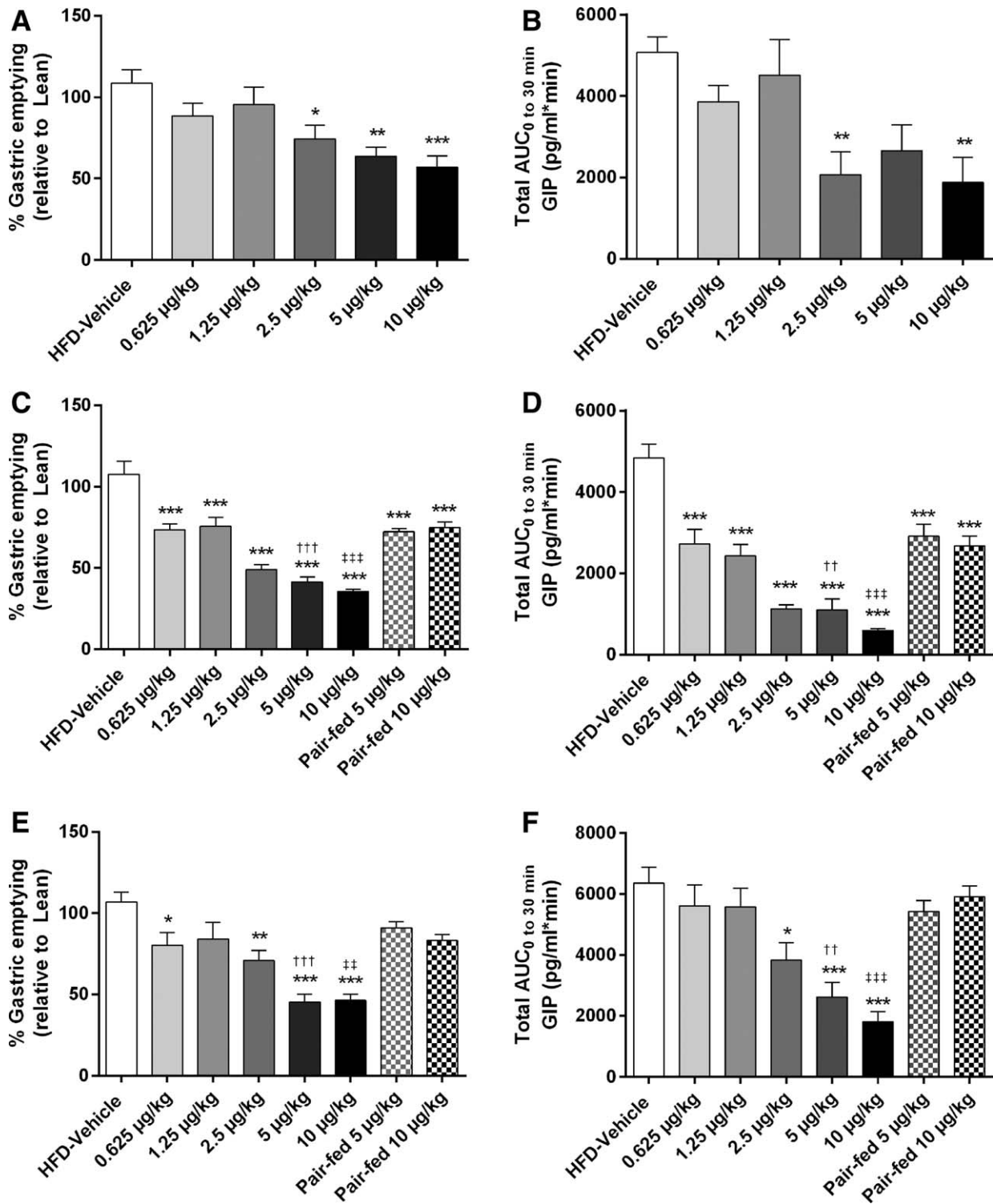
Figure 4 (**A**) Relative rates of gastric emptying measured 30 min after glucose challenge in the oral glucose tolerance test (OGTT) performed in treatment naive animals. (**B**) Area under the curve (AUC) of plasma levels of glucose-dependent insulinotropic peptide (GIP) during OGTT in treatment naive animals up to 30 min after glucose challenge. (**C**) Relative rates of gastric emptying measured 30 min after glucose challenge in the OGTT performed animals treated with KBP-042 for 3 weeks. (**D**) AUC of plasma levels of GIP during OGTT in animals treated for 3 weeks, up to 30 min after glucose challenge. (**E**) Relative rates of gastric emptying measured 30 min after glucose challenge in the OGTT performed animals treated with KBP-042 for 7 weeks. (**F**) AUC of plasma levels of GIP during OGTT in animals treated for 7 weeks, up to 30 min after glucose challenge. n = 10 for all groups except high-fat diet (HFD) vehicle (n = 12). Statistical analysis between groups 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. HFD vehicle. $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ vs. pair-fed 5 μg/kg. $\ddagger\ddagger P < 0.01$, $\ddagger\ddagger\ddagger P < 0.001$ vs. pair-fed 10 μg/kg. Data are expressed as mean ± SEM.
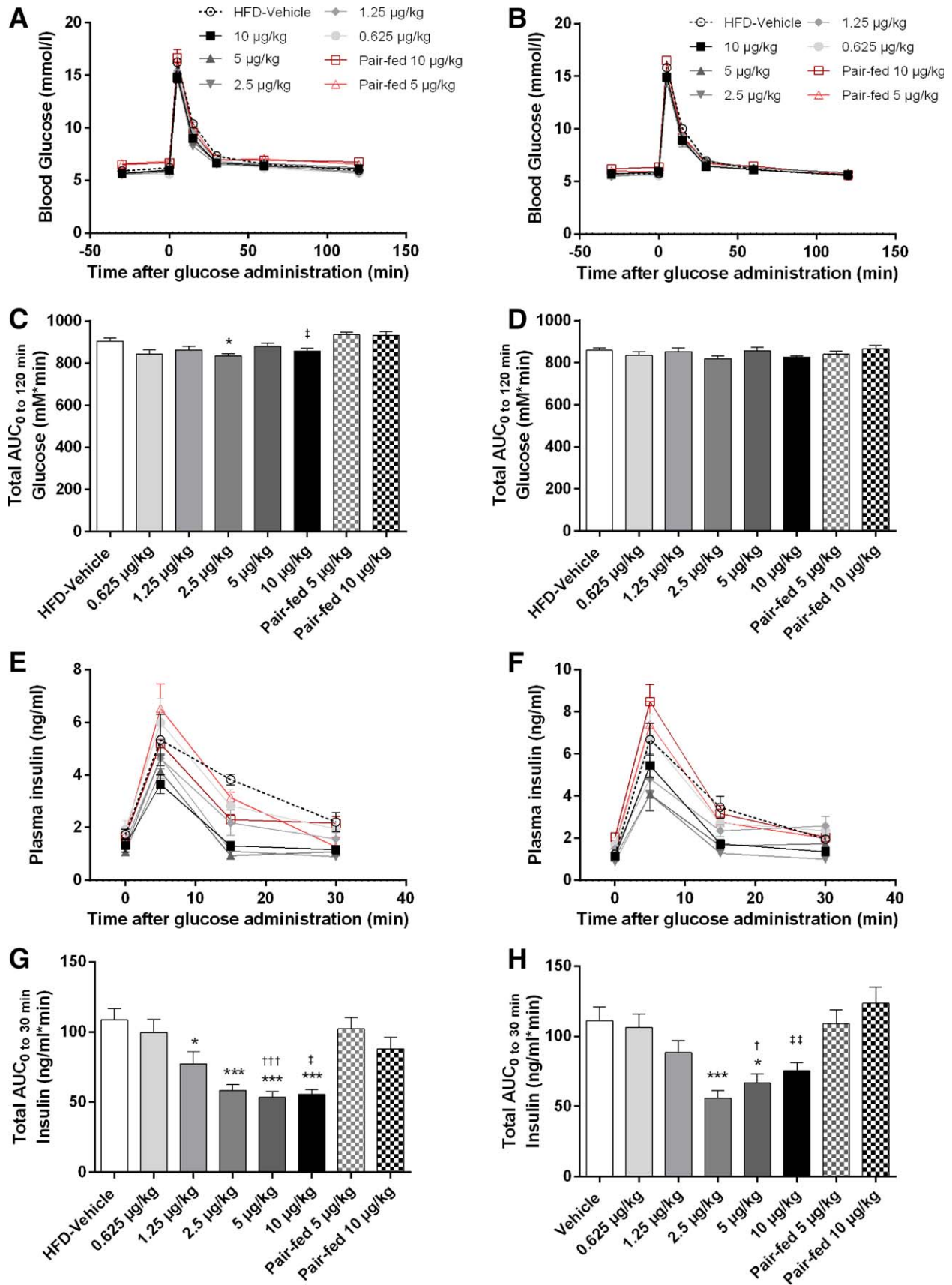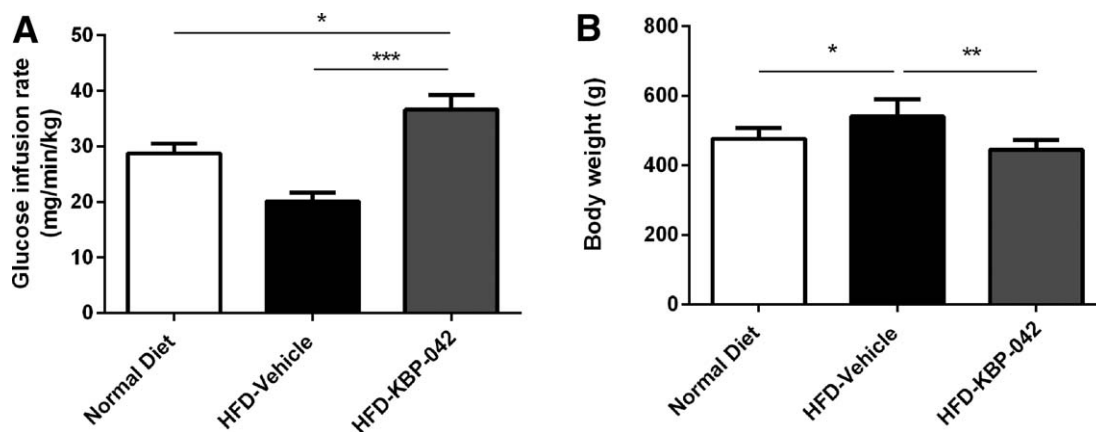
Figure 5.

Figure 6 (**A**) Glucose infusion rate (GIR) at steady state during hyperinsulinemic–euglycemic clamp when blood glucose was clamped at basal levels after 21 days of treatment. (**B**) Body weight at hyperinsulinemic–euglycemic clamp experiment day after 21 days of treatment. Statistical analysis between groups performed as a one-way ANOVA followed by Tukey's post hoc test with the following annotations: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are expressed as mean ± SEM.

significantly reduced insulin levels while maintaining glucose tolerance (Figure 5H).

KBP-042 improved whole body insulin sensitivity in the hyperinsulinemic–euglycemic clamp

A hyperinsulinemic–euglycemic clamp study was performed to address the effect of KBP-042 on insulin sensitivity. For this study, ND rats were compared with insulin-resistant HFD rats and 5 μg/kg KBP-042 treated HFD rats. Figure 6A shows GIR reduced by ~30% ($P = 0.057$) in the HFD group compared with ND. The treatment with KBP-042 led to a significant increase in GIR (82%, $P < 0.001$) compared with HFD vehicle. When KBP-042 treatment is compared with ND, GIR is increased with 27% ($P < 0.05$). As expected, body weight was increased after HFD for 10 weeks as compared with ND (Figure 6B), but treatment with KBP-042 for 21 days reduced weight with ~18%, and the body weight was not significantly different from the ND rats at the end of the study.

Discussion

In this study, KBP-042 induced a significant weight loss over a period of 8 weeks, *albeit* with dramatic reductions in food intake initially. Kaolin consumption was, however, only stimulated in a higher dose than used in this study, thus indicating the reduction in food intake was not due to illness. However, minor nausea in the rats cannot be excluded. The highest KBP-042 groups sustain the

weight loss (up to 20% compared with HFD vehicle) throughout the study, a phenomenon not seen in the pair-fed groups. The decreased food efficiency of the KBP-042-treated rats (2.5 μg/kg–10 μg/kg) and the large weight difference between treated and pair-fed rats, indicate increased energy expenditure. In general, amylin agonism blunts the reduction of energy expenditure that is normally caused by food restriction and weight loss, as well as changing RER (11,21), an indicator of fat utilization. Interestingly, amylin only increases energy expenditure when given as chronic infusion s.c. or i.c.v. (15,16,22), a finding likely related to short-lived activity of amylin (23). KBP-042 has a longer and more potent activation profile (17), despite a fast disappearance from plasma (<120 min) (18). However, energy expenditure, as well as potential fecal energy losses have to be formally assessed in future studies.

KBP-042 was able to significantly reduce TAG accumulation in the liver at both 2.5 μg/kg and 10 μg/kg. The reduction did not reach a significant level at 1.25 μg/kg and 5.0 μg/kg due to the relatively large individual variations in the hepatic TAG levels, but there is a tendency towards reduced hepatic TAG in these groups. Since ectopic deposition of lipids in the liver is related to increased insulin resistance, reducing the hepatic lipid-load could improve hepatic insulin sensitivity, hereby reducing gluconeogenesis in the fatty liver and increasing glucose tolerance (24). As of today, weight loss is the only remedy for ectopic lipid deposition, and KBP-042 serves as an excellent drug candidate to mediate this in an efficient manner. However, the extent to which a similar effect could be obtained by matching the weight loss remains to be explored. Importantly, the

Figure 5 (**A**,**B**) Intravenous glucose tolerance test (IVGTT) performed in animals treated for 3 weeks and 7 weeks, respectively, with either KBP-042 or saline. Animals were dosed s.c. at t = −30 and received i.v. glucose challenge at t = 0. (**C**) Area under the curve (AUC) 0 to 120 min for the IVGTT in panel A performed after treatment with KBP-042 for 3 weeks. (**D**) AUC 0 to 120 min for the IVGTT in panel B performed after treatment with KBP-042 for 7 weeks. (**E**) Plasma insulin levels during the IVGTT performed after 3 weeks of treatment. (**F**) Plasma insulin levels during the IVGTT performed after 7 weeks of treatment (legends as for panel A). (**G**) AUC for plasma insulin levels 0 to 30 min after glucose challenge in the IVGTT in panel A performed after treatment with KBP-042 for 3 weeks (legends as for panel B). (**H**) AUC for plasma insulin levels 0 to 30 min after glucose challenge in the IVGTT in panel B performed after treatment with KBP-042 for 7 weeks. n = 10 for all groups except high-fat diet (HFD) vehicle (n = 12). Statistical analysis between groups performed as a one-way ANOVA followed by Tukey's post hoc test with the following annotations: *$P < 0.05$, ***$P < 0.001$ vs. HFD vehicle. †$P < 0.05$, †††$P < 0.001$ vs. pair-fed 5 μg/kg. ‡$P < 0.05$, ‡‡$P < 0.01$ vs. pair-fed 10 μg/kg. Data are expressed as mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

analysis of the fatty acid composition of TAG further suggests that the fatty acid metabolism in the liver is unaltered, and the changes are an overall TAG reduction.

During acute OGTT, increases in plasma lactate and blood glucose were seen 30 min after administration of KBP-042, corresponding to previous studies showing acute hyperglycemia following acute administration of salmon calcitonin or rat amylin (25). This is likely explained by inhibition of insulin secretion, but also increased plasma lactate as seen in this study. This manifested as a tendency towards impaired glucose tolerance. Interestingly, the increase in plasma lactate was not present in animals treated chronically. In fact, chronic treatment led to improved oral glucose tolerance compared with both vehicle and pair-fed groups. Importantly, the improved glucose clearance was achieved with significantly lower plasma insulin levels, indicating improved insulin action. The improved glucose tolerance together with reduced liver TAG supports a general improved metabolism and insulin sensitivity. This is further supported by the reduction in adiposity, as plasma adiponectin is reduced in subjects who have obesity and related to for example, inflammation, insulin resistance, and energy metabolism (26,27), as well as type of phenotype in different fat depots (28). The observed increase in adiponectin is in alignment with the improvement in both glucose tolerance and insulin action as well as fatty acid removal from liver that KBP-042 induces (26,29-31). The reduced adiposity also manifested in lowering of plasma leptin, which corresponds well with previous demonstrations that KBP-042 increases the sensitivity towards leptin (18), a finding also seen with amylin (14,32).

IVGTT was performed to assess peripheral glucose homeostasis while circumventing the gastrointestinal system, which is obviously very affected by amylin agonism such as KBP-042 (33,34). Rats treated with KBP-042 maintained glucose tolerance with reduced insulin levels hence implying improved insulin sensitivity, *albeit* with an effect markedly lower than in the OGTT. This corroborates that KBP-042 has gastric emptying-independent effects on glucose tolerance. The reduced insulin levels both during IVGTT and OGTT could be explained by a direct KBP-042-mediated inhibition of both insulin and glucagon secretion directly in the islets of Langerhans (17), but maintaining or improving glycemia, glucose disposal rate, and insulin action after a significant weight loss is also well described in humans (5).

Plasma GIP levels and gastric emptying was assessed during the OGTT, and the rate of gastric emptying correlated to the GIP levels. In summary, KBP-042 reduces plasma incretin levels during OGTT, directly inhibits insulin and glucagon release from the islets of Langerhans (17), and reduces gastric emptying. These effects can also explain the reduced insulin levels in the OGTT, but not in the IVGTT. The reduced gastric emptying can mediate a beneficial effect on postprandial glucose levels, which along with fasting plasma glucose levels are very important factors in the reduction of risks related to hyperglycemia.

To formally assess the suggested increase in insulin action we performed a hyperinsulinemic–euglycemic clamp study. The reduced GIR seen in the HFD group compared with ND was expected since obesity is negatively correlated to insulin sensitivity and GIR (2). The large increase in GIR after treatment with KBP-042 illustrated the increase in insulin sensitivity. The KBP-042-induced weight loss could explain a large increase in GIR. However, here the rats treated

with KBP-042 had similar body weight to the ND, but with a significantly increased GIR. This could suggest that insulin sensitivity is increased beyond what would be expected from weight loss, although this has to be further tested in weight-matched animals receiving the same diet.

In conclusion, KBP-042 induced a sustained weight loss over 8 weeks in obese prediabetic rats but not in pair-fed animals, leading to reduction in adipose tissues, ectopic TAG deposition, improved glucose tolerance, and improved insulin action. The combination of a weight-reducing and insulin-sensitizing agent is to our knowledge unique. KBP-042 thus shows great promise for the treatment of type 2 diabetes and obesity due to its multiple beneficial effects on several aspects of the metabolic syndrome. O

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