**GLP-1/dexamethasone inhibits food reward without**

**inducing mood and memory deficits in mice**

Léa Décarie-Spain, BSc\*1, Alexandre Fisette, PhD\*2, Zhimeng Zhu3, PhD, Bin Yang3,4, PhD, Richard D. DiMarchi3,4, PhD, Matthias H. Tschöp2,5, MD PhD, Brian Finan3,4, PhD, Stephanie Fulton, PhD1#, Christoffer Clemmensen, PhD6#

**1**Montreal Diabetes Research Centre & CRCHUM, Université de Montréal, Quebec, Canada

**2**Institute for Diabetes and Obesity, Helmholtz Diabetes Center (HDC), Helmholtz Zentrum München & German Center for Diabetes Research (DZD), München-Neuherberg, Germany

**3**Department of Chemistry, Indiana University, Bloomington, IN, USA.

**4**Novo Nordisk Research Center Indianapolis, Indianapolis IN, USA

**5**Division of Metabolic Diseases, Department of Medicine, Technische Universität München, Munich, Germany.

**6**Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

\*These authors contributed equally

#Correspondence:
Christoffer Clemmensen; Novo Nordisk Foundation Center for Basic Metabolic Research, Blegdamsvej 3B, DK-2200 Copenhagen N, Denmark, Email: chc@sund.ku.dk

Stephanie Fulton; CRCHUM, 900 St-Denis, Montreal, QC, Canada, H1X 0A9, Email: stephanie.fulton@umontreal.ca

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**ABSTRACT**

Background: Pharmacotherapies targeting motivational aspects of feeding and palatable food reward, while sparing mood and cognitive function, represent an alluring approach to reverse obesity and maintain weight loss in an obesogenic environment. A novel glucagon-like peptide-1/dexamethasone (GLP-1/Dexa) conjugate, developed to selectively activate glucocorticoid receptors in GLP-1 receptor-expressing cells was shown to decrease food intake and lower body weight in obese mice. Here, we investigate if this novel drug candidate modulates the rewarding properties of food and if it affects behavioral indices of mood and memory.

Methods: C57Bl6 mice treated with the GLP-1/Dexa conjugate, GLP-1 or vehicle lever-pressed for HFHS food rewards in an operant task. Alterations in food-motivated behavior were also assessed following a HFHS diet withdrawal manipulation (switch to chow). The effects of repeated GLP-1/Dexa conjugate, GLP-1 or vehicle on free-feeding intake, body weight, anxiodepressive behaviors (elevated-plus maze, open field test & forced swim test), memory (novel object recognition) and mRNA expression of reward-relevant markers in the nucleus accumbens were also evaluated in mice fed a HFHS diet for 12 weeks.

Results: Mice treated with a GLP-1 analogue displayed a transient (4h) reduction in their motivation to lever press for HFHS reward, whereas treatment with equimolar doses of GLP-1/Dexa delivered a superior and sustained (20h) suppression of food-motivated behavior. GLP-1/Dexa also inhibited food reward following withdrawal from the HFHS diet. These benefits coincided with related transcriptional changes of dopaminergic markers in the nucleus accumbens. Importantly, repeated GLP-1/Dexa treatment during a HFHS diet caused weight loss without affecting anxiodepressive behavior and memory.

Conclusion: Via its actions to blunt the rewarding effects of palatable food without affecting mood and recognition memory, GLP-1-directed targeting of dexamethasone may serve as a promising and safe anti-obesity strategy.

Keywords: Obesity, food reward, appetite, GLP-1, co-agonist

1. **INTRODUCTION**

The prevalence of obesity continues to escalate worldwide and, in less than 40 years, obesity rates have doubled in more than 70 countries (Collaborators et al., 2017). Although the etiology of obesity is still incompletely understood, it is clear that ubiquitous access to palatable and energy-dense foods promotes overeating and excess body fat accumulation (Schwartz et al., 2017). Accordingly, therapies targeting hedonic and rewarding aspects of food intake might yield significant pharmacological efficacy to slow and/or reverse human obesity (Clemmensen et al., 2017). Importantly, in the pursuit of therapies targeting behavioral aspects of eating it is vital to ensure that weight loss efficacy is disconnected from off-target neuropsychiatric events.

Long acting glucagon-like peptide-1 (GLP-1) analogs produce significant weight loss in rodents and humans by suppressing food intake (Pi-Sunyer et al., 2015; Sisley et al., 2014). In line with the traditional understanding of energy homeostasis regulation, the effects of GLP-1 receptor (GLP-1R) signaling on feeding has predominantly been ascribed to receptor populations expressed in the hypothalamus and in the hindbrain (Hayes et al., 2011; Secher et al., 2014; Turton et al., 1996). However, GLP-1Rs are widely distributed across the CNS, including in regions known to be critical for feeding behavior (Heppner et al., 2015). In agreement, there is evidence to suggest that GLP-1R agonism alleviates obesity, in part, by reducing food reward (Alhadeff et al., 2012; Dickson et al., 2012; Dossat et al., 2011; Farr et al., 2016; ten Kulve et al., 2015; van Bloemendaal et al., 2015). However, the maximal efficacy of next generation GLP-1R agonists appears to be finite and limited to nearly ~10-15% body weight loss (O'Neil PM, 2018), and maximal dose limited due to adverse gastrointestinal events and elevations in heart rate (Dossat et al., 2011). Consequently, researchers are focusing on therapeutic strategies that can potentiate GLP-1 pharmacology without compromising safety (Finan et al., 2015).

Striving to boost the weight-lowering efficacy of GLP-1R agonism, we developed a pharmacological combinatorial approach based on the vision that certain peptide hormones might be suitable for selective tissue targeting of small molecules that provide independent, yet complementary metabolic benefits (Tschop et al., 2016). In a proof-of-principle study, GLP-1 was used as a shuttle to deliver estrogen actions to the CNS (Finan et al., 2012). More recently, we expanded this technology to couple GLP-1 with the glucocorticoid receptor agonist, dexamethasone (GLP-1/Dexa) (Quarta et al., 2017). GLP-1-directed targeting of glucocorticoid activity potently reverses diet-induced obesity and reverses central and systemic inflammation in mice, without exhibiting side-effects related to broad glucocorticoid signaling such as HPA axis inhibition. Notably, the anti-obesity effect of the GLP-1/Dexa conjugate was superior when the animals were maintained on a palatable high-fat, high sugar (HFHS) diet, suggesting that molecular integration of GLP-1 and glucocorticoid pharmacology might reverse obesity by altering certain aspects of food reward.

To test this hypothesis, we investigated the ability of GLP-1/Dexa to decrease food-motivated behavior. Given the link between weight loss pharmacotherapy and mood side effects (Dietrich et al., 2015), which is particularly evident with drugs affecting appetite (Dietrich and Horvath, 2012), we also assessed if chronic treatment with GLP-1/Dexa affected anxiodepressive behavior and memory. In comparison to GLP-1R mono-agonism, the GLP-1/Dexa conjugate displayed a superior impact on the long-term suppression of food-motivated behavior, without compromising certain mood-relevant and cognitive functions. Notably, GLP-1/Dexa counteracted the motivation to work for palatable high-calorie foods despite inducing a chronic negative energy balance, underscoring that this pharmacological approach might be particularly suitable for weight loss therapy in an obesogenic environment characterized by high food reward.

1. **MATERIAL AND METHODS**

**2.1 Animals and diets**

All procedures involving the use of animals were approved by the CRCHUM Animal Care Committee in accordance with Canadian Council on Animal Care guidelines. Eight weeks old C57BL/6J male mice from Jackson Laboratories were single-housedunder reverse cycle (lights off at 10am) in environmentally controlled rooms (22-24°C) with *ad libitum* access to water and food. Mice were fed standard chow or high-fat, high sugar (HFHS) diet (D12331; Research Diets, New Brunswick, NJ. 58% kcal from fat, 25.5% kcal from carbohydrates, and 16.4% kcal from protein) as indicated.

***2.2. In vivo* pharmacology**

The GLP-1/Dexa conjugate and its GLP-1 balanced analog were generated as previously described (Quarta et al., 2017). Of note, the GLP-1 analog (also used as the peptide vector for the Glp-1/Dexa conjugate) was shown to retain full potency at GLP-1 receptor and was protected from DPP-IV-mediated degradation (Finan et al., 2012). This GLP-1 analogue, referred to herein simply as “GLP-1 analogue,” has an aminoisobutyric acid at position 2 to provide protection from dipeptidyl peptidase-4 (DPP-IV) mediated degradation, a glutamic acid substitution at position 16 to support receptor potency, and the C-terminal extension derived from the GLP-1 paralog Exendin-4 to support aqueous solubility and serve as a spacer to the cysteine residue to which the dexamethasone moiety is conjugated. The “GLP-1 analogue” is the free cysteine equivalent of the GLP-1/Dexa conjugate. Mice were treated with subcutaneous injections of the different compounds (100 nmol/kg of weight in a volume of 5 uL/g of body weight) or vehicle (saline), either 4h (30 mins prior to the onset of the dark cycle) or 20h (in the second half of the light cycle) before behavioral testing, to respectively assess acute and prolonged effects. For long-term treatment, mice were injected subcutaneously daily for 11 days in the second half of the light cycle.

**2.3 Chronic treatment study and pair-feeding**

All 30 mice were fed a HFHS diet for 14 weeks. After the 12th week of diet, body weight-paired groups were formed within HFHS-fed mice and treated daily for 11 days with either GLP-1/Dexa, GLP-1 analogue alone, or vehicle. To dissociate the effects of food restriction from the benefits of GLP-1/Dexa, an additional group of pair-fed mice (n=10) was added to the long-term study. During the last 2 weeks of the long-term study, these HFHS-fed mice were food-restricted proportionally to the daily HFHS diet intake of mice receiving daily injections of GLP-1/Dexa (average of -32% of their daily caloric intake).

**2.4 Operant conditioning**

Mice (n=12) were trained to lever-press for 20mg HFHS food pellets (﻿Bio-Serv, Frenchtown, NJ, USA) on a progressive ratio (PR) schedule as described previously (Sharma et al., 2012). Briefly, chow-fed mice were food-restricted to maintain 90% of their body weight and trained daily in an operant task to press one of two levers (active versus inactive) to receive a HFHS pellet over the course of 5 weeks (2 weeks in fixed ratio 1 (FR1), 1 week in FR1 with a 5 secs time-out, 1 week in FR5 and 1 week in progressive ratio (PR). Once stable responding in the PR task was achieved (<20% variability in breakpoint over 3 consecutive days), all mice were provided with *ad libitum* access to food and tested until responding stabilized.

To evaluate the effects of the GLP-1/Dexa conjugate, GLP-1 analogue and vehicle control on food reward, a within-subject design was employed with random and balanced delivery of each drug over a 3-week period. The impact of each drug on food motivated responding was assessed over 1 week, consisting of baseline testing on Day 1, followed by a 3-day period of treatment testing and then a 3-day “washout” period during which mice returned to their former body weight. This 3-week protocol was carried out twice, first to test mice 4-h post-injection and then to test mice 20-h post-injection.

Mice previously trained for PR in the operant task were fed a HFHS diet for 6 weeks and transitioned to standard chow diet to induce a state of palatable dietary withdrawal which is reported to last for at least 2 weeks (Sharma et al., 2013). The ability of GLP-1/Dexa to impact food motivation in context of enhanced food craving, was assessed by measuring breakpoint ratio values after diet withdrawal (switch to chow) relative to baseline values during the 6th week of HFHS diet regimen. We used a within-subject design with counter-balanced treatment presentation.

**2.5 Behavioral testing**

Mice from the long-term study were subjected to a series of behavioral assessments on days 6-9 of the 11-day treatment period. All tests occurred at the end of the dark phase and were carried out one day apart following the sequence listed below.

*2.5.1 Forced swim test (FST)*

Animals were placed in a water-filled recipient (1600mL of water at 23 $\pm $ 2°C) for 6 mins, as described previously (Sharma et al., 2013). The proportion of immobility versus swimming time during the last 4 mins of the test was used to assess behavioral despair, while velocity during the first 2 mins was measured to screen for locomotor deficits.

*2.5.2 Elevated-plus maze (EPM)*

Mice were placed in the center of an elevated plus-shaped platform (﻿Med Associates, Inc., St Albans, VT, USA) and left free to explore for 5 mins, as described previously (Decarie-Spain et al., 2018; Sharma and Fulton, 2013). The proportion of entries into the open arms of the maze relative to the close ones and the center region was used as an indicator of anxiety-like behavior.

*2.5.3 Open field test (OFT)*

Animals were placed into the corner of a squared arena (40x40x40cm) and left free to explore for 5 mins, as described previously (Sharma and Fulton, 2013). The proportion of entries into the center region relative to the periphery and wall areas was used as an indicator of anxiety-like behavior. Total distance travelled was also measured to screen for locomotor deficits.

*2.5.4 Novel object recognition (NOR)*

Mice were placed in the same arena used for the OFT and 2 identical objects were presented for 5 mins. Mice went back in their respective home cage and 1h later, were put back in the arena where one of the objects was replaced by a new one. The proportion of time spent investigating the novel object relative to the old one was used as an indicator of recognition memory. Mice had to reach a criterion of 10 secs of investigation per trial to be included.

*2.5.5 Data analysis*

The FST, EPM and OFT were video recorded and analyzed with the EthoVision software. The NOR trials were video recorded and manually scored by timing investigation for each object. Investigation was defined as sniffing the object.

**2.6 Quantitative PCR**

One month following their last treatment, the same animals used for operant tasks and withdrawn from HFHS diet were sacrificed by decapitation under isoflurane 20h following a single injection with GLP-1/Dexa or vehicle. Brains were harvested and snap frozen in isopentane. Tissue punches of NAc (core and shell combined) were obtained from frozen coronal sections (200mm) and RNA extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was synthetized from 700ng of total RNA using random hexamers and M-MLV Reverse Transcriptase (Invitrogen). Real-time PCR was performed using the Rotor Gene SYBR Green PCR kit (Qiagen). Primers were designed using BLAST (U.S. National Library of Medicine) and synthesized by Integrated DNA Technologies, Inc. based on the sequences listed in Tab le 1. Data was extrapolated from standard curves and normalized to the housekeeping gene HPRT. Mean ± standard error of mean values for the co-agonist group are expressed in fold changes relative to vehicle normalized at 1.0.

**2.7 Statistical analyses**

Data were analyzed with GraphPad Prism 6 and are presented as mean ± standard error of the mean (SEM). To compare two groups, t-test were used, while one-way ANOVA (with repeated measures for within-subject design studies) with Bonferroni post-hoc test was used to compare three or more groups. A two-way ANOVA with repeated measures and Bonferroni post-hoc test was employed to assess the interaction of the GLP-1/Dexa conjugate treatment and time. Gene expression levels in the nucleus accumbens following injection of GLP-1/Dexa or vehicle were compared with an unpaired t-test. Fold change for food motivation following withdrawal and preference in the novel object recognition task were analyzed with a one sample t-test. Criterion for significance was set to p<0.05 in all comparisons.

1. **RESULTS**

**3.1 Food-motivated behavior is reduced by GLP-1/Dexa**

We first assessed the effects of a short-term 3-day treatment of GLP-1/Dexa on food-motivated behavior using both vehicle and a matched GLP-1 analog (herein referred to as “GLP-1 analogue”) as controls in a within-subject design experiment, with a washout period between the randomly assigned treatments that was sufficient to allow the mice to fully recover their basal body weight (Fig. 1a). A 3-day period of daily injections with GLP-1/Dexa induced a superior decrease in body weight compared to both vehicle and GLP-1 analogue-treated animals (-6.60% for GLP-1/Dexa versus +0.93% and -1.48% for vehicle and GLP-1 analogue, p<0.001 for both and p=0.076 for GLP-1 versus vehicle, Fig. 1b). Over the 3-day period, daily food intake was reduced by both GLP-1/Dexa and GLP-1 analogue relative to vehicle (-4.3kcal and -3.0kcal, respectively, p<0.005 for both, Fig. 1c). To examine the acute effect of GLP-1/Dexa on the motivation to work for palatable food reward, mice were exposed to the lever-press paradigm 4h after their daily injection. Both GLP-1/Dexa and GLP-1 analogue potently reduced the breakpoint ratio relative to vehicle treatment (-70%, 73%, respectively, p<0.01 for both, Fig. 1d), indicative of a strong suppression of food-motivated behavior. To assess whether GLP-1/Dexa and GLP-1 analogue effects were persistent over time, we repeated the experimental paradigm and exposed the animals to the lever-press paradigm 20h after their daily injection. In this context, GLP-1 analogue failed to influence food motivation, whereas the efficacy of GLP-1/Dexa was retained and significantly superior to both vehicle and GLP-1 control treatments (-45%, p<0.01 relative to vehicle, -30%, p<0.01 relative to GLP-1, (Fig. 1e). Of note, the baseline breakpoint ratios and preference for active lever did not vary across treatments or in time (Table S1). In summary, these experiments highlight a superior ability of the conjugate to suppress food motivated-behavior.

**3.2 GLP-1/Dexa alleviates increased motivation for food reward following high-fat, high sugar diet withdrawal**

Dieting leads to a well-described increased motivation toward palatable food. We therefore evaluated whether the effects of GLP-1/Dexa on food-motivated behavior measured previously could dampen the motivation for food reward after withdrawal from a HFHS diet (Fig. 2a). Mice receiving a short-term 3-day treatment of GLP-1/Dexa during the dietary withdrawal period lost more weight than when treated with vehicle (p<0.0001 on days 1, 2 and 3, Fig. 2b), an effect associated with vastly reduced food intake (p<0.0001 on days 1, 2 and 3, Fig. 2c). Importantly, whereas vehicle treatment was linked with high motivation to work for sucrose after HFHS withdrawal in a lever-pressing paradigm (as demonstrated via a significant increase in breakpoint ratio values relative to pre-withdrawal measurements, 1.85, p<0.05), GLP-1/Dexa treatment counteracted this surge in motivation (breakpoint ratio relative values, 1.02, p=N.S.) (Fig. 2d). The ability of GLP-1/Dexa to alleviate diet-induced food cravings despite being in a highly negative energy state underlines the potential of the compound for counteracting weight regain.

**3.3 Reward-related gene expression signature in the NAc is modulated by GLP-1/Dexa**

Given the effects of GLP-1/Dexa on food motivation were observed 20h after acute administration, we studied the transcriptional signature of dopamine and opioid-related molecules associated with the modulation of food reward by the nucleus accumbens (NAc) at the same time point. A single injection of GLP-1/Dexa reduced NAc gene expression of the dopamine receptor 1 (-40%, p<0.01), tended to lower levels of the short isoform of the dopamine receptor 2 (p=0.09), and decreased expression of the long isoform of the dopamine receptor 2 (-46.8% relative to vehicle, p<0.05) (Fig. 3). These findings were not accompanied by changes in gene expression for the rate-limiting enzyme for dopamine synthesis tyrosine hydroxylase or the dopamine transporter. As the opioid system tightly interacts with dopamine signaling in the NAc, opioid receptor gene expression was also investigated. Treatment with GLP-1/Dexa specifically decreased gene expression of the kappa opioid receptor (-31.0% relative to vehicle, p<0.05), while expression levels of the delta and mu receptors remained unchanged. Finally, glucocorticoid (GC) receptor expression was decreased by GLP-1/Dexa conjugate treatment (-13.6% relative to vehicle, p<0.05), while no differences were observed for the mineralocorticoid receptor. Altogether, these data suggest that systemic injection of GLP-1/Dexa can rapidly affect the expression of reward-related genes in the NAc.

**3.4 GLP-1/Dexa promotes greater weight loss without impairing cognitive or affective functions.**

High-fat feeding induces anxiodepressive-behaviors and cognitive impairments partly due to alterations in the CNS reward system (Sharma and Fulton, 2013; Volkow et al., 2013). In addition, anti-obesity therapies affecting the rewarding effects of food can also provoke deleterious mood and cognitive deficits (Binkley and Knowles, 2002; Taflinski and Chojnacka, 2000; Topol et al., 2010). In order to evaluate mood and cognitive functions of GLP-1/Dexa in the context of its anti-obesity properties, DIO mice received chronic treatment with GLP-1/Dexa and were exposed to a battery of behavioral tests (Fig. 4a).

Both GLP-1/Dexa and GLP-1 analogue elicited a reduction in obesity relative to vehicle-treated HFHS -fed controls, replicating relative efficacy of each compound as was previously published (Quarta et al., 2017). While 11-day treatment with GLP-1 analogue reduced body weight by 5.7% (p=0.05), GLP-1/Dexa induced a 11.3% weight loss (p<0.0001), and thus was significantly superior to GLP-1 analogue alone (p<0.05) (Fig. 4b-c). Although vehicle-treated mice that were pair-fed to GLP-1/Dexa-treated mice induced a significant weight loss (-8.5%, p<0.001), it did not reach the efficacy of GLP-1/Dexa treatment, thereby highlighting the food intake-independent beneficial effect of the compound. The GLP-1/Dexa conjugate had a superior effect on lowering overall caloric intake relative to GLP-1 analogue and vehicle treated mice (Fig. 4d-e).

GLP-1/Dexa had no impact on anxiety-like behavior as assessed via several behavioral tests performed during days 6-9 of the treatment period. While GLP-1/Dexa did not influence depressive behavior relative to vehicle treatment, immobility time in the FST was enhanced by pair-feeding relative to GLP-1 analogue and GLP-1/Dexa treatments (p<0.05). This indicates greater levels of behavioral despair (Fig. 4f), an effect not due to change in swimming ability as evidenced by unaltered velocity (Fig. 4g). No differences were found in frequency of entries into the open arms of the EPM (Fig. 4h), or in the center zone of the OFT (Fig. 4j). In addition, overall distance travelled was unchanged in both the EPM (Fig. 4i) and the OFT (Fig. 4k). Only pair-fed animals displayed increased preference for the novel object in the NOR (Fig. 4l) suggesting a reversal of the memory recognition deficit induced by HFHS diet. However, these results show that GLP-1/Dexa can potently induce weight loss without dampening cognitive or affective functions.

1. **DISCUSSION**

Encouraged by the idea that pharmacological targeting of food reward in combination with canonical anorectic and thermogenic pathways will provide superior efficacy to reverse obesity, we investigated the effects of a novel GLP-1/Dexa conjugate on food-motivated behaviors in mice. We demonstrate that GLP-1/Dexa has a potent and sustained effect on inhibiting palatable food reward in lean mice, and treatment counteracts the heightened food motivation of obese mice switched from a palatable high-calorie diet to a low-calorie diet. Finally, we establish that the weight-lowering benefits of GLP-1/Dexa are dissociated from any adverse effects on working memory and anxio-depressive behavior.

In agreement with a large body of work, our results confirm that GLP-1R agonism potently inhibits food reward (Hayes and Schmidt, 2016; Mietlicki-Baase et al., 2014). In comparison with GLP-1 analogue, treatment with equimolar doses of GLP-1/Dexa exhibited a superior and sustained effect to dampen food reward. Importantly, this larger effect on weight loss and food motivation was observed in different feeding paradigms, with increased efficacy of GLP-1/Dexa over GLP-1 analogue alone in animals either with free access to palatable food or in animals in a period of withdrawal from palatable food, where motivation toward palatable food is heightened. These findings substantiate recent work by Quarta *et al.* (Quarta et al., 2017) and suggest that the anti-obesity efficacy of GLP-1/Dexa is linked to the ability to inhibit hedonic overeating. However, increased drug exposure cannot fully be discounted as a contributing factor to the sustained effects. The acute transcriptional changes of dopaminergic genes in the NAc in response to GLP-1/Dexa treatment suggest that GLP-1-directed targeting of glucocorticoid actions may blunt food reward through modulation of canonical mesolimbic reward signaling.

Exogenous glucocorticoids have previously been linked to enhanced memory consolidation and contextual learning of food cues via hippocampal GCs (de Quervain et al., 2017; Zorawski and Killcross, 2002). Conversely, impairments in working memory have also been reported following glucocorticoid treatment, emphasizing possible dual modulatory effects on memory (Barsegyan et al., 2010; de Quervain et al., 2017). Our findings suggest that GLP-1R-selective targeting of dexamethasone appropriately exploits the dual pharmacodynamics exhibited by this hormonal pairing on food-motivated behaviors, while circumventing potential glucocorticoid-induced adverse events on recognition memory. While GC influence on memory is much more associated with hippocampal actions, GC receptors are also expressed in the perirhinal network which is deemed to underlie recognition memory function tested here (Laugero et al., 2002). It should be noted that in contrast to caloric restriction, which elicited an improvement in object recognition memory, GLP-1/Dexa treatment did not enhance recognition memory in DIO mice, despite potently lowering body weight. However, experimenter implemented food restriction is not the same as intrinsic decreases in food intake. Enhanced memory in response to caloric restriction may be an evolved positive adaptation to optimize foraging skills under food scarcity. We speculate that the drug-induced negative energy state is not biologically perceived as a potential starvation risk.

GLP-1R has a widespread expression profile in the brain and GLP-1R signaling in the VTA (Dickson et al., 2012; Hernandez et al., 2018; Schmidt et al., 2016), NAc (Dickson et al., 2012; Mietlicki-Baase et al., 2014), lateral hypothalamus (Lopez-Ferreras et al., 2018) hippocampus (Hsu et al., 2015; Hsu et al., 2017), NTS (Alhadeff and Grill, 2014; Alhadeff et al., 2017; Richard et al., 2015) and parabrachial nucleus (Alhadeff et al., 2014; Richard et al., 2014) can modulate food intake, behavior and reward. Previously we found that GLP-1/Dexa delivers potent anti-inflammatory actions in hypothalamic nuclei (Quarta et al., 2017). Others have shown that GLP-1-mediated targeting of the nuclear hormone estrogen signals through the supramammillary nucleus (Vogel et al., 2016) and the dorsal raphe nuclei (Cao et al., 2014) to inhibit food reward. Interestingly, infusion of corticosterone into the fourth ventricle attenuates cocaine self-administration via GLP-1R signaling in the VTA (Schmidt et al., 2016). Thus, coordinated delivery of dexamethasone to multiple discrete GLP-1R positive neuronal populations might underlie the herein observed effects on food-motivated behavior, but altered biodistribution such as enhanced CNS penetrance may also be involved.

Although further studies are needed to determine the anatomical and molecular mechanisms driving the benefits of targeted dexamethasone on food reward, we demonstrate that the GLP-1/Dexa drug conjugate has a safe anxio-depressive profile in mice. The type 1 cannabinoid receptor (CB1R) inverse agonist Rimonabant which was discontinued due to severe adverse psychiatric effects (Christensen et al., 2007), serves as a reminder to meticulously ensure that the pharmacological benefits on food-motivated behaviors are appropriately uncoupled from adverse effects on anxiety and mood; which are frequently linked to neuropharmacology (Dietrich and Horvath, 2012). Some reports suggest that dexamethasone can induce depression-like behavior in mice (Wrobel et al., 2014), and that dexamethasone has a dose-dependent biphasic effect on anxiety via opioidergic neurons (Vafaei et al., 2008). Importantly, GLP-1-mediated targeted delivery of dexamethasone avoids these potential adverse effects on anxio-depressive behaviors. This likely reflects the restricted biodistribution governed by GLP-1R targeting, but could also reflect the relatively low systemic concentrations of dexamethasone introduced by the equimolar pairing with GLP-1.

 There are several limitationsto our study. As already pointed out, it remains enigmatic if the benefits of GLP-1/Dexa on food reward are mediated directly via GLP-1R positive neurons in the mesolimbic reward system or, for example, if GLP-1/Dexa acts via glutamatergic neurons in the circumventricular organs as a gateway to access behavioral neurocircuits (Adams et al., 2018). Furthermore, although we provide evidence for a superior efficacy of GLP-1/Dexa relative to GLP-1 mono-therapy on food reward, the lack of mono-therapeutic controls in every experimental condition preclude us from making broader conclusions pertaining to pharmacological synergy. Finally, we must acknowledge the limitations of assessing off-target effects on stress, anxiety and different types of memory in mice. Although we believe these findings are a step in the right direction, an expansion of the safety profile of GLP-1/Dexa is necessary prior to its clinical progression. In context, because GLP-1 treatment in humans is particularly dose-limited by adverse events such as nausea and vomiting, future studies should carefully address the aversion profile of GLP-1/Dexa. Finally, as obesity is linked to an abundance of palatable food options, laboratory high-fat feeding and DIO models have a number of limitations with respect to mimicking the complexities of human overeating (Johnson and Wardle, 2014; Kleinert et al., 2018). Notably, the ability of GLP-1R agonism to inhibit food intake in rodents may be facilitated by the consistent composition of the diet (Mella et al., 2017). To fully unmask the capacity of GLP-1/Dexa to inhibit feeding and food reward it would be advantageous to use cafeteria-like diets with a variety of palatable, energy-dense foods (Ellacott et al., 2010; Sclafani and Springer, 1976). Moreover, the conditions of access to palatable food can trigger different types of behavioral adaptations (Di Segni et al., 2014) (Di Segni et al., 2014). Pharmacological treatment of obesity and hyperphagia might thus show unequal weight loss efficacy according to the type of feeding paradigm.

One of the central challenges in obesity treatment is that the bulk of individuals eventually regain most of the lost weight. It has been argued that weight loss and weight loss maintenance may be dissimilar biological challenges that require different pharmacology (Clemmensen et al., 2017). Targeted delivery of nuclear hormones may be an unconventional strategy to induce long-lasting structural changes that could counter the biological drive that favors weight regain. However, whether or not such a mechanism explains the sustained effect of GLP-1/Dexa to inhibit food reward requires further investigations.

1. **CONCLUSIONS**

Collectively, the present data demonstrate that targeted delivery of dexamethasone by GLP-1 potently inhibits palatable food reward and protects against weight rebound in the context of a negative energy balance. Importantly, GLP-1/Dexa induces benefits on food-motivated behavior that are uncoupled from several known adverse effects on anxiety, despair and working memory. Future studies are needed to delineate the central mechanisms of action of GLP-1/Dexa and to determine the translational value of this novel anti-obesity drug candidate.

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M.H.T has served as member of the Novo Nordisk advisory board and SAB member of ERX Pharmaceuticals. The Institute for Diabetes and Obesity cooperates with Novo Nordisk and Sanofi-Aventis. B.F. and R.D.D are currently employees of Novo Nordisk. All other authors declare no competing interests.

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**FIGURE LEGENDS**

**Figure 1. GLP-1/Dexa reduces food-motivated responding in a sustained manner.**

(a) Experimental timeline design. Purple arrows represent injections. (b) Body weight loss relative to baseline over 3-day treatment with vehicle (black), GLP-1 (orange) and GLP-1/Dexa (green). (c) Average daily caloric intake of chow over 3-day treatment. (d) Breakpoint ratios for high-fat, high sugar (HFHS) rewards 4h following injection. (e) Breakpoint ratios for HFHS rewards 20h post-injection. One-way ANOVA, repeated measures, Bonferroni post hoc; (n=12/group). \*\*p<0.01, \*\*\*p<0.005, \*\*\*\*p<0.001

**Figure 2. GLP-1/Dexa alleviates heightened food reward following withdrawal from a high-fat, high sugar diet.**

(a) Experimental timeline. (b) Body weight following daily injections with vehicle (black) and GLP-1/Dexa (green), (c) Cumulative caloric intake of chow diet with daily injections. (d) Relative breakpoint ratios for food rewards following withdrawal from the HFHS diet. Two-way ANOVA, repeated measures with Bonferroni post hoc (c, d) and one-sample t-test at 1.0 (d); (n=12/group). \*p>0.05, \*\*\*\*p<0.001.

**Figure 3. Expression of reward-related genes in the nucleus accumbens is modulated by GLP-1/Dexa.**

NAc gene expression for dopamine receptor 1 (D1r), dopamine receptor 2 short isoform (D2rsh), dopamine receptor 2 long isoform (D2rlg), tyrosine hydroxylase (TH), dopamine transporter (DAT), delta opioid receptor (Dor), kappa opioid receptor (Kor), mu opioid receptor (Mor), glucocorticoid receptor (Gr) and mineralocorticoid receptor (MR) following a single injection of vehicle (black) or GLP-1/Dexa (green) 20h prior to sacrifice. Two-tailed t-test (n=5/group); \*p<0.05, \*\*p<0.01.

**Figure 4.** **GLP-1/Dexa promotes weight loss without impairing cognitive or affective functions in diet-induced obese mice.**

(a) Experimental timeline. (b-c) Change in body weight and (d-e) cumulative caloric intake over 11-day period with daily s.c. injections of vehicle (black), GLP-1 (orange), GLP-1/Dexa (green) and vehicle mice pair-fed to GLP-1/Dexa (magenta) on HFHS diet. Effects on behavioral despair, anxiety-like behavior and cognition assessed by (f-g) forced swim test, (h-i) elevated plus maze, (j-k) open field test and (l) novel object recognition on days 6-10 of treatment period. One-way ANOVA, Bonferroni post-hoc (n=10/group); except (l) one-sample t-test, t=1.0. \*p<0.05, \*\*p<0.01, \*\*\*p<0.005, \*\*\*\*p<0.001.