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Graphical abstract

Exenatide once weekly for alcohol use disorder investigated in a randomized, placebo-controlled clinical trial

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Abstract:

Background

Alcohol use disorder (AUD) is a chronic, relapsing brain disorder that accounts for 5% of deaths annually, and there is an urgent need to develop new targets for therapeutic intervention. The glucagon-like peptide-1 receptor agonist exenatide reduces alcohol consumption in rodents and nonhuman primates, but its efficacy in patients with AUD is unknown.

Methods

In a randomized, double-blinded, placebo-controlled clinical trial, treatment-seeking AUD patients were assigned to receive exenatide (2 mg subcutaneously) or placebo once weekly for 26-weeks, in addition to standard cognitive-behavioral therapy. The primary outcome was reduction in number of heavy drinking days. A subgroup also completed fMRI and SPECT brain scans.

Results

A total of 127 patients were enrolled. Our data revealed that although exenatide did not significantly reduce the number of heavy drinking days compared to placebo, it significantly attenuated fMRI alcohol cue-reactivity in the ventral striatum and septal area, which are crucial brain areas for drug reward and addiction. In addition, the dopamine transporter availability was lower in the exenatide group compared to the placebo group. Exploratory analyses revealed that exenatide significantly reduced heavy drinking days and total alcohol intake in a subgroup of obese patients (BMI>30 kg/m^2). Adverse events were mainly gastrointestinal.

Conclusions

This first randomized controlled trial (RCT) on the effects of a GLP-1 receptor agonist in AUD patients provides new important knowledge on the effects of GLP-1 receptor agonists as a novel treatment target in addiction.

Trial registration

EudraCT: 2016-003343-11 and ClinicalTrials.gov: NCT03232112

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Introduction

Alcohol use disorder (AUD) is an essential contributor to the burden of global disease (1). In Denmark, the cumulative all-cause ten-year mortality risk is almost 30% after a first-time hospital contact due to an alcohol problem (2). Only three medications are approved by the Food and Drug Administration (FDA) to treat AUD; disulfiram, naltrexone, and acamprosate (3). About 40% of patients treated for AUD relapse within the first three years (4), and new targets for therapeutic interventions are urgently needed for this devastating chronic disease (1,3).

The endogenous glucagon-like peptide 1 (GLP-1) is a 30 amino acid peptide hormone produced in the intestinal L-cells in response to food intake (5), as well as in the nucleus tractus solitarius of the medulla oblongata (6). GLP-1 stimulates insulin secretion, inhibits glucagon secretion, and notably, dampens appetite and food intake (5). GLP-1 receptor agonists are approved by the European Medicines Agency (EMA) and FDA to treat type 2 diabetes and obesity (7). Since drugs of abuse and alcohol activate the same reward system that underlies food reward (8), it is conceivable that appetite-regulating peptides such as GLP-1 target areas associated with reward and addiction. In support for this hypothesis, several studies have reported expression of GLP-1 receptors in brain areas associated with reward and addiction (6,9–16). Furthermore, treatment with GLP-1 receptor agonists reduce alcohol intake and decrease relapse-like alcohol drinking in nonhuman primates (17) and rodents (18). In humans, a recent study reported that the GLP-1 receptor 168Ser allele variant was associated with increased alcohol intake in humans (19). However, the effects of a GLP-1 receptor agonist on alcohol consumption in humans remain unknown. To this end, we performed a randomized, placebo-controlled clinical trial lasting 26 weeks plus a long-term six-month follow-up to evaluate the efficacy of the once-weekly GLP-1 receptor agonist exenatide (Bydueron®) at a dose of 2 mg in patients diagnosed with AUD

according to DSM-5. In total, 127 treatment-seeking AUD patients, who had a minimum of five heavy drinking days, i.e., 60/48 grams of alcohol or more per day (men/women) in the past 30 days, were included. Since the pharmacodynamic and pharmacokinetic of a GLP-1 receptor agonist in patients with AUD has not been investigated, we chose a dosing regimen consistent with established tolerability and efficacy in treatment of type 2 diabetes, i.e. exenatide, 2mg subcutaneously once weekly. Importantly, exenatide crosses the blood brain barrier (20), and a similar dosing regiment, i.e. 2 mg subcutaneously once weekly, has recently shown efficacy in other neuropsychiatric disorders including nicotine dependence (21) and Parkinson's disease (20) suggesting a central engagement, possibly mediated, at least in part, by dopamine signaling (22).

The primary endpoint was reduction in heavy drinking days, recorded with the time-line follow-back method (23). A subgroup of the patients had a functional Magnetic Resonance Imaging (fMRI) scan and a single-photon emission computerized tomography (SPECT) scan performed at baseline and at week 26. Using the fMRI technique, we investigated whether exenatide once-weekly would reduce alcohol cue reactivity in brain areas involved in drug reward and addiction, and in top-down regulation of impulsivity (24), as pre-clinical- and clinical evidence suggests that GLP-1 receptor stimulation may be associated with improved cognitive impairment (25). By use of the SPECT scan, we measured the availability of the striatal dopamine transporter (DAT), a key modulator of extracellular dopamine. Dopamine plays a pivotal role in the neurobiological underpinnings of reward (26), and a large body of evidence suggests that brain dopamine homeostasis changes following chronic alcohol intake (27).

Results

Characteristics of the patients

From 7th of August 2017 to 1st of October 2019, 152 patients were screened for eligibility, and 127 patients were enrolled; 62 were randomly assigned to the exenatide group, and 65 were assigned to the placebo group (Figure 1). Overall, the two treatment groups were balanced with respect to baseline characteristics (Table 1). All patients were Caucasians, with a mean age of 52 years. The majority of the patients were men (60%). On average, they had 17 heavy drinking days and an overall alcohol intake of 2400g of pure alcohol over the last month, and 80% fulfilled the criteria for severe AUD, i.e., more than five symptoms, according to DSM-5 (see baseline characteristics- and flowchart for the patients included in the brain-imaging sub-study in Appendix 1 and 2). Of the 127 patients included a total of 58 patients completed the trial, i.e., participated in the last follow-up after 26 weeks of treatment; 25 patients finished prematurely, i.e., participated in a final examination before 26 weeks of treatment. Fifty-five patients participated in the long-term six-month follow-up visit (Supplemental Figure 1), with the last visit held on the 10th of October 2020. The mean (SD) number of injections in the exenatide group was 22.6 (2.2) and in the placebo group 22.1 (2.8) (Supplemental Table 1). There was no difference (p=0.46) between the two groups in time to trial discontinuation (Figure 2). In addition, 25 healthy controls matched with gender, age, and educational status of the patients included were recruited for the fMRI substudy.

Efficacy

For both groups, the number of heavy drinking days (Table 2, Figure 3) and total alcohol intake (Table 2) were strongly reduced, but there were no significant differences between the two groups. The exenatide group had a reduction in BMI of 0.95 (95% CI-1.6 to -0.3, p=0.006), HbA1c of 1.6 mmol/mol (95% CI -2.8 to -0.4, p=0.011) and a worsening in DUDIT-score of 0.96 points (95%

Cl 0.7 to 1.3, p<0.001) relative to the placebo group (Table 2). There were no group differences for FGF21, PEth, or bonemarkers (Supplemental Table 2), life quality measurements; SF-36 (Supplemental Table 3), SCL-92 (Supplemental Table 4) or cognition (Supplemental Table 5). Exenatide once weekly increased urine oxidative stress parameters: 8-oxoGUO of 0.24 nmol/mmol creatinine (95% Cl 0.04 to 0.44, p=0.022) and 8-oxodG of 0.43 nmol/mmol creatinine (95% Cl 0.15 to 0.72, p=0.003) relative to placebo (Supplemental Table 2). In the exenatide group, the plasma level of exenatide was 45.6 pmol/L (95% Cl 16.5 to 74.7, p=0.003), and the overall anti-exenatide antibody binding was 16.1% (95% Cl 6.9 to 25.3, p=0.002) relative to the placebo group (Supplemental Table 2).

Exploratory analyses

Exenatide once weekly did not reduce the number of heavy drinking days in the prespecified subgroup analyses (baseline heavy drinking days, severity of DSM-5 criteria, and geography) (Supplemental Table 6). However, an exploratory subgroup analysis (Supplemental Table 7) including BMI-subgroups, revealed that in obese patients with a BMI >30 kg/m^2 (n=30), exenatide reduced heavy drinking days by 23.6 percentage points (95% Cl -44.4 to -2.7, p=0.034) (Figure 4) and reduced total alcohol intake per 30 days by 1205 grams (95% Cl -2206 to -204, p=0.026) relative to placebo (Figure 5). In patients with a BMI <25 kg/m^2 (n=52), treatment with exenatide increased number of heavy drinking days by 27.5 percentage points (95% Cl 4.7 to 50.2, p=0.024) relative to the placebo group. However, in this subgroup (BMI <25 kg/m^2) the total alcohol intake did not differ between treatment groups. Other exploratory posthoc subgroup analyses were performed to investigate if there were sub-groups responding differently than others on the intervention. However, no significant differences were observed with respect to gender, baseline

craving (PACS score), baseline AUDIT score, baseline number of days without alcohol, baseline total alcohol consumption, fMRI-subgroup (n=22), and SPECT-subgroup (n=16).

Besides the exploratory subgroup analyses, we also looked at the reduction in WHO Risk Drinking Levels (28). Both groups reduced their risk drinking levels, but there was no significant difference between the two groups (Supplementary Table 6).

To explore if there were a correlation between change in HbA1c and change in Heavy drinking days, the Pearson correlation coefficient was computed in the imputed dataset (n=127) to assess linear relationship. Here, we found a weak negative correlation between the two variables, r(12755) = -.27, p = .001. We also found a weak negative correlation between changes HbA1c and changes in total alcohol intake, r(12755) = -.36, p = .001.

Six months long-term follow-up

There was no difference between the two groups at the six-month follow-up after exenatide or placebo discontinuation (Table 2, Supplemental Table 8), except for a higher AUDIT-score (5.1 points, 95% Cl 0.9 to 9.3, p=0.02) in the original exenatide group (adjusted from the end of treatment) compared to the placebo group.

fMRI Alcohol cue-reactivity

The predefined region of interest (ROI) masks was acquired from the WFU PickAtlas. The analyses revealed a statistically significant interaction between treatment and time on the fMRI response in all three ROIs; ventral striatum [F(1,31) = 4.744, p=0.037, partial $\eta 2$ = 0.133]; dorsal striatum [F(1,31) = 6.124, p =0.019, partial $\eta 2$ =0.165]; and putamen [F(1,31) = 4.730, p=0.037, partial $\eta 2$ = 0.132], indicating reduced cue-reactivity after 26 weeks of treatment with exenatide compared to placebo. The ROI analysis in the caudate and nucleus accumbens did not reveal any

significant effects (Figure 6A). At week 26, the cue-induced activity in ventral striatum was significantly lower in the exenatide group compared to placebo (M=-0.176, SE=0.075, p = 0.025). However, in the dorsal striatum (M=-0.142, SE=0.076, p = 0.073) and in the putamen (M=-0.123, SE=0.084, p =0 .153) no significant differences were observed. At baseline, cue-induced activity did not differ between the treatment groups. Within the exenatide group, cue-induced activity was significantly reduced from baseline to week 26 in ventral striatum (M=-0.254, SE=0.116, p = 0.044) and in dorsal striatum (M=-0.351, SE=0.156, p = 0.039), but not in putamen (M=-0.405, SE=0.202, p =0.063). Within the placebo group, we found no statistically significant differences (Figure 6B).

At baseline, the exploratory whole-brain analysis showed no significant difference in cue reactivity between the placebo group and the exenatide group. When comparing cue reactivity in all patients with healthy controls, significant differences were found in the left superior-, and middle frontal gyrus, caudate, and insula (p=0.001). However, at the 26 weeks rescan, these differences were no longer significant. At the week 26 assessment, cue-induced activation was significantly reduced in the exenatide group compared to the placebo group in the following brain areas (Appendix 1: Supplemental Table 13); left caudate nucleus and septal area (Figure 7A), and right middle frontal gyrus (Figure 7B). There was no significant change in cue reactivity in the placebo group at the rescan, but the exenatide group showed a significant reduction in cue-induced activation in the temporal lobe, hippocampus, and parahippocampus (rescans per-protocol; Appendix 1: Supplemental Table 14, Supplemental Figure 5; rescans per-protocol including premature rescans: Supplemental Table 15, Supplemental Figure 6).

Subjective craving results - ALCUE

The analysis showed a significant difference at baseline between the healthy controls and the patients $p<0.001$ ((mean±SD) healthy controls = 8.8 ±15.96, placebo group = 33.5 ± 26.9, exenatide group 30.6 ± 28.6). At the 26 weeks follow-up this was no longer significant, $p = 0.50$ ((mean±SD) healthy controls = 8.8 ±15.96, placebo group = 13.6 ± 12.0, exenatide group 14.8 ± 23.07) and there were no significant difference between the exenatide and the placebo-group ($p= 0.980$).

fMRI Spatial working memory

The voxel-wise analysis showed a significant reduction in the exenatide group at the week 26 rescan compared to placebo in response to the 2-back>1-back task in two clusters in the right frontal pole and right superior frontal gyrus, within the dorsolateral prefrontal cortex ROI (Figure 8 & Appendix 1: Supplemental Table 16). The additional right dlPFC ROI analysis showed no significant change in the exenatide group at week 26 compared to placebo in task-related activations ($F(1,31)$, $p=0.122$, partial $\eta 2 = 0.076$). The reduction in task-related neuronal activations in the exenatide group occurred in the absence of change in cognitive performance on the SCIP ($p=0.93$).

SPECT Dopamine transporter availability

After adjustment for age, there were no significant differences comparing baseline DAT availability of the patients with AUD and healthy controls in striatum $F(1,62)=0.474$, $p=0.494$, caudate $F(1,62)=1.160$, $p=0.286$, and putamen $F(1,62)=0.005$, $p=0.944$ (Figure 9A)

At the week 26 re-scan, DAT availability in striatum, caudate, and putamen was significantly lower in the exenatide group compared to the placebo group (striatum $F(1,13)=4.978$, $p=0.044$; caudate $F(1,13)=8.066$, $p=0.014$; putamen $F(1,13)=6.571$, $p=0.024$) (Appendix 2: Supplemental Table 18, Figure 9B).

Safety

Gastrointestinal symptoms, bodyweight loss, fatigue, and injection site reactions were the most common adverse events reported, and the incidence was higher in the exenatide compared to the placebo group (nausea, 37.1% vs. 15.4%; decreased appetite, 24.2% vs. 9.2%; vomiting, 22.6% vs. 7.7%; overall weight loss, 67.7% vs. 40.0%; fatigue, 12.9% vs. 4.6%, and injection site reaction, 41.0% vs. 0.0%) (Table 3). The gastrointestinal side-effects reported lasted up until the first five weeks of treatment, and the weight loss continued throughout the trial. The injection site reactions were typically small nodules of 1-2 centimeters, hard, mobile, skin-colored, and were reabsorbed within six weeks, leaving no scar. Serious adverse events were reported almost equally between the two groups (exenatide 24.2% vs. placebo 18.5%), and there were no cases of acute pancreatitis or elevation of pancreas enzymes above upper limits. One patient in the exenatide treatment group committed suicide seven weeks after withdrawal from the trial. One patient in the placebo group was found dead after being hospitalized three times in one week for alcohol withdrawal symptoms.

Discussion

This is the first RCT investigating the effects of exenatide in AUD patients. Treatment with exenatide once weekly was not superior to placebo in reducing the number of heavy drinking days in the prespecified analysis. The negative results could reflect the characteristics of the AUD patients included in our RCT. Data from preclinical trials showed that high alcohol-consuming animals decreased their alcohol intake significantly more than low alcohol-consuming animals when treated with a GLP-1 receptor agonist (29,30). In the present trial, 80% of the patients fulfilled the DSM-5 criteria for severe AUD. However, their severity profile, based on baseline alcohol intake and heavy drinking days (Table 1), was less severe than observed in other AUD pharmacotherapy trials (31,32). Another explanation could be that the potent placebo response could have masked a possible beneficial effect of exenatide (Figure 3-4). The observed potent placebo response could be due to the standardized cognitive behaviour therapy (CBT) against AUD (38) offered to all participants in the study, but it could also be due to the less severity profile of the AUD patients included, which is typically linked to a higher placebo response (33). Large placebo responses are also reported in other clinical AUD trials and shown to be negatively correlated with the treatment intervention effect sizes (34).

Predefined fMRI brain- ROI analysis found a reduced alcohol cue-reactivity in the exenatide group compared to the placebo group in the ventral striatum, a region that plays a pivotal role in addiction and relapse (Figure 6). This finding is important because it implies that AUD subjects treated with exenatide lose the incentive salience of alcohol-associated cues. The exenatide-induced reduction in cue-reactivity in the septal area (35) observed in the whole-brain analysis (Figure 7A) is particularly intriguing as this is an area connected to reward (15), and a brain area where GLP-1 receptors are highly expressed (6). These findings are in accordance with a central effect of

exenatide as mentioned in the introduction. Future fMRI studies investigating the effects of GLP-1 receptor agonists on alcohol-cue induced activation should include the septal area as a region of interest (ROI).

Impairments in cognitive processes related to executive function in AUD patients (36), may negatively influence clinical outcomes due to deficits in self-regulation (37). In the fMRI spatial working memory test, we found reduced cue-reactivity in the dorsal prefrontal cortex in the exenatide group compared to the placebo group, possibly indicating a reduced effort to maintain cognitive performance (38).

The SPECT sub-study revealed no significant differences in DAT availability at baseline between the AUD patients and healthy controls, which is in accordance with some earlier findings (39), but in discordance with others (40). After 26 weeks of treatment, the analysis revealed a significant reduction of DAT in the striatum, caudate, and putamen in the exenatide group, compared to placebo, which might compensate for the decreased dopamine activity previously reported in AUD patients (41). Notably, this effect is most likely not acutely induced since no change in DAT availability was observed after acute treatment with exenatide in healthy volunteers (42).

Even though the results from the exploratory post hoc BMI subgroup analysis are preliminary, we think they are of substantial interest because overlapping dysfunctional brain circuits are observed in individuals who suffer from obesity or addiction (8), and deranged GLP-1 signaling is also reported in obese individuals (43). In addition, an fMRI study in obese vs. lean individuals showed that exenatide infusions “normalized” the brain response to a food-paradigm in obese patients with a BMI > 30 kg/m^2 compared to lean individuals (44). Moreover, several GLP-1 receptor agonists have recently been approved to treat obesity (BMI>30 kg/m^2), and other compounds are under

development (7). The reason why the number of heavy drinking days was increased in the subgroup of exenatide-treated patients with a BMI < 25 kg/m^2 compared to placebo-treated patients could be that those lean individuals treated with exenatide experienced a larger decrease in blood sugar (45), and this might be associated with increased alcohol craving (46).

The significant increase in urinary oxidative stress markers in the exenatide group was previously reported in type 2 diabetes patients treated with exenatide (47), but the clinical significance of rising levels of urinary stress parameters 8-oxoGuo and 8-oxodG is currently unknown (48). Notably, increased urinary oxidative stress parameters in patients with type 2 diabetes is associated with increased mortality risk (49), and the clinical impact of these biomarkers should be further investigated.

GLP-1 receptor agonists have shown beneficial skeletal effects in rodents (50). However, in the present trial, no differences in bone turnover markers were observed between groups, indicating that bone-related adverse effects are not of concern in this patient population.

Both the exenatide group and the placebo group exhibit an overall reduction in DUDIT-score after 26 weeks of treatment. However, the exenatide group had a significantly higher DUDIT-score compared to placebo after 26 weeks of treatment (Table 2). An exclusion criterion was a diagnosis of any active substance use disorder (SUD) except for nicotine. Individuals who had a DUDIT score > 6 (men), >2 (women) were screened according to ICD-10 SUD criteria and, if diagnosed with SUD, excluded from the trial. Only four of the 25 included participants with a positive DUDIT score (range between 1-22 points) finished per protocol. This is essential information for a follow-

up study, where it may be relevant to exclude all individuals with a positive baseline DUDIT-score to increase study compliance.

The previously reported safety profile of exenatide once weekly is consistent with the present safety data. Our most significant safety concern was the risk of pancreatitis in patients with AUD (3) combined with the associated risk of exenatide treatment (51,52). Importantly, none of the patients experienced a rise in blood amylase above upper limits or developed pancreatitis. Surprisingly, the injection site reactions to exenatide were a bigger problem for the patients due to unexpected concerns from their relatives, who might have been unaware of their AUD diagnosis. This led to a 6.5 % withdrawal rate specifically due to injection site reactions in our AUD trial compared with only 0.5% in exenatide treated patients with type 2 diabetes (53). The gastrointestinal (GI) side effects, which are well-recognized but typically transient (54) was in the exenatide group (44.1%) higher than reported in diabetes trials (55,56). Also, 23.6% of placebo-treated patients experienced GI side-effects, indicating that this group of patients may have a GI vulnerability (57). Only a single RCT has investigated the effects of pre-treatment with anti-emetics, reporting a significant reduction in nausea and vomiting in exenatide-treated healthy subjects (58).

Large drop-out rates are often observed in AUD intervention trials (59) and the present study is - with a drop-out of 54.3% - no exception. Although our sensitivity analysis (Supplemental Table 9) confirmed the robustness of the results even with imputations of missing data, the present drop-out rate (69 out of 127) remains a concern when evaluating the reproducibility and reliability of the findings. Weekly visits for 26 weeks might have been a contributory factor. However, in accordance with the EMA guidelines (60), we chose a study duration time of 26 weeks to see if

there was a sustained treatment effect, lasting longer than the 12 weeks often reported for alcohol RCTs (61).

The approved 2 mg dosing regimen for diabetes patients is reported as the maximally efficacious dose for glucose control, reduction in body weight, and tolerable side effects (62). Our data also shows that AUD patients obtain the same incretin response as diabetes patients with respect to improved glycemic control, weight loss, and side effects. Also preclinically, the standard exenatide dose used in preclinical food reward trials (63) has shown effects in preclinical alcohol self administration experiments (64,65). We did report a central effect in the brain imaging sub-studies, but of course, we cannot rule out that the standard dose given, was too low to elicit a reduction in number of heavy drinking days. However, the mean plasma exenatide level in this study were four times as high as reported as the minimal effective concentration in humans ≈ 50 pg/ml (66). Also, due to safety concerns in this vulnerable group of patients, we did not raise the dose above the registered dose for treatment of type 2 diabetes.

Previous studies in diabetes patients have reported that while 45% of individuals receiving exenatide generate low-titer anti-exenatide antibodies (67), there is no apparent correlation between antibody titers and the effect of exenatide on mean HbA1c (55,67). To the best of our knowledge, there is also no evidence of altered exenatide clearance in AUD patients. The renal elimination of exenatide (5) is an advantage in this group of patients, who typically have a heightened risk of hepatic injury (1).

One would expect a correlation between reduced brain alcohol cue reactivity and alcohol consumption. However, this was not the case in the present study, neither for the whole group of patients (n=127) nor for the subgroup of patients that were fMRI-scanned (n=22) or SPECT-

scanned (n=16). The sample size of the fMRI BMI-subgroups with BMI<25 (n=7) or BMI>30, (n=5), was too small to further explore, if the overall fMRI striatal responses were correlated with heavy drinking days in the overweight or obese subgroups. Only a few RCTs on AUD patients, including fMRI measurements at baseline and follow-up, have been performed (68), and most studies have been underpowered or have to varying study populations to report significant clinical treatment effects (69).

Materials and Methods

Trial design

This randomized, placebo-controlled, double-blinded clinical trial was conducted out of four alcohol outpatient clinics in Copenhagen, Denmark. The main trial comprised a 26-week treatment period investigating the primary- and secondary endpoints. To evaluate the potential long-term effects, a single follow-up visit was conducted six months post-treatment (24). A subgroup of the participants also underwent a functional Magnetic Resonance Imaging (fMRI) scan and a Single-photon Emission Computed Tomography (SPECT) DAT scan at baseline and after 26 weeks of treatment.

The study protocol, statistical analysis plan and de-identified individual participant data, except raw fMRI-, MRS-data, and alcohol dictionaries, will be available at the Mendeley database (70) . Data will be available at publication, and access criteria are a methodologically sound proposal with an approved aim directed to the corresponding author, and requestors will have to sign a data access agreement. Data will be available for five years.

Patients

All potential participants received oral and written information about the project. Before signing the written consent form, the alcohol breath concentration had to be below 0.5‰, which is the same limit as driving a motor vehicle in Denmark (71). Eligible patients were 18-70 years of age, diagnosed with AUD according to DSM-5 and alcohol dependence according to ICD-10 and treatment-seeking. Inclusion criteria required a minimum of five heavy drinking days, i.e., 60/48 grams of alcohol or more per day (men/women), in the past 30 days, measured by use of the Time-Line Follow Back (TLFB) method (72). Key exclusion criteria included severe mental disorder, other drug use disorder, a history of diabetes, pancreatitis, alcohol withdrawal seizures, and current

treatment with drugs against alcohol dependence (disulfiram, acamprosate, naltrexone, and nalmefene). Full in- and exclusion criteria are listed in Supplemental Table 10. The healthy controls included in the fMRI-sub study (n=25) were matched by sex, age, and educational level. All patients were recruited from outpatient alcohol treatment facilities in the suburbs of Copenhagen or through our project webpage, and healthy controls, via the project webpage. No patients were involved in setting the research question, planning the study, interpreting-, or writing up the results. The results of the trial and the assigned intervention will be disseminated to all patients and healthy participants.

Procedures

The randomization was stratified in terms of sex, age (+/- 40 years of age), and number of heavy drinking days at baseline (four strata), and the patients were randomly assigned 1:1 by REDCap (73), to receive exenatide once weekly (Bydureon®), 2 mg or placebo subcutaneously. The weekly injections were administered by an unblinded project nurse who did not participate in any assessments or behavioral treatment sessions. No randomization was performed in the imaging subgroup, as all eligible patients were invited to participate.

Patients who participated in the brain imaging sub-study were scanned before receiving the first injection and again after 26 weeks of treatment. Throughout the trial, patients received the assigned treatment blindfolded by an unblinded nurse at the outpatient clinic to whom they also delivered their weekly alcohol diary. Patients were assessed by blinded project staff at the time of screening, at week 4, 12, 20, 26 (end of the main trial), and at the long-term six-month follow-up visit (Supplemental Table 11, Supplemental Figure 2). At every assessment, weight, somatic symptoms, or diseases since the last visit were recorded, and safety blood samples were collected. In case medical assistance was needed, a 24-hour phone line was available. As a safety precaution due to

earlier associations of pancreatitis caused by GLP-1 receptor agonist treatment (74), blood pancreas amylase was measured at all assessments. Participants with initial severe GI side-effects received injections every second week for the first six weeks to reduce GI-symptoms. All harms were recorded up until ten weeks after termination of the intervention e.g week 26.

Throughout the trial, all patients received the assigned treatment as an add-on to standard AUD behavioral treatment, which included therapy sessions every second week, with a combination of motivational interviewing (MI), cognitive therapy, and family therapy with a blinded therapist. Patients discontinuing the trial after a minimum of eight weeks were encouraged to participate in a premature final visit and rescan. Only patients completing the week 26 visit (premature + per protocol) were invited for the long-term six-month follow-up visit.

The healthy fMRI control group was assessed for eligibility before brain imaging at the Neurobiology Research Unit at Rigshospitalet, Copenhagen, Denmark. See Appendix 1 for full details of the fMRI sub-study and Appendix 2 for the SPECT sub-study.

Outcomes

The primary endpoint was change in heavy drinking days, from baseline to week 26, as recorded by the TLFB-method. Secondary endpoints included changes in total alcohol consumption; number of days with no alcohol consumption; Penn Alcohol Craving Scale (PACS) score; Alcohol Use Disorders Identification Test (AUDIT) score; Drug Use Disorders Identification Test (DUDIT) score; Screen For Cognitive Impairment in Psychiatry (SCIP) test; Fagerströms Test for Nicotine Dependence; blood gamma-glutamyl transferase (GGT); blood alanine aminotransferase (ALAT), blood phosphatidyl-ethanol (PEth), mean cell volume (MCV); glycemic control parameters (HbA1c), body weight, blood pressure, heart rate. Moreover, measures of health and life quality; Short Form Health Survey (SF-36), Symptom Checklist (SCL-92); SPECT DAT

specific binding ratio (BPnd); fMRI BOLD signal change and change in subjective craving assessed with an alcohol cue-reactivity task; change in top-down regulation assessed with a fMRI spatial working memory task; change in heavy drinking days at six-month follow-up. Additional methodological details regarding the analysis of blood- and urine samples are available in the supplementary text.

Statistical analysis

The study was designed to have 90% power to detect a 28-percentage point treatment difference between the two groups with an estimated drop-out of 40%. We planned to include 114 patients, but due to a 60% drop-out, we extended enrolment until the 1st of October 2019 or until 144 patients were included, whichever came first. All continuous outcomes were analyzed with an ANOVA adjusted for baseline until the last observational endpoint, and missing data were imputed with the use of multiple imputations in the mice package (75) in R software version 3.6.0 (76), method = "pmm" (predictive mean matching), and the number of imputed datasets = 100.

No adjustment for covariates was performed. SCIP data were analyzed with a linear mixed model, adjusted for benzodiazepine intake at the time of the assessment. DUDIT data were analyzed with a censored regression model due to zero-inflated values. An exploratory subgroup analysis based on the World Health Organization (WHO) body mass index (BMI) categories (77) was performed to see if the effect of the treatment was related to baseline BMI. The statistical analysis plan was uploaded to ClinicalTrials homepage (78) and the dataset was locked before any analysis were performed. All statistical analyses, except the post hoc analysis regarding exenatide plasma levels, were performed blinded. The hypothesis-test was two-sided, the level of statistical significance was 5%, and a confidence interval of 95%. All efficacy and safety analyses were performed after

the intention-to-treat principle. Analyses were performed with the R software version 3.6.0 (76). See appendix 1 and 2 for the complete statistical method for the fMRI- and SPECT analyses.

Study approval

The protocol was approved by the Danish Ethics Committee of the Capital Region, Copenhagen, Denmark (H-17003043), the Danish Medical Agency (2017014028), and the Danish Data Protection Agency (RHP-2017-029). The trial was monitored by an independent study monitor (GCP unit, Copenhagen, Denmark). Protocol modifications performed after trial commencement are shown in Supplemental Table 12. All participants provided written informed consent prior to study inclusion. The funding sources and the manufacturer of exenatide once weekly (Bydureon®, AstraZeneca), had no influence on the trial design or data analysis. The trial was conducted according to the Helsinki declaration, and the authors assume responsibility for the accuracy of data, analysis, and overall fidelity to the trial protocol. Trial registrations; ClinicalTrials.gov: NCT03232112 and EudraCT: 2016-003343-11.

Author contributions:

Conceptualization: AFJ, TV

Data curation: MKK, CE

Formal analysis: MKK, AFJ, CE (statistical power analysis & statistical analysis plan); MKK & CE (clinical data); MKK, MEJ (SPECT data); JM (N-back task fMRI); PMF, MKK, AL (ALCUE fMRI), MPG (FGF-21); HEP (urine oxidative stress parameters); NRJ (plasma PINP, CTX, TRAP-5b); JJH (exenatide- and antibody plasma levels); MB (plasma PEth levels)

Funding acquisition: AFJ, MKK

Investigation: MKK, MEJ, NLD, MM, CFJ, AMJ, VZ; GTK (SPECT scans)

Methodology: AFJ, TV, MKK, MEJ, KWM, HB, NV, GMK, UB

Project administration: AFJ (sponsor investigator), MKK, MEJ

Software: SVK (ALCUE fMRI); PMF (ALCUE fMRI adaptation to E-prime)

Validation: MKK, AFJ, CE (clinical data); MKK, AL, SVK, PMF (ALCUE fMRI-data); MKK, JM (N-back task fMRI data); MKK, MEJ, GKT (SPECT data); MPG (FGF-21); HEP (urine oxidative stress parameters); NRJ (plasma PINP, CTX, TRAP-5b); JJH (exenatide- and antibody plasma levels); MB (plasma PEth levels)

Visualization: MKK (clinical data); MKK, MEJ (SPECT DATA); JM (N-back task fMRI data); SVK, MKK, AL (ALCUE fMRI-data)

Writing – original draft: MKK; JM (N-back task fMRI)

Writing – review & editing: All authors

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Supplementary Materials

Methods: analysis of serum, plasma, and urine samples

Supplemental Figure 1 to 3

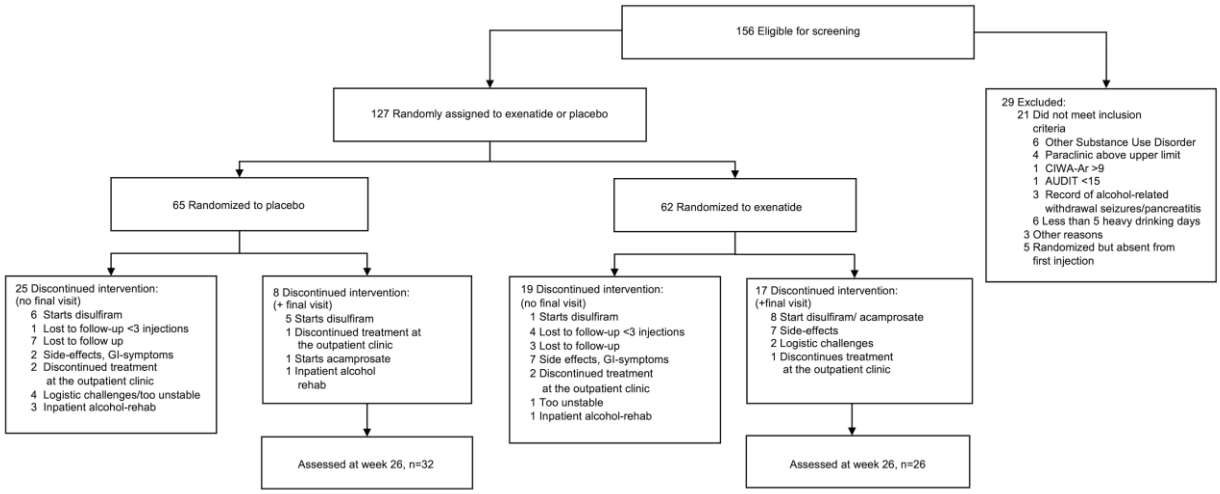
Supplemental Table 1 to 12

Appendix 1: fMRI Alcohol cue-reactivity (ALCUE) and Spatial working memory (N-back task)

Appendix 2: SPECT (single photon emission computed tomography scan)

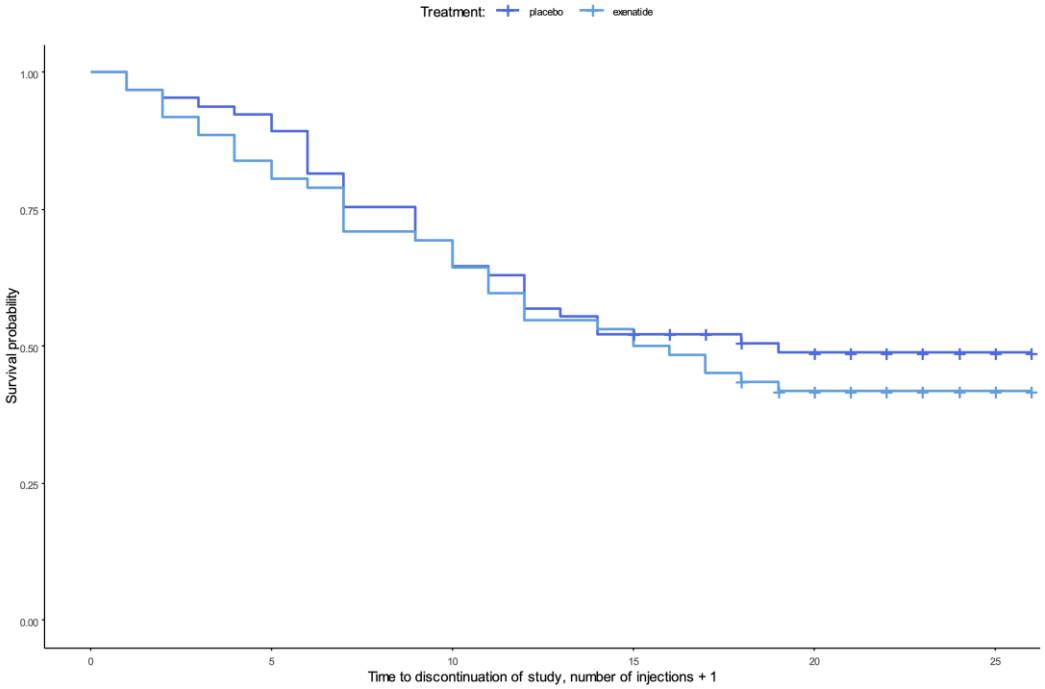
Figures

Figure 1: CONSORT flow diagram



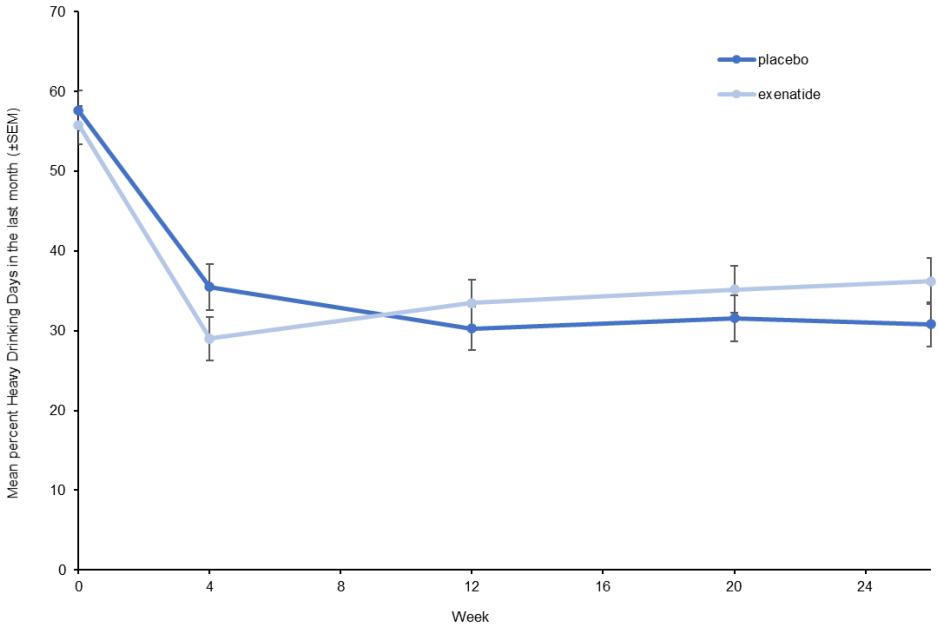
Study diagram of the patient flow according to CONSORT 2010 statement. Details regarding initial meetings and ineligibility for screening can be found in Supplemental Figure 3, and a flowchart for the six-month follow-up can be found in Supplemental Figure 1. AUDIT denotes Alcohol Use Disorders Identification Test and CIWA-Ar, Clinical Institute Withdrawal Assessment of Alcohol Scale, Revised. Of the 127 patients included in the study, 65 patients were randomized to exenatide 2 mg once weekly, and 62 patients were randomized to placebo. Thirty-two patients from the exenatide group, and 26 patients from the placebo group completed the study after 26 weeks of trial participation.

Figure 2: Withdrawal / lost to follow up Kaplan Meyer survival curve



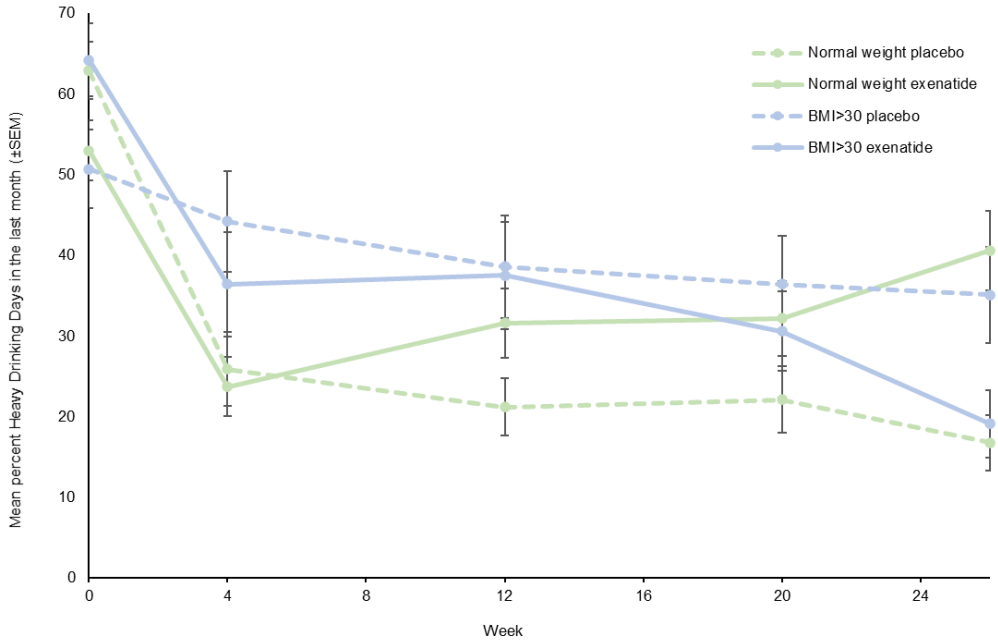
Kaplan Meyer survivalcurve. The time to discontinuation was not significantly different in the two groups (p = 0.46). Input data is the number of injections +1 because patients were registered as discontinued in the week after the last injection was received. All patients included (n=127).

Figure 3: Reduction in Heavy Drinking Days



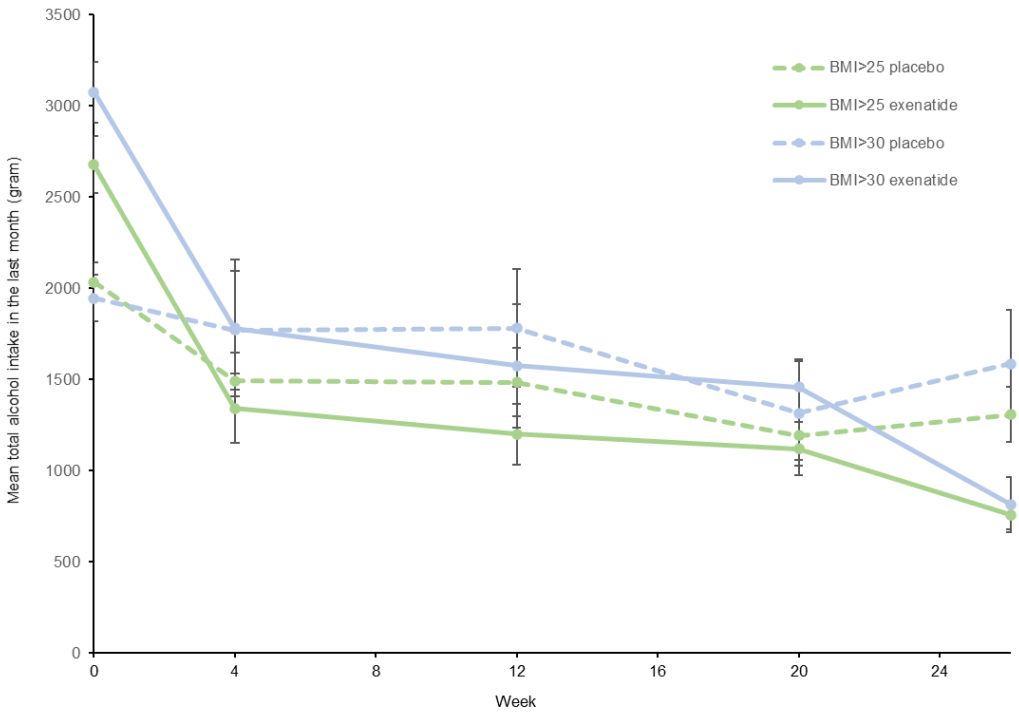
Mean percent Heavy Drinking Days in the last 30 days, measured with the Time-Line Follow Back (TLFB) method, at all assessments (week 0, week 4, week 12, week 20, week 26). Data were analyzed with an ANOVA adjusted for baseline, and missing data is imputed with the use of multiple imputations as described in the text (n=127). Data represent mean ± SEM.

Figure 4: Reduction in Heavy Drinking Days - BMI subgroups



Exploratory analysis af mean percent Heavy Drinking Days in the last 30 days, measured with the Time-Line Follow Back (TLFB) method, at all assessments (week 0, week 4, week 12, week 20, week 26) within the BMI subgroups. Normal weight (n = 52), BMI > 30 (n = 30). Only significant findings from Supplementary Table 7 are included. Data were analyzed with an ANOVA adjusted for baseline, and missing data is imputed with the use of multiple imputations as described in the text. Data represent mean ± SEM.

Figure 5: Reduction total alcohol intake - BMI subgroups



Exploratory analysis af mean total alcohol intake in the last 30 days, measured with the Time-Line Follow Back (TLFB) method, at all assessments (week 0, week 4, week 12, week 20, week 26) within the BMI subgroups. BMI>25 (n = 75), BMI > 30 (n = 30). Only significant findings from Supplementary Table 7 is included. Data were analyzed with an ANOVA adjusted for baseline, and missing data is imputed with the use of multiple imputations as described in the text. Data represent mean ± SEM.

Figure 6: fMRI ALCUE ROI results

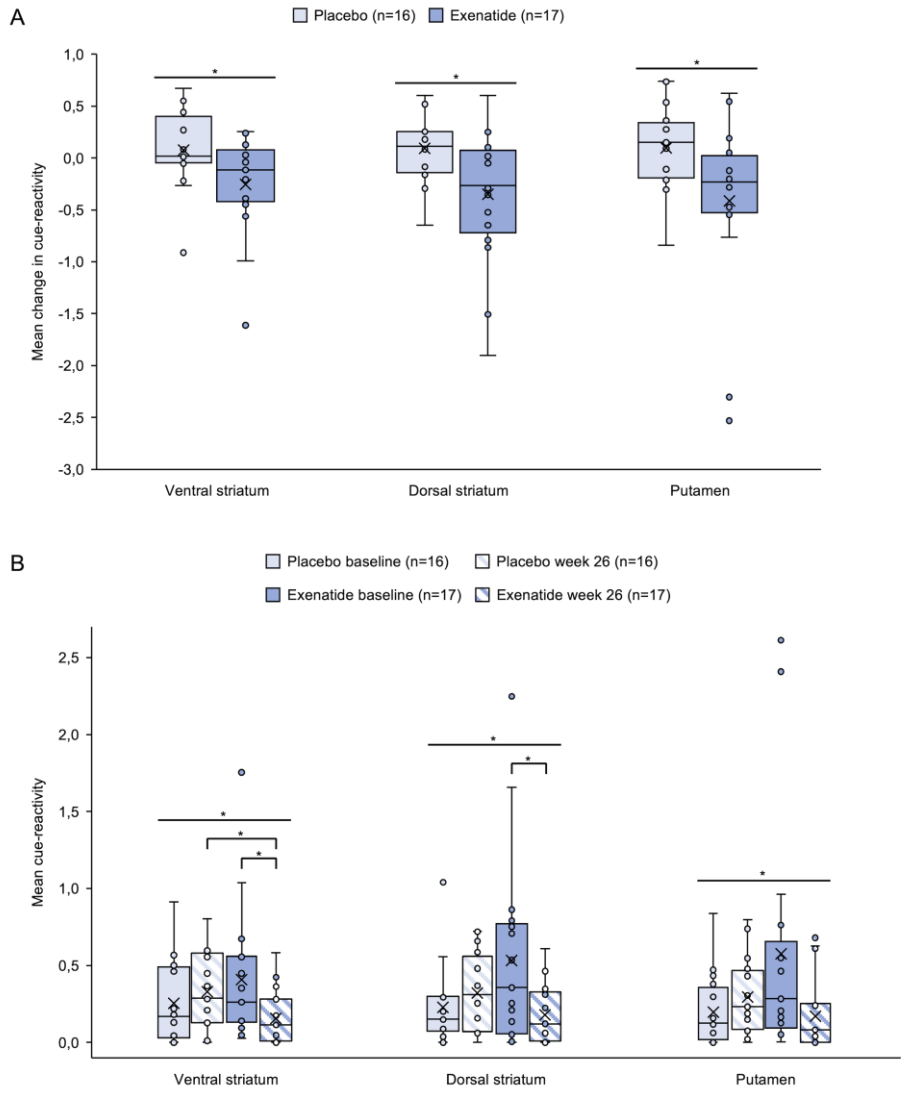
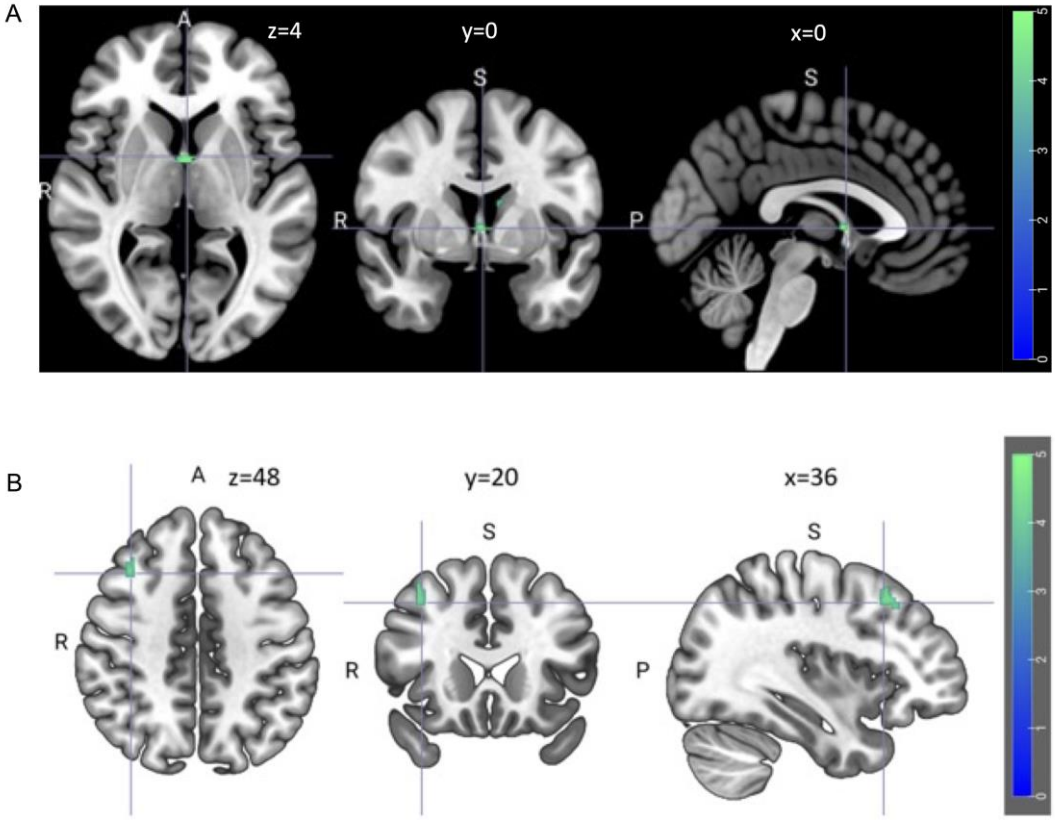


Figure 6A: Key fMRI findings showed reduced cue-reactivity after 26 weeks of treatment with exenatide compared to placebo. Analysis revealed statistically significant interaction between the treatment and time on fMRI response in all three ROIs; ventral striatum [F(1,31) = 4.744, p =0.037, partial η2 =0.133]; dorsal striatum [F(1,31) = 6.124, p = 0.019, partial η2 =0.165]; putamen [F(1,31) = 4.730, p =0.037, partial η2 =0.132].*p<0.05.

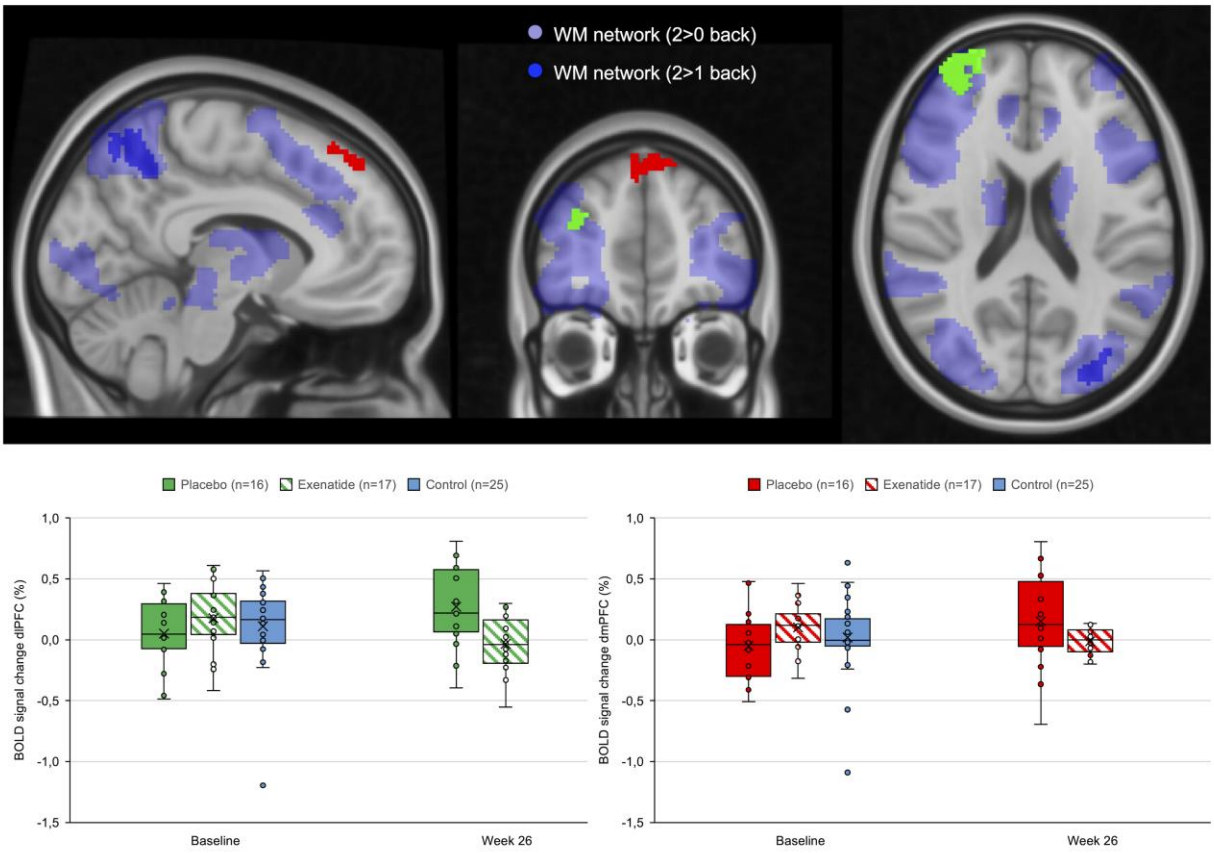
Figure 6B: In more detail, we found that at week 26, cue-induced activity was significantly lower in ventral striatum after treatment with exenatide compared to placebo (M=-0.176, SE=0.075, p = 0.025), but not in dorsal striatum (M=-0.142, SE=0.076, p = 0.073) nor in putamen (M=-0.123, SE=0.084, p =0 .153). At baseline cue-induced activity did not differ significantly between groups. Within the exenatide group, cue-induced activity was significantly reduced from baseline to week 26 in ventral striatum (M=-0.254, SE=0.116, p = 0.044) and in dorsal striatum (M=-0.351, SE=0.156, p = 0.039), but not in putamen (M=-0.405, SE=0.202, p =0.063). Within the placebo group, no statistically significant differences were found. (A+B) ROI data were analyzed using a repeated-measures ANOVA, including factors group and time and an independent sample T-test comparing groups (placebo and exenatide). Placebo n=16, exenatide n=17. (A & B) Boxes represents upper and lower quartiles, the line represents the median, and the X represents the mean. Horizontal lines indicate significant interactions bwteen treatment and time (*p<0.05), and brackets indicate significant simple effects (*p<0.05).

Figure 7: fMRI ALCUE wholebrain results



Reduced cue-induced activation in the exenatide group compared to the placebo group after 26 weeks of treatment in the left caudate nucleus and septal area (x,y,z coordinates = 0, 0, 4) (Panel A) and right middle frontal gyrus (x,y,z coordinates = 36, 20, 48) (Panel B). A two-sample t-test were performed for the post-hoc analyses to compare groups (placebo, exenatide) and within a group across time (placebo/exenatide: T1, T2). Contrast alcohol - neutral stimuli, combined voxel-wise- [P < 0.001] and cluster-extent threshold [k >= 101 voxels], corresponding to pFWE < 0.05 (n=22).

Figure 8: fMRI spatial working memory task (N-back task)



The exenatide group showed a reduction at follow-up in the response to 2-back>1-back task compared to the placebo group (2-way mixed effect ANOVA; placebo n=16, exenatide n=17, control n=25) in two prefrontal clusters (frontal pole x,y,z=34, 54, 20, corrected $p<0.002$; superior frontal gyrus x,y,z=4, 46, 46, corrected $p<0.001$). Boxes represents upper and lower quartiles, the line represents the median, and the X represents the mean.

Figure 9: SPECT DAT results combined

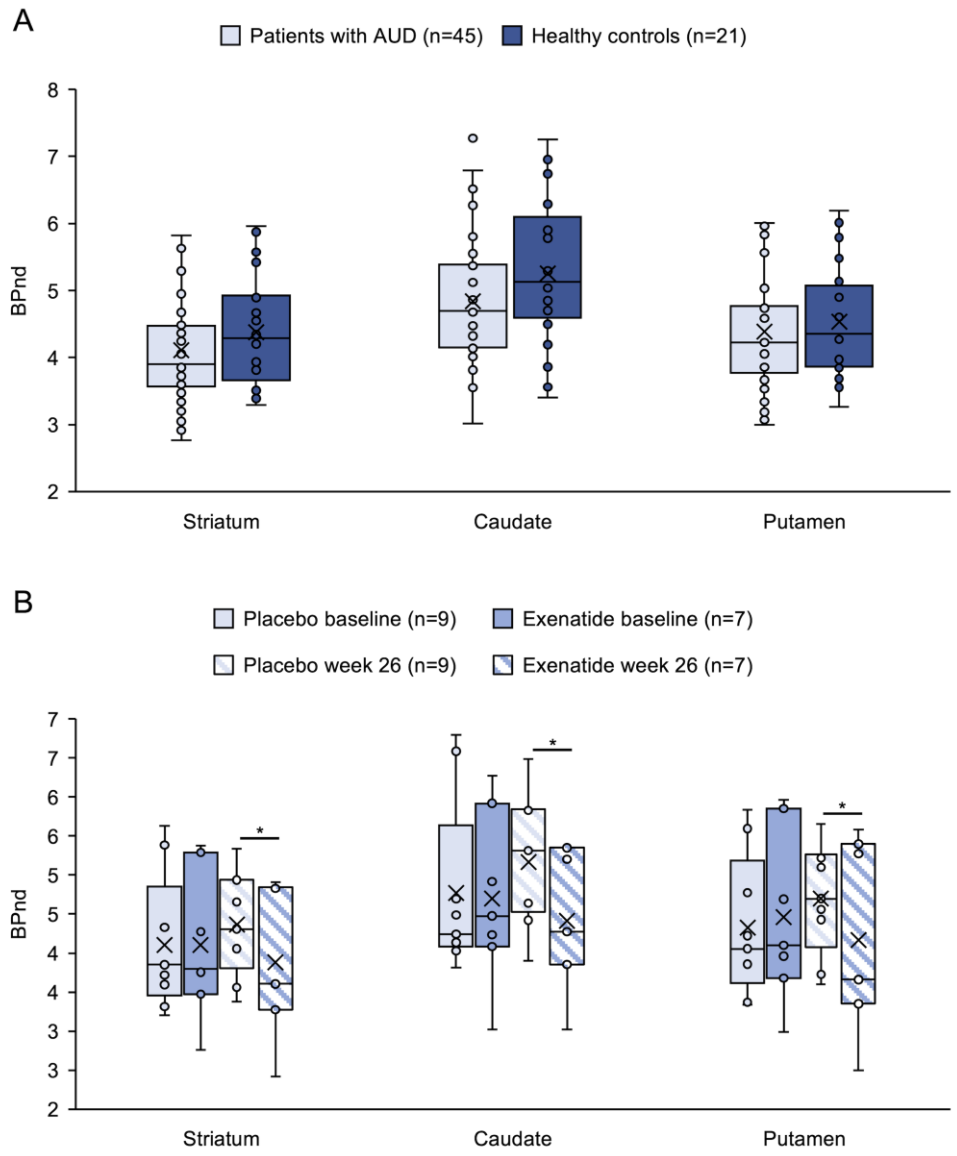


Figure 9A: Baseline DAT availability in striatum, caudate, and putamen in AUD patients did not differ from healthy controls. Data were analyzed with a one-way analysis of covariance (ANCOVA), adjusted for baseline DAT availability; healthy controls n=21, patients at baseline n=45. *p<0.05.

Figure 9B: At the week 26 re-scan, DAT availability in striatum, caudate, and putamen was significantly lower in the exenatide group compared to the placebo group (striatum $F(1,13)=4.978$, $p=0.044$; caudate $F(1,13)=8.066$, $p=0.014$; putamen $F(1,13)=6.571$, $p=0.024$), Data were analyzed with an ANCOVA adjusted for age; Placebo n=9, exenatide n=7. *p<0.05. (A &B) Boxes represents upper and lower quartiles, the line represents the median, and the X represents the mean.

Table 1. baseline demographic and clinical characteristics

Characteristics		Placebo (n= 65)	Exenatide (n=62)
Sex [a]	Male, No. %	39 (60.0%)	37 (59.7%)
Age	Mean (SD)	52.5 (10.0)	52.1 (10.8)
	[a]Under 40 years of age, No. %	7 (10.8%)	8 (12.9%)
	[a]40 years of age and above, No. %	58 (89.2%)	54 (87.1%)
Social status	Cohabitation/married, No. %	36 (55.4%)	32 (51.6%)
	Missing, No. %	1 (1.5%)	0 (0.0%)
Job	Job, No. %	31 (47.7%)	30 (48.4%)
	Missing, No. %	19 (29.2%)	19 (30.6%)
Education	Lower secondary school, No. %	7 (10.9%)	9 (14.5%)
	Upper secondary school, No. %	3 (4.7%)	2 (3.3%)
	Vocational education/short-cycle higher education, No. %	22 (34.4%)	25 (40.3%)
	Medium cycle higher education/Higher education, No. %	32 (50.0%)	26 (41.9%)
AUDIT	Mean (SD)	25.9 (5.2)	25.6 (5.7)
ICD10 Alcohol dependence	3 symptoms, No. %	14 (21.5%)	14 (22.6%)
	4 symptoms, No. %	16 (24.6%)	17 (27.4%)
	5 symptoms, No. %	16 (24.6%)	20 (32.3%)
	6 symptoms, No. %	19 (29.3%)	11 (17.7%)
DSM-5 Alcohol use disorder	Mild (2-3 symptoms), No. %	4 (6.2%)	7 (11.3%)
	Moderate (4-5 symptoms), No. %	7 (10.7%)	5 (8.1%)
	Severe (>5 symptoms), No. %	54 (83.1%)	50 (80.6%)
Heavy drinking days	Mean (SD)	17.3 (8.5)	16.7 (8.2)
Heavy drinking days, Randomization-strata	[a] 5-11 heavy drinking days, No. %	22 (33.8%)	22 (35.5%)
	[a] 12-17 heavy drinking days, No. %	13 (20.0%)	13 (21.0%)
	[a] 18-23 heavy drinking days, No. %	10 (15.4%)	10 (16.1%)
	[a] 24-30 heavy drinking days, No. %	20 (30.8%)	17 (27.4%)
Days without alcohol consumption/30 days	Mean (SD)	9.92 (7.9)	9.11 (7.3)
Total alcohol consumption (gram of alcohol/30 days)	Mean (SD)	2430 (1860)	2370 (1580)
Weight (kg)	Mean (SD)	82.1 (15.4)	82.8 (18.9)
BMI (kg/m^2)	Mean (SD)	26.7 (4.6)	26.7 (5.2)
HbA1c (mmol/mol)	Mean (SD)	33.2 (3.9)	34.9 (4.1)

Baseline demographic and clinical characteristics. Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; ICD-10, International Classification of Diseases, Tenth Revision; DSM-5, Diagnostic and Statistical Manual of Mental Disorders; BMI, body mass index (calculated as weight in kilograms divided by height in square meters); HbA1c, glycated hemoglobin, [a]randomization strata

Table 2. Change in End Points from baseline to week 26

Characteristic	Placebo group n = 65	Exenatide group n = 62	Estimated treatment difference, exenatide vs placebo (95% Cl)	p-value
Clinical, mean (95% Cl)				
Self-reported drinking/alcohol scales				
Heavy drinking days, pp[a] (Primary endpoint)	-26.8 (-34.4 to -19.2)	-19.6 (-27.4 to -11.8)	6.0 (-7.4 to 19.4)	0.37
Total alcohol consumption, g/30 days	-1313 (-1586 to -1039)	-1304 (-1584 to -1024)	-42.0 (-507.7 to 423.7)	0.86
Days without alcohol consumption,pp[a]	20.6 (13.1 to 28.1)	11.3 (3.6 to 18.9)	-10.5[b] (-2.6 to 23.4)	0.11
PACS	-7.3 (-8.8 to -5.8)	-5.4 (-7.0 to -3.9)	1.2 (-1.7 to 4.0)	0.42
AUDIT	-8.2 (-10.0 to -6.5)	-7.0 (-8.8 to -5.1)	1.1 (-2.9 to 5.0)	0.59
Alcohol biomarkers				
PEth, µmol/l	-0.03 (-0.3 to 0.2)	-0.09 (-0.3 to 0.2)	-0.13 (-0.7 to 0.4)	0.64
Liver/pancreas parameters				
ALAT, U/L	-7.9 (-13.7 to -2.1)	-3.7 (-9.7 to 2.2)	-2.5 (-14.3 to 9.4)	0.68
GGT, U/L	-16.5 (-45.0 to 12.0)	-13.6 (-42.8 to 15.6)	-5.4 (-58.2 to 47.4)	0.84
MCV, fL	-1.3 (-2.1 to -0.5)	-1.8 (-2.6 to -1.0)	-0.6 (-2.1 to 1.0)	0.45
Pancreas type Amylase, U/L, n=110	-0.4 (-2.5 to 1.6)	4.1 (2.0 to 6.3)	4.2 (-6.1 to 8.4)	0.054
P-Amylase, U/L, n=17	32.5 (20.4 to 44.5)	18.3 (6.9 to 29.7)	-11.5 (-32.3 to 9.3)	0.30
Clinal measures				
Bodyweight, kg	-0.5 (-1.8 to 0.9)	-2.9 (-4.3 to -1.5)	-2.4 (-5.0 to 0.2)	0.07
Systolic blood pressure, mm Hg	-4.2 (-7.6 to -0.9)	-4.3 (-7.7 to -0.8)	-0.3 (-7.0 to 6.4)	0.93
Diastolic blood pressure, mm Hg	0.2 (-2.2 to 2.6)	-1.9 (-4.4 to 0.6)	-2.3 (-9.1 to 4.4)	0.32
Pulse, p	2.4 (0.1 to 4.7)	5.0 (2.6 to 7.4)	2.2 (-2.6 to 6.9)	0.36
Waist circumference, cm	-1.5 (-3.2 to 0.3)	-3.5 (-5.3 to -1.7)	-1.8 (-5.1 to 1.5)	0.27
BMI	-0.06 (-0.4 to 0.3)	-1.01 (-1.4 to -0.6)	-0.95 (-1.6 to -0.3)	0.006
Glucose metabolism				
Glycaemic control parameters HbA1c, mmol/mol	1.4 (0.8 to 2.0)	-0.7 (-1.3 to -0.1)	-1.6 (-2.8 to -0.4)	0.011
Rating scales				
DUDIT[i]	-8.3 (-8.9 to -7.8)	-7.3 (-7.7 to -6.9)	1.0 (0.7 to 1.3)	>0.001
Fagerstrom Test for Nicotine Dependence, n=73[c]	-0.4 (-1.1 to 0.2)	-0.2 (-0.8 to 0.4)	0.4 (-0.6 to 1.4)	0.44
SF-36, general health[d+f]	12.3 (9.3 to 15.2)	7.9 (4.9 to 10.9)	-2.0 (-7.7 to 3.7)	0.48
SCL-92, Total scale[e+h]	-0.4 (-0.5 to -0.3)	-0.2 (-0.3 to -0.1)	0.1 (-0.1 to 0.3)	0.38
Six-month follow-up				
Heavy drinking days (pp[a]) from baseline, n=43	-5.6 (-8.4 to -2.7)	-3.2 (-5.9 to -0.5)	2.6 (-1.3 to 6.5)	0.18
Heavy drinking days (pp[a]) from end of treatment, n=43	-1.8 (-4.6 to 0.9)	0.5 (-2.1 to 3.0)	2.5 (-1.2 to 6.3)	0.19
SCIP (baseline to follow-up)				
SCIP total[g+j], n = 127	-	-	0.2 (-3.5 to 3.9)	0.93
SCIP (week 4 to follow-up)				

SCIP total[f+g], n = 111	-	-	-0.9 (-4.9 to 3.2)	0.67

Change in End Points from baseline to week 26. ALAT denotes Alanine aminotransferase; AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index (calculated as weight in kilograms divided by height in square meters); DUDIT, Drug Use Disorders Identification Test; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; MCV, Mean cell volume; PACS, Penn Alcohol Craving Scale; Peth, Phosphatidylethanol; SCIP, Screen for Cognitive Impairment in Psychiatry test; SCL-92, Symptom Checklist, and SF-36, Short-Form Health Survey. [a]pp = percentage points, [b]a reduction, indicates fewer 0-days, [c]all individuals who reported smoking during the 26 weeks, [d] scores range from 0-100 with a high score defining a more favorable health state, [e]scores range from 0-4, with lower scores indicating higher quality of life, [f]Supplemental Table 8 for full details of the sub-scales, [g]linear mixed model, adjusted for intake of benzodiazepine at the time of the assessment, [h]Supplemental Table 9 for full details of the sub-scales, [i]a censored regression model was used for this analysis due to zero-inflated values, scores range from 0-44 with a higher score indicating substance use disorder, [j]Supplemental Table 10 for full details of the sub-scales.

Table 3. Adverse events/Serious adverse events

		Placebo n=65	Exenatide n=62
no. of patients/total no. (%)			
Serious adverse events	Any serious adverse events	8 (18·5)	11 (24·2)
	Hospitalisations due to withdrawal symptoms (in total)	6 (9·2)	9 (14·5)
	Hospitalised due to withdrawal symptoms (individuals)	4 (6·2)	4 (6·6)
	Death	1 (1·5)	0 (0·0)
	Suicide (7 weeks after the end of participation)	0 (0·0)	1 (1·6)
	Acute appendicitis	0 (0·0)	1 (1·6)
	Suicidal behavior	1 (1·5)	0 (0·0)
Adverse events/Adverse reactions	Weight loss from baseline - overall[a]	26 (40·0)	42 (67·7)
	0-2 kg	13 (20·0)	17 (27·4)
	2-4 kg	10 (15·4)	7 (11·3)
	> 4 kg	3 (4·6)	18 (29·0)
	Weight gain from baseline[a]	31 (47·7)	12 (19·3)
	Nausea	10 (15·4)	23 (37·1)
	Injection site reactions	0 (0·0)	26 (41·9)
	Loss of appetite	6 (9·2)	15 (24·2)
	Vomiting	5 (7·7)	14 (22·6)
	Upper-respiratory-tract infection	9 (13·8)	8 (12·9)
	Elevated alanine transaminase (ALAT)	8 (12·3)	5 (8·1)
	Fatigue	3 (4·6)	8 (12·9)
	Generalized itching	7 (10·8)	2 (3·2)
	Changes in stool pattern	5 (7·7)	3 (4·8)
	Gastroenteritis	3 (4·6)	3 (4·8)
	Headache	4 (6·2)	1 (1·6)
	Dizziness	2 (3·1)	3 (4·8)
	Reflux	2 (3·1)	3 (4·8)
	Diarrhea	3 (4·6)	1 (1·6)
	Muscle weakness	1 (1·5)	2 (3·2)
	Rash	0 (0·0)	3 (4·8)
	Other pain	0 (0·0)	3 (4·8)
	Muscle-pain	1 (1·5)	1 (1·6)
	Lower urinary tract infection	0 (0·0)	1 (1·6)
	abdominal pain	1 (1·5)	0 (0·0)
	Worsening in anxiety level	0 (0·0)	1 (1·6)
	Miscellaneous	19 (29·2)	19 (30·6)

Events reported minimum once per participant. [a]Weight-loss and weight-gain are presented according to numbers of individuals with weight change at last visit, rather than several individual events across all time points.