Anti-obesity effects of the novel long-acting amylin analogue ZP4982 in high-fat diet fed rats

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Background and aim

Amylin is a peptide co-secreted with insulin from pancreatic β-cells in response to meal ingestion. Amylin plays important roles in the control of food intake and in the regulation of postprandial blood glucose levels, and it has inhibitory effects on gastric emptying, and pancreatic glucagon secretion. Here we investigated the anti-obesity effects of the long-acting amylin analogue ZP4982 in high-fat diet (HFD) fed rats.

Materials and methods

Study 1 and 2: High-fat fed (overweight) rat model - Rats maintained on a HFD for 2 weeks prior to the study

Study 1 was conducted with male Sprague Dawley (SD) rats (~9 weeks of age). Rats were allowed to self-select between a low-fat diet (LFD; standard chow, 11 % of total energy from fat, Altromin 1324, Brogaarden A/S, Gentofte, Denmark) and a HFD (60% of total energy from fat, D12492, Research Diet Inc., New Brunswick, USA) from Day -3 and remained on this regimen for the duration of the study. Rats were treated for 4 weeks with the long acting amylin analogue ZP4982 (30 nmol/kg, s.c., QW).

In Study 2, male SD rats (~9 weeks of age) were treated for 4 weeks with ZP4982 (1, 5 and 30 nmol/kg, s.c., QW), ZP4982 (5 nmol/kg, s.c., every 4th day), or the GLP-1 analogue, liraglutide (50 nmol/kg, s.c., bid).

Sudy 3: High-fat fed (obese) rat model - Rats maintained on a HFD for > 12 weeks prior to the study

Male SD rats (~25 weeks of age) were treated for 4 weeks with ZP4982 (0.8 and 5 nmol/kg, s.c., QW), or liraglutide (50 nmol/kg, s.c., bid). A group of rats were kept on normal chow (Altromin 1324, Brogaarden A/S, Gentofte, Denmark) and served as age control group.

Metabolic endpoints were assessed: body weight, food and water intake, blood glucose, plasma insulin, liver fat content (MR scanning) and body fat depot weight.

Results: Efficacy on human calcitonin receptor (hCTR), human Amylin receptor 1 (AMYR1) and AMYR3

Peptide	hCTR EC50 (cAMP; nM)	hCTR/RAMP1 EC50 (cAMP; nM)	hCTR/RAMP3 EC50 (cAMP; nM)
ZP4982	0.06±0.012 (n=6)	0.26±0.097 (n=3)	0.22±0.0063 (n=2)

Table 1. In-vitro efficacy
EC50 on the hCTR, hAMYR1 (CTR/RAMP1), and AMYR3 (CTR/RAMP3),measured as cAMP accumulation in COS-7 cells stably expressing the individual receptors. Amylin receptor phenotype are heterodimers of CTR and Receptor Activity Modifying Proteins (RAMPs). Data are shown as mean EC50±SD.

Results: Effects of the long-acting amylin analogue ZP4982 on food preference (Study 1)

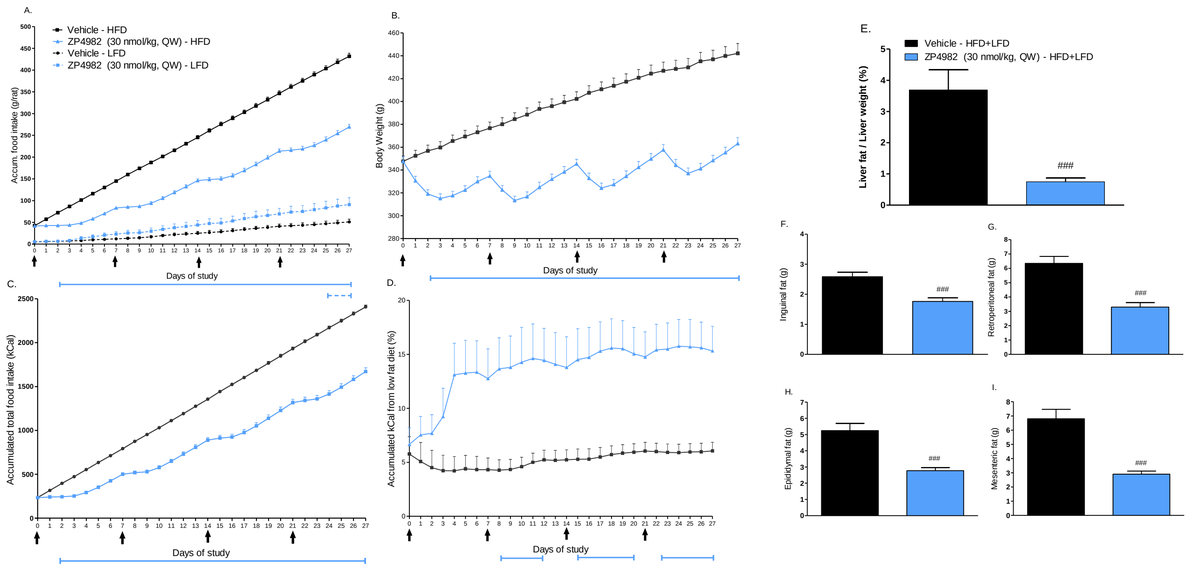

Figure 1. Effects of 4 weeks treatment with vehicle or ZP4982 on food intake, body weight, food preference, liver fat and body fat mass. A) Accumulated HFD and LFD food intake (g), B) Body weight (g), C) Accumulated total calorie intake (kcal, both diets), and D) Accumulated calorie intake from LFD (kcal), E) Terminal liver fat (%), F) Inguinal fat depot (g), G) Retroperitoneal fat depot (g), H) Epididymal fat depot (g), and I) Mesenteric fat depot (g). Data are mean values + SEM (n=6-10/group). Data were compared by 2-way ANOVA followed by Bonferroni post tests or by unpaired t-test, ###p < 0.001 vs. vehicle. The arrows below the graphs represent dosing schedule. The lines below the graphs represent significant difference (p < 0.05) vs. vehicle.

Results: Effects of ZP4982 on the development of obesity in high-fat fed rats (Study 2)

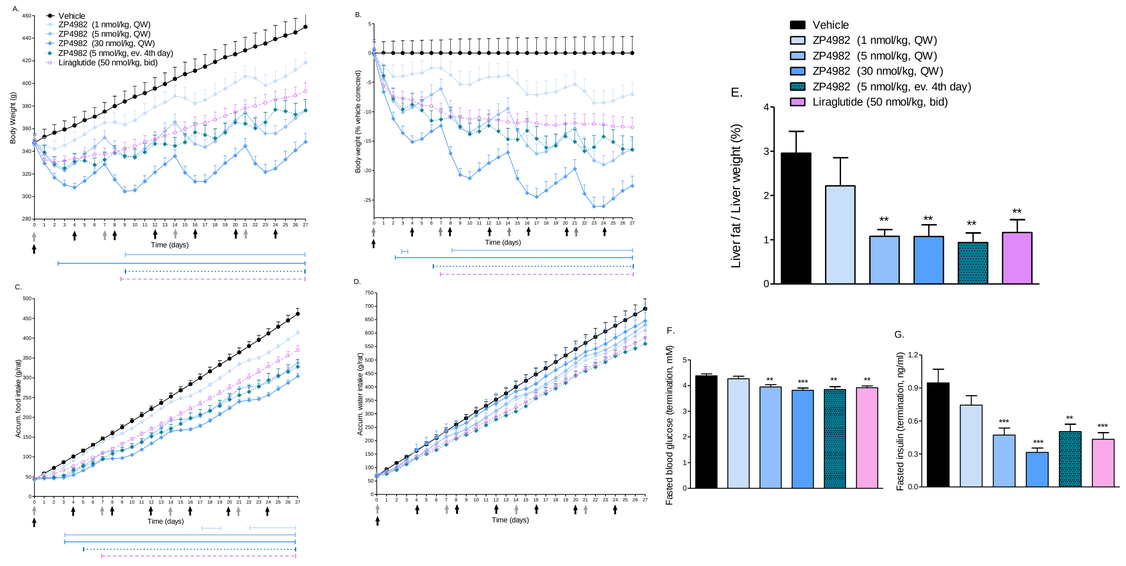

Figure 2. Effects of 4 weeks treatment with vehicle, ZP4982, or liraglutide on body weight, food and water intake, liver fat, blood glucose and plasma insulin levels. A) Body weight (g), B) Relative body weight changes (%, vehicle corrected), C) Accumulated food intake (g/rat)), D) Accumulated water intake (g/rat)), E) Terminal liver fat (%), F) Terminal fasted blood glucose levels (mmol/L), and G) Terminal fasted plasma insulin levels (ng/ml). Data are mean values + SEM (n=10/group). Data were compared by 2-way ANOVA followed by Bonferroni post tests or by 1-way ANOVA followed by Bonferroni post tests, **p < 0.01, ***p < 0.001 vs. vehicle. The arrows below the graphs represent dosing schedule. The lines below the graphs represent significant difference (p < 0.05) vs. vehicle.

Results: Effects of ZP4982 on adiposity in DIO rats (Study 3)

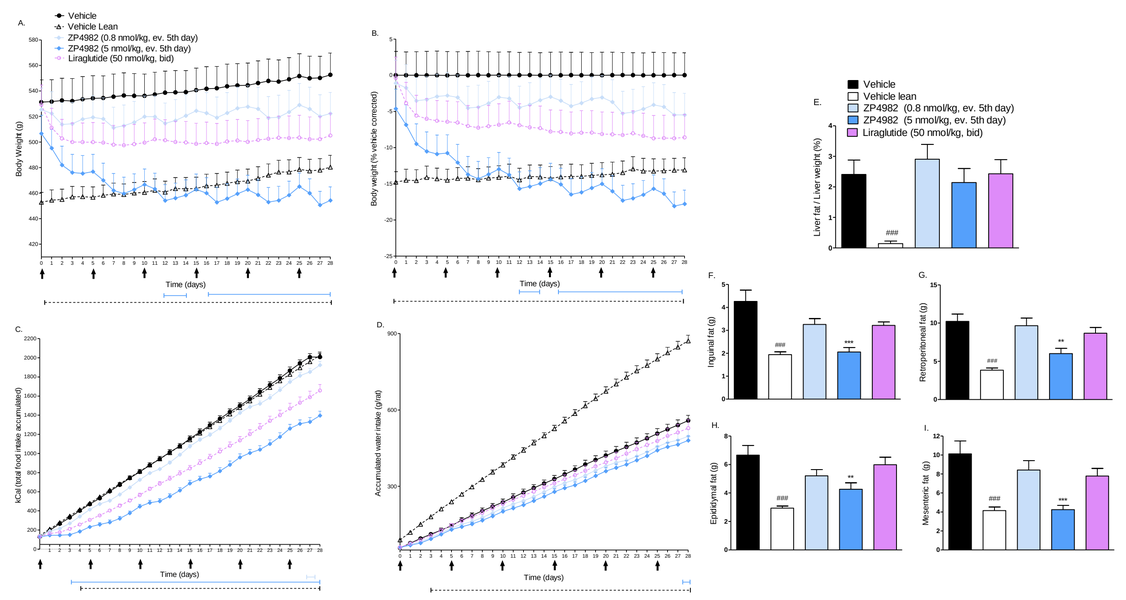

Figure 3. Effects of 4 weeks treatment with vehicle, ZP4982, or liraglutide on body weight, food and water intake, liver fat and body fat mass. A) Body weight (g), B) Relative body weight changes (%, vehicle corrected). C) Accumulated food intake (g/rat)), D) Accumulated water intake (g/rat)), E) Terminal liver fat (%), F) Inguinal fat depot (g), G) Retroperitoneal fat depot (g), H) Epididymal fat depot (g), and I) Mesenteric fat depot (g). Data are mean values + SEM (n=10/group). Data were compared by 2-way ANOVA followed by Bonferroni post tests or by 1-way ANOVA followed by Bonferroni post tests, **p < 0.01, ***p < 0.001 vs. vehicle, or by unpaired t-test, ###p < 0.001 vs. vehicle. The arrows below the graphs represent dosing schedule. The lines below the graphs represent significant difference (p < 0.05) vs. vehicle.

Conclusion

Treatment with the novel long-acting amylin analogue ZP4982 caused

- **Changes in food preference towards foods low in fat**
- **Inhibition of food intake**
- **Potent and sustained body weight reduction**

We conclude that long-acting amylin analogues may provide an attractive option for the treatment and/or prevention of obesity and obesity-related comorbidities.