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Liraglutide pharmacotherapy reduces body weight and improves glycemic control in juvenile obese / hyperglycemic male and female rats

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Abstract

Aims: Maternal and early-postnatal consumption of high-fat/high-sucrose diet (HFSD) dramatically alters the offspring phenotype, predisposing the progeny to have reduced sensitivity to satiation signals, increased body weight, and blood glucose impairments. Recent therapeutics for treating adult obesity have focused on targeting the glucagon-like-peptide-1 (GLP-1) system. Here, we examine whether the GLP-1 receptor agonist liraglutide could be used in juvenile male and female rats as an anti-obesity/diabetic pharmaceutical to prevent not only adolescent obesity/hyperglycemia, but also prevent early-adult onset obesity.

Material and Methods: Pregnant dams were fed either standard chow or HFSD from gestational day 2, throughout pregnancy and lactation. Offspring were weaned on to the respective maternal diet. Juveniles received daily subcutaneous injection of liraglutide (50 µg/kg, from postnatal day [PND]30-PND40 and 200 µg/kg from PND40-PND60) or vehicle. Food intake, body weight and glycemic levels were evaluated across the experimental period.

Results: Chronic liraglutide administration in juveniles prevented body weight gain in males and retained a normo-glycemic profile in both male and female rats.

Conclusion: These preclinical data suggest that maternal and early-life consumption of HFSD increases caloric intake, body weight gain and hyperglycemia, a collective set of unwanted metabolic effects that appear to be treatable in juveniles with liraglutide pharmacotherapy intervention.

INTRODUCTION

The worldwide prevalence of adolescence obesity has markedly increased over the past three decades in Europe and the USA (1–3). Obesity during adolescence results in numerous physiological and psychological comorbidities that perpetuates into adulthood (3, 4). Adding further complexity to the etiology of the disease, obese adults are more likely to foster obese children, thus passing the disease onto the next generation (5). Therefore, effective prevention and more aggressive treatment strategies to treat adolescent obesity are urgently

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needed. To this end, there are a number of behavioral, surgical and pharmacologically-FDA-approved treatments for adult obesity that should be explored as potential interventions for juvenile obesity. Among the recently FDA-approved anti-obesity medications for adults, liraglutide, an agonist of the glucagon-like peptide-1 receptor (GLP-1R), exerts its food-intake suppressive effects by activating GLP-1Rs in the central nervous system (CNS) (6–9). While attention has largely focused on treating adult obesity and diabetes with liraglutide pharmacotherapy, given the limited profile of potential life-threatening adverse events with liraglutide, it is surprising how little is known with regard to the ability of liraglutide to treat/prevent obesity and diabetes in juvenile populations (10, 11).

In this manuscript we exploit the developmental overnutrition hypothesis, which suggests that maternal obesity and early-life exposure to high caloric diet predisposes offspring to altered energy balance and consequential increase risk for adiposity in adulthood (12–14). While genetic and environmental factors both affect the development of obesity (15–17), several studies have shown that *in utero* and early-life exposure to a high-fat/-sucrose diet (HFSD) produces long-term metabolic effects on offspring with regard to energy balance and glycemic control (18–20). Here, we sought to utilize a rodent model that mimics a classic human phenomenon of HFSD exposure during pregnancy that drives hyperphagia and weight gain in offspring to determine whether subsequent juvenile intervention with liraglutide could treat and prevent the onset of obesity and chronic hyperglycemia during adolescences and early adulthood.

Animal models have proven invaluable to investigate the effects of maternal diet on offspring phenotype. Here we exposed pregnant rat dams to a HFSD to facilitate *in utero* and early-life exposure of the offspring to HFSD resulting in a dramatic increase in body weight gain, caloric intake and glucose intolerance in the offspring. As very recent clinical trials have begun to assess the safety and the tolerability of liraglutide pharmacotherapy in children and adolescents (ClinicalTrials.gov Identifier: NCT02696148 and ClinicalTrials.gov Identifier: NCT01541215), the current set of preclinical findings will have direct translational relevance showing that the GLP-1R analog liraglutide could be effective to treat obesity and hyperglycemia in juveniles.

MATERIALS AND METHODS

Animals:

Pregnant female Sprague-Dawley rats (Charles River Laboratories, USA) were received on gestation day 2 (GD2). Animals were individually housed in conventional plexiglass cages with beta-chip bedding, ad libitum access to food and water in a temperature and humidity-controlled environment on a 12-hour/12-hour light-dark cycle. Pregnant rats were divided into two groups according to their diet throughout gestation and lactation: standard chow diet (3% fat, 7% sugar; Purina Rodent Chow 5001; $n=4$; Ralston Purina Company, St. Louis, MO) or high-fat/high-sucrose diet (HFSD; 60% kcal from fat, 20% sugar, $n=4$; Research Diet Inc, New Brunswick, NJ). All dams were started on their diets upon arrival on GD2. Body weight and food intake were measured every 48h until parturition. Females were allowed to deliver spontaneously. Within 24h of delivery the litter size was assessed, pups weighted and sexed. The day of parturition is post-natal day (PND)-0. On PND1, litters were

culled to 12 each. Pups and dams were weighted every 48h during suckling period. At weaning, on PND21, all pups were removed from the dams and single-housed in hanging-wire cages. Pups remained on the respective maternal diet (chow or HFSD) for the duration of experimental testing. Body weight, food intake, and kaolin (aluminum silicate; Research Diet Inc, New Brunswick, NJ) intake (as a proxy measure of pica; ingestion of non-nutritive substances in response to visceral malaise (21–23)) is measured daily for the entire duration of the experiment. All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals.

Drugs:

Liraglutide (gift of NovoNordisk, Denmark) was dissolved in 0.1M PBS (pH 7.4) for systemic injections. Liraglutide is a selective GLP-1R agonist in humans and rodents(9, 24–27).

Effects of chronic daily administration of liraglutide in juvenile male and female rats on body weight gain and food intake.

The experiment was designed to determine whether chronic liraglutide administration in male and female juvenile rats is effective to achieve a normalization of weight gain and suppression of hyperphagia of HFSD, without disrupting regular growth and without inducing nausea/malaise during adolescence. To this end, male and female rats ($n = \sim 6$ per group/gender/diet), from PND30-PND40 (a period corresponding to childhood in rats) received daily subcutaneous (S.C.) injections of vehicle (1mL/kg) or liraglutide (50 μ g/kg); followed by daily S.C. injections of vehicle or liraglutide (200 μ g/kg) from PND40-PND60 (a time period corresponding to pre-puberty and adolescence in rats). These doses were chosen from our preliminary studies and the literature (28–31). Animals were habituated to S.C. injections for at least one week before the beginning of the treatment. Injections were given at the beginning of the dark onset and body weight, food and kaolin intake were recorded daily, throughout the 4 weeks of experimentation. Food and kaolin intake analyses recorded every 24h beginning at dark cycle onset accounted for crumb spillage.

Effects of liraglutide on glycemic control during an oral glucose tolerance test (OGTT) in juvenile male rats:

At the beginning of the experiment (to acquire baseline measurements), *during* the experiment (PND50; data not shown) and *at the end* of the chronic drug administration (PND60), animals were deprived of food for 2h before testing. Shortly before the onset of the dark phase, water was also removed from the cage. A small drop of blood was collected from the tail tip and analyzed for blood glucose (BG) concentrations using a standard handheld glucometer (AccuCheck, TheraSense, Alameda, CA) as previously reported (32). For the OGTT performed *prior* to the liraglutide treatment and after the baseline BG reading, animals received oral gavage of glucose (2g/kg of 25% glucose solution). Subsequent BG readings were taken at 20, 40, 60 and 120 minutes after glucose gavage. After the final BG reading food and water were returned. For the OGTTs *during* and *at the end* of the liraglutide treatment: after the baseline BG reading ($t = -30$ min), each rat received their assigned SC injection of liraglutide (50 or 200 μ g/kg) or vehicle (1mg/kg sterile saline

solution). Thirty minutes later ($t=0\text{min}$), BG was measured and each rat received an oral gavage of glucose (2g/kg of 25% glucose solution). Subsequent BG readings were taken at 20, 40, 60 and 120 minutes after glucose gavage. After the final BG readings, food, water and kaolin were returned.

Statistical methods:

All data are expressed as mean $\pm$ S.E.M, male and female rats were analyzed separately. All data were entered in Excel version 16.10 and subsequently subjected to statistical analysis using GraphPad Prism Software version 7.0 (San Diego, CA, USA). Statistical significance was set at $P < 0.05$. Statistical evaluation of the data was carried out using One- or Two- way ANOVA with Tukey post-hoc analysis (One-way) or Sidak post-hoc analysis (Two-way) between control and treatment groups in cases in which statistical significance was established.

RESULTS

Dams: body weight and energy intake across gestation and lactation.

Pregnant dams were exposed to chow or HFSD beginning at gestational day 2 and maintained on the respective diets during pregnancy. During gestation and lactation body weight of both chow and HFSD-maintained dams increased similarly, with no significant difference (Figure 1A). The same observation was made for energy intake during gestation (Figure 1 B, C); while during lactation cumulative caloric intake was significantly higher in females maintained on HFSD compared to chow-controls (Figure 1D; Student t-test, $*P < 0.05$).

Offspring: litter size and growth curves during lactation and weaning.

Litter size of HFSD-maintained dams did not significantly differ in body weight compared to chow control (Figure 2). In addition, the female:male sex ratio, an imbalance of which could contribute to litter birthweight differences, was not significantly different between groups. After litter size was culled to twelve pups in all litters (7 males and 5 females for chow; 5 males and 7 females for HFSD), individual pup weights were recorded every 8 days during lactation and weaning, prior to the beginning of the treatment. Progressively, pups of both sexes exposed pre- and post-natally to HFSD became heavier than controls (Figure 2 A, B; $P < 0.0001$; Two-way ANOVA). Post hoc analysis revealed differences in body weight during lactation at PND10 and PND18, and during weaning (PND26).

Chronic liraglutide treatment effectively suppresses excess body weight gain in juvenile male rats exposed pre- and post-natally to HFSD.

While numerous studies have described the caloric intake and body weight suppressive effects of liraglutide in adult rodents and humans [see (25, 26, 28, 33–35) for example], a direct comparison of the chronic food intake and body weight suppressive effects of liraglutide in juvenile animal models or humans is still lacking. We therefore aimed to determine whether daily liraglutide treatment is effective in curbing excessive body weight gain in juvenile male and female rats maintained on a HFSD without affecting the normal growth curve or inducing behavioral measures of nausea/malaise (Figure 3 and supplement

figure 1). During peri-adolescence (PND30–40), liraglutide treatment did not affect body weight gain, irrespective of diet. Chronic treatment with liraglutide significantly reduced body weight gain during late adolescence (PND40–50) and young adulthood (PND50–60) in juvenile male rats maintained on HFSD, compared to vehicle-controls (* $P < 0.05$, Two-way ANOVA, Figure 3A, black line). Importantly, the same dose of daily liraglutide injections did not affect body weight gain in chow-fed animals; suggesting that, during this critical period of development, liraglutide pharmacotherapy does not interfere with normal/regular growth but can be effective at treating excessive body weight gain driven by maintenance on a HFSD.

The food intake suppressive effects of chronic liraglutide treatment were evaluated in the same juvenile rats with *ad libitum* access to chow or HFSD (expressed in Kcal from food; $n \sim 6$ per diet/gender/group) over the 4 weeks of treatment. During peri-adolescence (PND30–40), late adolescence (PND40–50), through young adulthood (PND50–60), while rats maintained on HFSD showed an increased caloric intake compared to their chow counterpart, liraglutide treatment did not reduce feeding, irrespective of diet ($P < 0.05$: Two-way ANOVA, Figure 3B, C, D). Finally, chronic treatment with liraglutide during late adolescence/young adulthood, significantly reduced body weight gain in juvenile rats maintained on HFSD compared to vehicle ($P < 0.05$, Two-way ANOVA, Figure 3 E, F, G).

Together, our results demonstrate that chronic treatment with liraglutide in juvenile male rats significantly reduced body weight gain in animals maintained on HFSD compared to control ($P < 0.05$, Two-way ANOVA, Figure 3). Moreover, daily liraglutide treatment did not affect body weight or feeding behavior in chow-fed juvenile animals, suggesting that liraglutide-based pharmacotherapy in juveniles does not affect regular growth.

Liraglutide has potent beneficial effects on glycemic control in juvenile male rats maintained on HFSD.

As previously reported (36), rats pre- and post-natally maintained on HFSD are hyperglycemic compared to their chow counterpart, (Figure 4A). Area under the curve (AUC) analyses revealed that maintenance on HFSD results in hyperglycemia compared to chow maintained juvenile rats (Figure 4 B, C: Two-way ANOVA, $P < 0.05$). Daily liraglutide (200 $\mu\text{g/kg}$) treatment significantly reduced blood glucose concentration during the OGTT (main effect of drug, $F_{3,120} = 32.29$, $P < 0.0001$; drug x time interaction, $F_{15,120} = 32.29$, $P = 0.0068$; Figure 4D). Posthoc analyses showed that liraglutide significantly suppressed BG at 20 minutes after glucose gavage (*vs* vehicle, $P < 0.0001$) in both HFSD-maintained and chow-fed juvenile rats. Moreover, liraglutide significantly lowered BG at 20, 40 and 60 minutes after glucose gavage in chow-fed animals compared to chow-fed controls ($P < 0.05$). Interestingly, injection of liraglutide also reduced BG concentrations prior to oral glucose gavage ($t = 0$, $P < 0.05$). Area under the curve (AUC) analyses revealed that liraglutide produced a greater magnitude of suppression in BG concentrations post-glucose load compared to saline vehicle injections in HFSD-maintained juvenile rats (Figure 4 E, F: Two-way ANOVA, $P < 0.05$).

Daily liraglutide treatment does not significantly suppress body weight in juvenile female rats exposed pre- and post-natally to HFSD.

Chronic liraglutide treatment during adolescence (PND30–50) through young adulthood, (PND50–60) fail to significantly affect body weight gain (Figure 5 A, E, F, G) or food intake (Figure 5 B, C, D), irrespective of diet. Of importance, is the observation that in adult rats, females became less obese than males. Since liraglutide mainly reduced body weight gain by reducing the excessive body weight gain from HFSD exposure (*i.e.*; adiposity), it is possible that the absence of a significant decrease in body weight is due to an initial overall lower fat content in the juvenile female.

Liraglutide significantly improves glycemic control in juvenile female rats maintained on HFSD.

As previously reported (36), rats pre- and post-natally maintained on HFSD are hyperglycemic compared to their lean counterpart (Figure 6). Daily liraglutide treatment significantly reduced BG at 20 minutes after glucose gavage (*vs* vehicle, $P < 0.0001$) in HFSD-maintained, but not in chow-fed female juvenile rats. Interestingly, injection of liraglutide also reduced BG levels prior to oral glucose gavage ($t = 0$, $P < 0.05$) in HFSD-maintained female rats compared to controls. Area under the curve (AUC) analyses revealed that liraglutide had hypoglycemic effects on post-glucose load compared to saline in HFSD-maintained juvenile female rats (Figure 6 E, F: Two-way ANOVA, $P < 0.05$). Our data demonstrate that chronic liraglutide treatment in juvenile females is effective to rescue the HFSD-induced hyperglycemia.

DISCUSSION

There is overwhelming evidence that nutritional status *in utero* and during early-life is able to modify energy balance regulation later in life (18–20). As our obesogenic environment continues to drive increasing frequency of maternal obesity, the risk for adolescent obesity and associated metabolic diseases is of severe medical and economic concern. Safe and effective treatment options are therefore needed to treat childhood obesity and prevent the onset in adulthood. To this end, while the most compelling solution involves a combinatorial approach of lifestyle modifications combined with socioeconomic and educational policy changes, it is undeniable that adolescents born from obese mothers are at greater risk simply due to the biological predispositions placed on them early in life and require further medical intervention. Here, we provide a complementary set of translational data showing that liraglutide treatment in juvenile rats is able to rescue the obese phenotype without producing negative impacts on normal body weight growth. Additionally, we show that liraglutide also improves glycemic tolerance in juvenile hyperglycemic male and female rats maintained on a HFSD. While we did observe a greater impact of liraglutide on male offspring compared to females, we believe this may be due in part to the greater divergence in body weight observed in male *vs.* female juvenile rats maintained on a HFSD, as similar improvements were observed for both sexes on glucose tolerance.

The idea of developmental programming, proposed by Baker *et al.* in the 1990s, has been extensively demonstrated in animals and human studies. Surprisingly however, very little is

known about the consequences of maternal and early-life exposure to a diet highly rich in fat and sugar on the developing offspring. While several studies have described the metabolic effects of maternal obesity on offspring (13, 37–39), the effects of overnutrition without the confounding factor of maternal obesity are still missing. Therefore, to investigate the effect of maternal overnutrition rather than obesity, we maintained our dams on HFSD starting at GD2 and then throughout pregnancy. Consistent with previous reports in which dams were exposed to HFSD only during gestation (36), body weight did not differ significantly between dietary groups throughout pregnancy, suggesting that HFSD-dams might have increased energy expenditure during pregnancy (40). Beginning in lactation, the HFSD-dams began to significantly increase their energy intake, suggesting that maternal lactation capacity is a primary determinant of nutrient supply in rodents. Further, these data might also indicate that lactation itself, the macronutrient constituents in the milk, or some other lactation-associate factors may exert a stronger effect on the offspring body weight phenotype compared to the *in-utero* contribution. Supporting this notion, it is worth noting that at birth, male and female pups did not differ in body weight, suggesting that maternal exposure to HFSD does not result in an immediate obese phenotype. However, as early as PND10 body weight significantly diverged and continue to increase in both male and female rats exposed pre- and post-natally to HFSD compared to chow pups. Future studies, using cross-fostering will be valuable in teasing apart the *in-utero* vs. postnatal timescale that drives offspring obesity.

An important observation of the current set of data is that daily liraglutide administration to juvenile rats maintained pre- and post-natally on chow did not interfere with regular body weight growth. Thus, were these data to be translated into future clinical trials, any potential weight reductions seen in obese children would therefore not be a consequence of some unwanted impact on normal growth curves of the child, presumably not affecting the gain of lean body mass. While the effects of liraglutide on body weight reduction in juvenile female rats maintained on HFSD was not as robust in magnitude as what was observed in males, a trend in decreasing body weight gain is clearly present and grows in magnitude during the late adolescence/young adulthood time period (*i.e.*; PND50-PND60). A potential explanation for the lack of efficacy of liraglutide in juvenile females, could be the initial starting lower body weight of the female rats. Liraglutide treatment is effective as early as PND40 in juvenile male rats, and their body weight is already ~300g at that time point. Juvenile female rats maintained pre- and post-natally on HFSD, reached ~300g only towards the end of the experimental period (PND60), when a clear trend in decreasing body weight is present in the HFSD-liraglutide treated female HFSD group. These findings suggest that body weight at the beginning of the treatment might be critical. Nonetheless, it is important to note that chronic liraglutide treatment was still able to reduce the HFSD-induced hyperglycemia in both juvenile male and female rats, irrespective to the difference in body weight. Future studies are certainly warranted to also assess the impact of chronic liraglutide treatment in juveniles on fasting and post-prandial insulin and glucagon plasma concentrations.

Several lines of preclinical evidence in rodents demonstrate that GLP-1R analogs, such as liraglutide, inhibit food intake via action in the CNS(7, 41, 42). GLP-1R are present in nuclei of the hypothalamus and brainstem (*e.g.*; dorsal-vagal-complex), as well as nuclei of the mesolimbic reward system that are all associated with the control of food intake (7, 41,

42). While the CNS penetrance appears to slightly differ for GLP-1R analogs (26, 43–45), previous research has shown that systemically injected liraglutide is able to cross the blood-brain barrier and act centrally by binding to GLP-1R in the hypothalamus and hindbrain (46). In addition, systemic GLP-1R analogs may also signal to appetite centers in the brain through activation of GLP-1R expressed on vagal afferent neurons (29). We speculate that these putative sites-of-action for liraglutide would not differ in juvenile animals compared to adults, however, such studies are certainly warranted in the future.

Collectively, these results provide novel evidence that liraglutide pharmacotherapy during critical periods of juvenile development is effective to curb excessive body weight gain and to retain a normo-glycemic profile without causing nausea/malaise, in juvenile rats maintained on a HFSD diet. As childhood obesity rates continue to rise in Western societies, and given that liraglutide is FDA-approved treatment for obesity in adults, it seems critical to conduct multiple clinical trials in obese children and adolescents. To this point, these preclinical data provide compelling evidence to support the hypothesis that liraglutide may be a useful pharmacotherapy for treating obesity and T2DM in juvenile populations without causing any impact on normal growth curves.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Body weight and energy intake of dams during gestation and lactation.

Body weight of dams maintained on chow or HFSD was recorded every 48h during gestation and lactation. Data are expressed as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet.

(B) Energy intake (Kcal) of dams maintained on chow or HFSD was recorded every 48h during gestation and lactation ($P < 0.05$; Student t-test). (C) During gestation there is no significant difference in feeding behaviour in chow-fed vs HFSD-maintained dams. (D) During lactation caloric intake is significantly higher in dams maintained on HFSD compared to chow-control ($P < 0.05$; Student t-test). Data are expressed as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet.

Figure 2. Growth curves of the offspring.

Body weight of the pups (A) male and (B) female from dams fed control-chow or HFSD.

Male and female pups weaned on HFSD are significantly heavier than their respective chow-controls (Males: $P=0.0003$ at PND10; $P < 0.0001$ at PND18 and PND26 ; Two-way ANOVA, posthoc test. Female: $P < 0.0001$ at PND10, PND18 and PND26 ; Two-way ANOVA, posthoc test) at PND 10, 18 and 26. Data are expressed as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet; PND= post-natal day.

Figure 3. Effects of chronic liraglutide administration on body weight and energy intake in juvenile male rats.

(A) Daily liraglutide administration during late adolescence (PND40–50) through young adulthood (PND50–60) significantly reduced body weight in rats maintained on HFSD compared to vehicle-controls (* $P < 0.05$; Two-way ANOVA). Chronic liraglutide administration during early post-natal stages (PND30–60) did not affect regular growth in chow-fed rats. Daily liraglutide treatment during (B) peri-adolescence (PND30–40), (C) late adolescence (PND41–50) and young adulthood (PND51–60) has no effect in reducing

feeding, irrespective to diet. Young rats pre- and post-natally maintained on HFSD have increased caloric intake compared to their chow counterpart at all time points ($P < 0.05$; Two-way ANOVA). (E) During PND30–40, daily liraglutide treatment has no effect on body weight change. Vehicle-HFSD-maintained rats have increased body weight compared to chow-fed animals ($P < 0.05$; Two-way ANOVA). (F) Control rats pre- and post-natally maintained on HFSD are significantly heavier than vehicle-treated chow-fed rats. Chronic liraglutide treatment does not affect body weight change in chow-fed rats during late adolescence (PND41–50), while effectively reduced body weight change in HFSD-maintained rats ($P < 0.05$; Two-way ANOVA). (G) Chronic liraglutide treatment significantly decreased body weight change in HFSD-maintained rats, but not in chow-fed during young adulthood (PND51–60) ($P < 0.05$; Two-way ANOVA). Different letters within each figure significantly differ from each other. Data are expressed as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet; PND= post-natal day.

Figure 4. OGTT prior to, and the end of, chronic liraglutide treatment in juvenile male rats. (A) OGTT performed prior to the beginning of the liraglutide treatment in juvenile male rats. Animals maintained on HFSD are hyper-glycemic compared to chow-fed rats (* $P < 0.05$; Two-way ANOVA). The total area under the curve (AUC) is significantly higher in juvenile rats maintained on HFSD at (B) 60min (* $P < 0.001$; t-test) and (C) 120 min (* $P < 0.001$; t-test), indicating a hyperglycemic state in rats fed pre- and post-natally on HFSD compared to their chow-fed counterpart. (D) Chronic treatment with liraglutide significantly improved glucose tolerance in juvenile rats maintained on HFSD compared to their vehicle-

treated counterparts (Chow Liraglutide *vs* HFSD vehicle: * $P < 0.05$; Chow vehicle *vs* Chow liraglutide: \$\$\$ $P < 0.001$; Chow vehicle *vs* HFSD vehicle: $P < 0.05$; Chow vehicle *vs* HFSD liraglutide: &&& $P < 0.001$; HFSD vehicle *vs* HFSD liraglutide: ### $P < 0.001$; Two-way ANOVA). Area under the curve (AUC) analyses (**E**) from 0 to 60 minutes and (**F**) from 0 to 120 minutes (*i.e.*; post-glucose load) show that liraglutide reduce AUC in HFSD-maintained rats ($P < 0.05$; Two-way ANOVA). Different letters within each figure significantly differ from each other. Data are given as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet.

Figure 5. Effects of chronic liraglutide administration on body weight and food intake in juvenile female rats.

(A) Daily liraglutide administration during adolescence (PND30–50) through young adulthood (PND50–60) does not reduce body weight in juvenile female rats, irrespective to the diet. (B) Daily liraglutide treatment during peri-adolescence (PND30–40) has no effect in reducing feeding, irrespective to diet. Young rats pre- and post-natally maintained on HFSD have increased caloric intake compared to their chow counterpart ($P < 0.05$; Two-way ANOVA). (C) During late adolescence (PND41–50) and (D) young adulthood (PND51–60),

chronic liraglutide treatment does not reduce feeding in chow-fed nor HFSD-maintained rats. Daily liraglutide treatment does not decreased body weight change (**E**) during peri-adolescence (PND30–40), nor (**F**) during late adolescence (PND41–50), nor (**G**) during young adulthood (PND51–60). Different letters within each figure significantly differ from each other. Data are expressed as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet; PND= post-natal day.

Figure 6. OGTT prior to, and the end of, chronic liraglutide treatment in juvenile female rats. (A) OGTT performed prior to the beginning of the liraglutide treatment in juvenile female rats. Animals maintained on HFSD are hyper-glycemic compared to chow-fed rats (* $P < 0.05$; Two-way ANOVA). The total area under the curve (AUC) is significantly higher in juvenile rats maintained on HFSD at (B) 60min (* $P = 0.0004$; t-test) and (C) 120 min (* $P = 0.0002$; t-test), indicating a hyperglycemic state in rats fed pre- and post-natally on HFSD compared to their chow-fed counterpart. (D) Chronic treatment with liraglutide significantly improved glucose tolerance in juvenile rats maintained on HFSD compared to their vehicle-

treated counterparts (HFSD vehicle *vs* HFSD liraglutide: * $P < 0.05$; Chow liraglutide *vs* HFSD vehicle: # $P < 0.05$; Two-way ANOVA). Area under the curve (AUC) analyses (**E**) from 0 to 60 minutes and (**F**) from 0 to 120 minutes (*i.e.* post-glucose load) show that liraglutide reduce AUC in HFSD-maintained rats ($P < 0.05$; Two-way ANOVA). Different letters within each figure significantly differ from each other. Data are given as mean $\pm$ S.E.M. HFSD= high-fat sucrose diet.