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Original Article

Effects of liraglutide and sibutramine on food intake, palatability, body weight and glucose tolerance in the gubra DIO-rats

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Aim: To validate the gubra DIO-rats as a useful animal model of human obesity.
Methods: The gubra diet-induced obesity (DIO) rat model was based on male Sprague-Dawley rats with *ad libitum* access to regular chow and a palatable diet rich in fat and sugar. To evaluate the versatility of the gubra DIO-rats as a valid model of human obesity syndrome, the efficacy of 2 weight loss compounds liraglutide and sibutramine with different mechanisms of action were examined in 7-month-old gubra DIO-rats. Liraglutide (200 μg/kg, sc) was administered bi-daily, and sibutramine (5 mg/kg, *po*) was administered once daily for 23 d.
Results: Both the compounds effectively reduced the food intake, body weight and total fat mass as measured by nuclear magnetic resonance. Whereas the 5-HT reuptake inhibitor/5-HT receptor agonist sibutramine reduced the intake of both chow and the gubra-diet, the GLP-1 analogue liraglutide predominantly reduced the intake of the highly palatable diet, indicating a shift in food preference. Sibutramine lowered the insulin sensitivity index, primarily via reductions in glucose-stimulated insulin secretion.
Conclusion: This animal model responds well to 2 weight loss compounds with different mechanisms of action. Moreover, the gubra DIO-rat can be particularly useful for the testing of compounds with potential effects on diet preference.

Keywords: obesity; diet-induced obese rats; food preference; glucose tolerance; glucagon-like peptide-1 (GLP-1); 5-hydroxytryptamine (5-HT); sibutramine; liraglutide

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Introduction

Obesity has become one of the leading causes of death in the industrialized world and has become an increasing health concern for children[1, 2]. Currently, the only efficacious and lasting treatments for weight loss are surgical interventions, and there is an enormous unmet medical need for novel anti-obesity drugs. Hence, the availability of animal models that predictably reflect human obesity syndrome is important. Although the genetic models of obesity (*eg*, the *ob/ob* and *db/db* mice, Zucker *fa/fa* rats, Agouti mice, and MC4 knockout mice) have contributed significantly to our understanding of the molecular physiology of food intake and energy homeostasis[3, 4], the fact that human obesity is a complex interplay between environment (food, exercise) and genetics (multiple genes)[5, 6] requires animal models that reflect this complexity.

One such polygenic model is the diet-induced obese (DIO) and diet-resistant (DR) outbred rat models[7–10]. In the Levin DIO-rats, phenotypic differences in body weight gain, adiposity, and insulin sensitivity are only expressed between the outbred substrains when they are placed on diets of moderate fat, sucrose, and caloric content[11]. A major difference, however, between this experimental design and obesity in humans is that a large aspect of human susceptibility to obesity may not depend on the ability to resist weight gain when force-fed a high-fat diet. Rather, it may hinge on individual differences in the propensity to choose high-fat foods[12–14].

The gubra DIO-rat model is based on male Sprague-Dawley (SPD) rats fed a highly palatable fat- and sugar-rich diet (HPFS diet) composed of equal amounts of the chocolate spread Nutella, peanut butter and powdered chow. The rats also have access to standard pelleted chow, and hence the model allows for assessment of diet preference. Compared to other cafeteria diets, the gubra diet is easy to dispense and measure, and does not change from day-to-day[15]. The diet promotes voluntary hyperphagia that results in rapid weight gain and increases fat pad mass.

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To validate the gubra DIO-rat as a useful animal model of human obesity, we examined the body weight changes as well as the ingestive responses and feeding behaviors of the well-known anti-obesity agent sibutramine and the once-daily human glucagon-like peptide-1 (GLP-1) analog liraglutide for 23 d in this animal model. Sibutramine is a sympathomimetic medication that was available for long-term treatment, and the most recent drug to be withdrawn from the market due to a side effect of increased risk of cardiovascular events. Liraglutide is a long-acting GLP-1 analog available for the treatment of type 2 diabetes that has also shown clinically relevant weight loss following treatment in both diabetic[16] and non-diabetic obese patients[17].

Materials and methods

Compounds

Sibutramine (molecular weight=334.40 g/mol) was obtained from AH Diagnostics (Aarhus, Denmark). Liraglutide (molecular weight=3751.20 g/mol) was obtained from Novo Nordisk, Maaloev, Denmark.

Animals

Twenty male Sprague-Dawley rats were purchased from Taconic (Denmark). All animal experiments were conducted in accordance with internationally accepted principles for the care and use of laboratory animals, and were approved by the Danish Committee for animal research (license 2008/561-1565). The animals were housed in a standard 12-h light/dark cycle (lights on, 6:00 AM; lights off, 6:00 PM) at a room temperature of 20–22 °C and relative humidity of 50%–60%. All animals had free access to water.

Upon arrival at six weeks of age, the rats were offered a two-choice diet consisting of a standard rodent chow (Altromin #1324, Brogaarden, Denmark) and the gubra diet, a high palatable high-fat high-sugar diet made up of a paste (1:1:1) of chocolate spread (Nutella, Ferrero, Italy), peanut butter (Skippy, Unilever, USA) and powdered regular rodent chow (Altromin #1324, Brogaarden, Denmark). The rats were housed two per cage for 26 weeks (until stratification d -7). From d -7 to d 23, rats were housed one per cage. The rats had *ad libitum* access to chow, the gubra diet and water unless otherwise stated.

Drug treatment

On d -7, the rats were stratified into the following three groups based on body weight (n=6; two outliers were discarded): Vehicle (0.5% hydroxypropyl methylcellulose, Sigma, St Louis, MI); sibutramine (5 mg/kg, *po*); or liraglutide (200 μg/kg, sc). All compounds were freshly prepared and administered for 23 d via oral gavage/subcutaneous injections. Vehicle and sibutramine were administered orally once daily 2 h into the light phase, whereas liraglutide was administered subcutaneously bi-daily, two and 10 h into the light phase, respectively. The first dose was given on d 0. The liraglutide dosing was gradually increased over the first four days from 50 to 200 μg/kg. Body weight and food intake were measured daily and bi-weekly, respectively, from days 0 to 23.

Non-invasive whole body composition

Whole body composition was analyzed weekly by non-invasive EchoMRI-900 (EchoMRI, USA). The scanner (QMR systems) measured whole body fat and lean tissue mass. During the scanning procedure, the rat was placed in a restrainer for approximately one minute.

Oral glucose tolerance test (OGTT)

An OGTT was performed on d 21. Animals were mildly fasted, with access to only 50% of their daily energy requirements in the 16 h preceding the test. The amount of food administered on the day prior to the OGTT was calculated for each individual animal as the mean of the two preceding measurements of food intake. Compounds were administered 45 min prior to administration of the oral glucose load. The OGTT was carried out at 8:00 AM. Blood samples for blood glucose and plasma insulin analyses were collected from the sublingual capillaries (capillaries perforated using 23-gauge needle) at t=-60, 0, 15, 30, 60, 120, and 240 min prior to and after the oral glucose load of 2 g/kg body weight glucose (glucose: 500 mg/L, Frescenius Kabi, Sweden). The baseline blood sample (-60) was additionally analyzed for total plasma triglycerides, total cholesterol and free fatty acids (NEFA-C). Glucose and insulin area under the curve (AUC) calculations were determined as total AUC based on data from the -60 to 240 min measurements. The insulin sensitivity index (ISI) was estimated according to Matsuda and DeFronzo[18] using the following equation: $10.000/\sqrt{[(FBG*FPI)*(AUC\text{-}G*AUC\text{-}I)]}$, where FBG and FPI are fasting glucose and insulin, respectively, and AUC is the area under the curve for glucose and insulin measurements. Following the OGTT, the animals had unrestricted access to food. Two days after the OGTT, the animals were euthanized by CO_2 anesthesia. Trunk blood was collected and body white adipose tissue compartments were removed and weighed. Fat deposit weight analyses included mesenterial, retroperitoneal (right), epididymal (left) and subcutaneous inguinal (left) fat.

Blood chemistry analyses

Whole blood glucose

Blood was collected in 10-μL heparinized glass capillary tubes and immediately suspended in buffer [(0.5 mL of glucose/lactate system solution (EKF-diagnostics, Germany)], and then analysed for glucose using a BIOSEN c-Line glucose meter (EKF-diagnostics, Germany).

Insulin

A total of 100 μL of blood was collected in heparinized tubes. Plasma was separated and insulin was measured in duplicate for each data point using an ultrasensitive ELISA (Mercodia AB, Sweden).

Plasma FFA (NEFA-C)

A total of 200 μL of blood was collected in EDTA tubes con-

taining 1% NaF and the plasma was separated. The FFA content was measured using the autoanalyzer Cobas C-111 with a commercial kit (Abbott, USA).

Cholesterol and triglycerides

A total of 200 μL of blood was collected in heparinized tubes and the plasma was separated. Total triglycerides and cholesterol were measured using the autoanalyzer Cobas C-111 with a commercial kit (Roche Diagnostics, Germany).

Statistical evaluation

All data were imported into Excel 5.0 spreadsheets and subsequently subjected to relevant statistical analyses using GraphPad Prism 5.0 software. The results are presented as the mean±SEM unless otherwise stated. Statistical evaluation of the data was carried out using a one-way or a repeated measure two-way analysis of variance (ANOVA) with appropriate *post-hoc* analysis between control and treatment groups in cases where statistical significance was established ($P<0.05$; Bonferroni).

Results

Body weight analysis

Both sibutramine and liraglutide significantly reduced body weight and relative body weight changes (Figures 1A–1B). Whereas sibutramine induced weight loss stabilized after approximately two weeks of treatment (leading to a 12% drop in body weight), the liraglutide-treated rats continued to display weight loss, reaching a 15% body weight loss on the final day of the experiment (Figure 1).

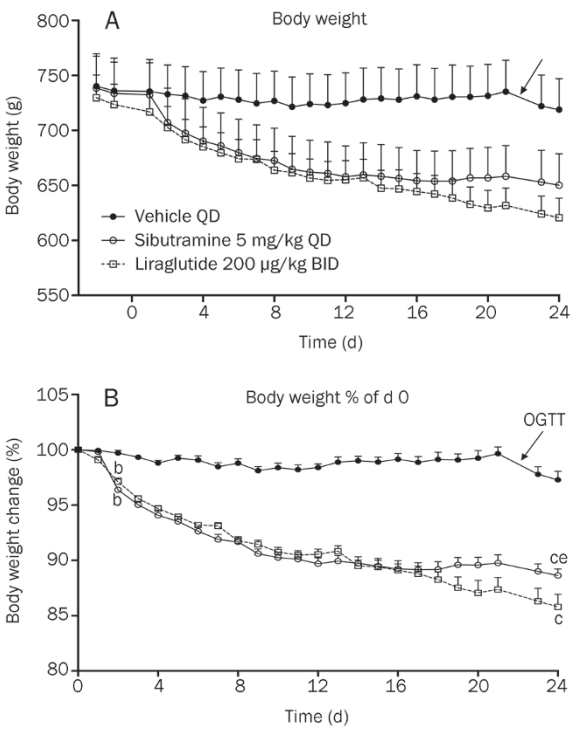

Figure 1. (A) body weight (g) and (B) body weight change (%) of gubra DIO rats treated with vehicle, sibutramine or liraglutide following a 23-d drug administration period. $^{b}P<0.05$, $^{c}P<0.01$ *vs* vehicle group. $^{e}P<0.05$ *vs* liraglutide. Data are means±SEM. $n=6$.

Food and water intake analyses

Both treatment regimens resulted in an acute drop in the intake of the gubra diet (Figures 2A–2F). Sibutramine also reduced the intake of the chow diet (Figures 2B, 2D). Liraglutide-treated rats, however, increased their intake of chow, indicating a shift in food preference from the very palatable gubra diet to chow (Figures 2B, 2D). Both treatment regimens showed unaffected water intake throughout the study period, with volumes similar to vehicle-treated rats at approximately 20–25 mL/day (data not shown).

Glucose tolerance

An OGTT was performed on day 21 (Figure 3). In the glucose tolerance test, data from an age-matched group of chow-fed SPD rats were included for comparison (Figures 3A, 3B). Although the gubra-DIO-rats do not display overt hyperglycemia during the glucose tolerance test, glucose levels in these animals were elevated compared to those in the age-matched chow-fed rats (Figures 3A, 3B). Whereas liraglutide reduced the area under the glucose curve (AUC glucose) significantly compared to vehicle, no difference in AUC was observed in sibutramine-treated rats (Figure 3B). Sibutramine, however, seemed to inhibit insulin secretion, resulting in a reduced AUC insulin (Figure 3D). Although not significant, both liraglutide- and sibutramine-treated rats tended to have lower insulin levels at the baseline (semi-fasted state) sample obtained at the -60 min time point in the OGTT (Figure 3C). As a measure of whole body insulin sensitivity, the insulin sensitivity index was calculated according to Matsuda and DeFronzo[18]. Insulin sensitivity was significantly higher in the sibutramine-treated rats compared to the vehicle-treated rats, whereas no effect on insulin sensitivity was observed following liraglutide treatment (Figure 3E). Triglyceride levels tended to be lower in both liraglutide- and sibutramine-treated rats, but not to a statistically significant extent. Likewise, no significant effects on cholesterol or free fatty acids were observed following sibutramine or liraglutide treatment, although the former tended to increase cholesterol levels compared to vehicle treatment (Table 1).

Body fat mass

Weekly measurements of whole body composition demon-

Table 1. Plasma lipids (triglyceride, cholesterol, and free fatty acids) measured in the semi-fasted state 60 min prior to glucose load on the day of the experimental OGTT following 21-d drug administration. Data are means±SEM. $n=6$. $^{b}P<0.05$ *vs* Liraglutide.

	Vehicle	Sibutramine	Liraglutide
Triglyceride (mmol/L)	1.186±0.16	1.002±0.14	0.778±0.12
Cholesterol (mmol/L)	2.916±0.15	3.350±0.14^{b}	2.756±0.11
FFA (μmol/L)	277.60±37.27	327.17±25.46	278.52±16.44

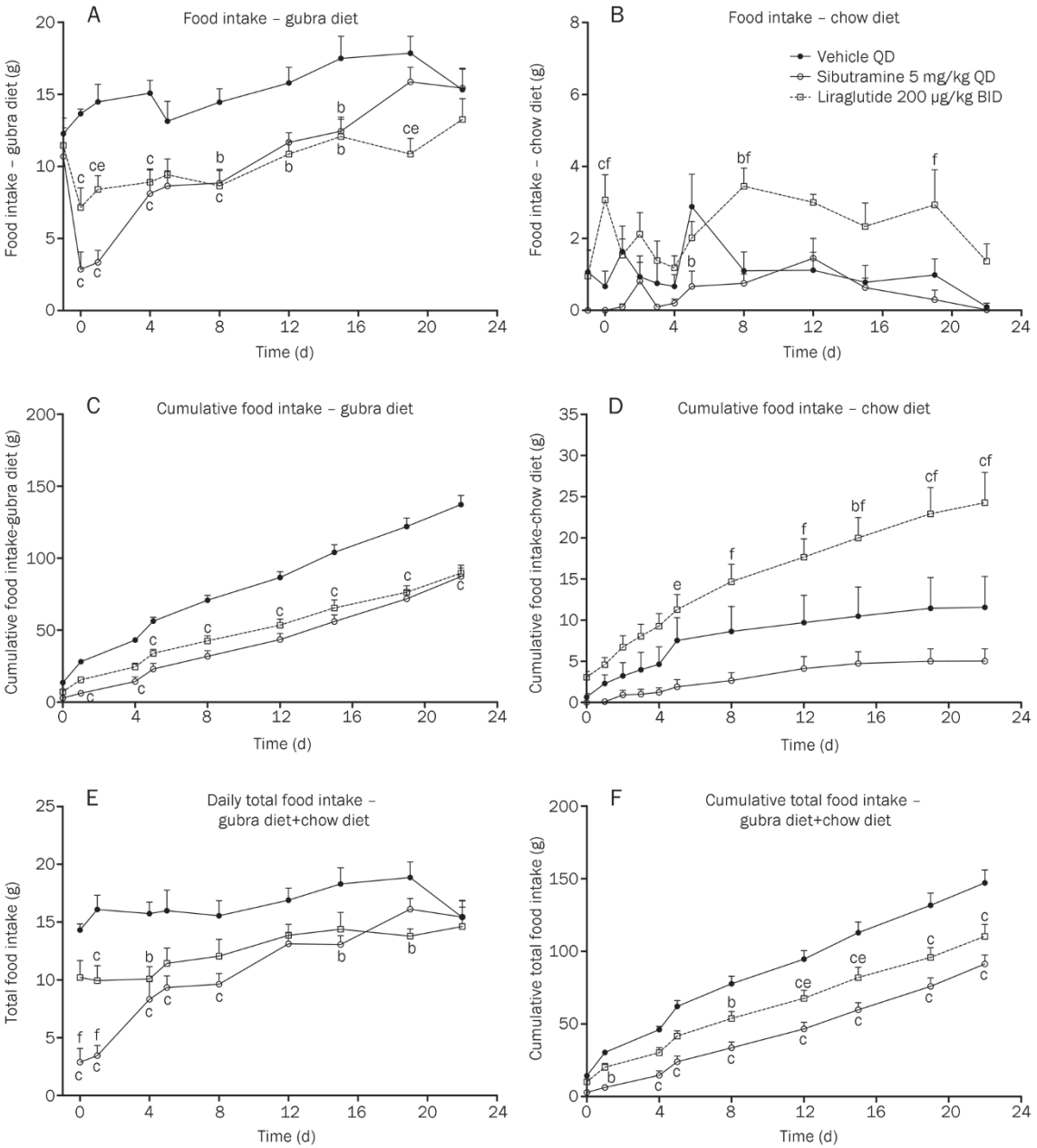

Figure 2. (A, B) daily, (C, D) cumulative intake and (E, F) total daily/cumulative intake of HPFS gubra diet and regular rodent chow (g), respectively, following a 23-d drug administration period. $^{b}P<0.05$, $^{c}P<0.01$ *vs* vehicle group. $^{e}P<0.05$, $^{f}P<0.01$ *vs* sibutramine. Data are means±SEM. $n=6$.

strated a significant reduction in total fat mass in both treatment groups (Figure 4A). No significant changes were seen in lean mass (Figure 4A). The slight initial drop in lean body mass was most likely due to the effects of the compounds on total body water. Both compounds reduced the size of all fat deposits (Figure 4B), with the most marked changes seen in the retroperitoneal and mesenterial fat pads.

Discussion

In this study, we present a detailed description of the weight gain curve, food intake and body fat composition of the gubra DIO-rat. The gubra DIO-rat has a normal Sprague-Dawley background, but displays overt obesity when fed a highly palatable fat- and sugar-rich diet for more than 14 weeks. The gubra-DIO-rats did not develop frank diabetes, but displayed elevated OGTT glucose levels compared to the age-matched chow-fed rats. We demonstrated that two different weight loss compounds, sibutramine and liraglutide, led to a 12%–15% body weight loss in this animal model, and perhaps more interestingly, that liraglutide treatment led to a shift in diet preferences with an increased craving for chow.

Dietary-induced obese animal models have played a key role in the screening of novel compounds for effects on food intake and/or body weight for many years. Thus, the first

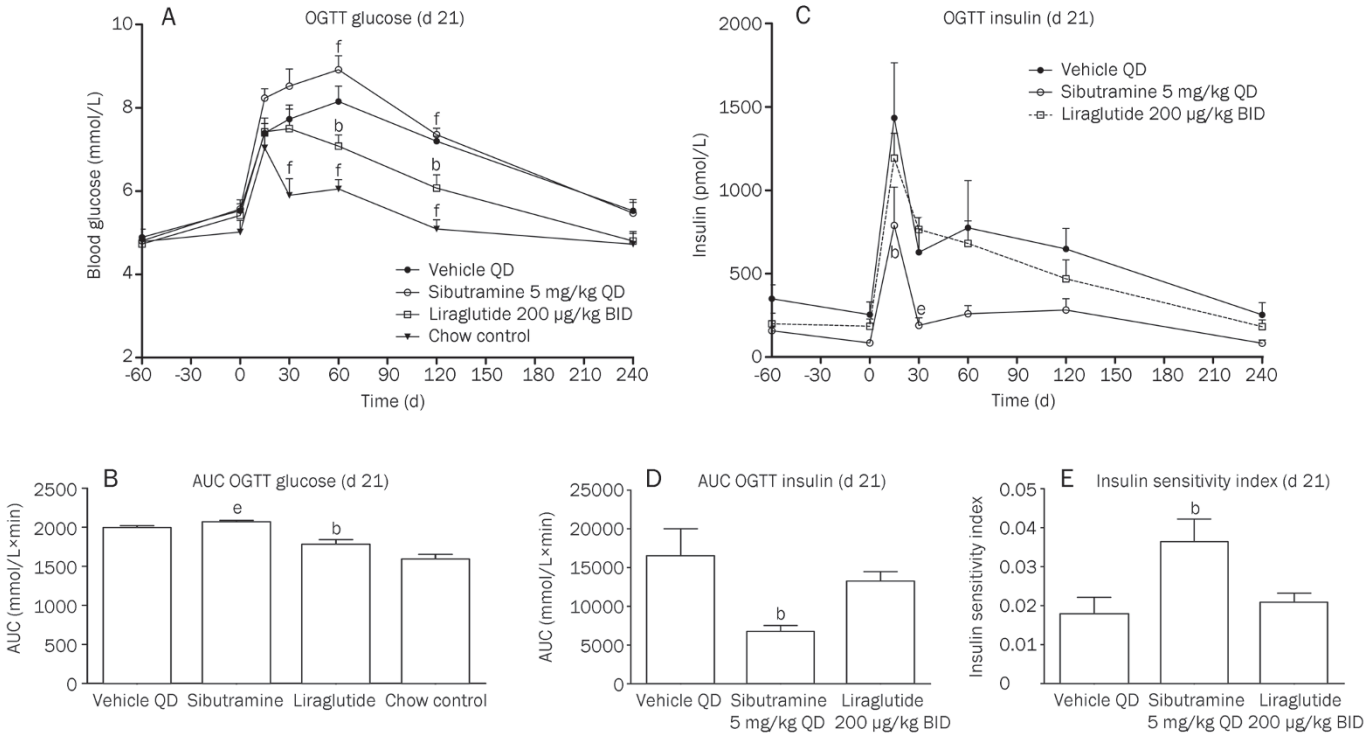

Figure 3. (A, B) blood glucose and (C, D) plasma insulin responses to an OGTT following 21-day drug administration. Rats were semi-fasted 16 h before the OGTT. Compounds were administered 45 min prior to the per oral glucose load (2 g/kg). (E) Insulin sensitivity index (CISI) calculated according to formula of Matsuda and DeFronzo, 1999. $^{b}P<0.05$ *vs* vehicle group. $^{e}P<0.05$ *vs* liraglutide. Data are means±SEM. $n=6$.

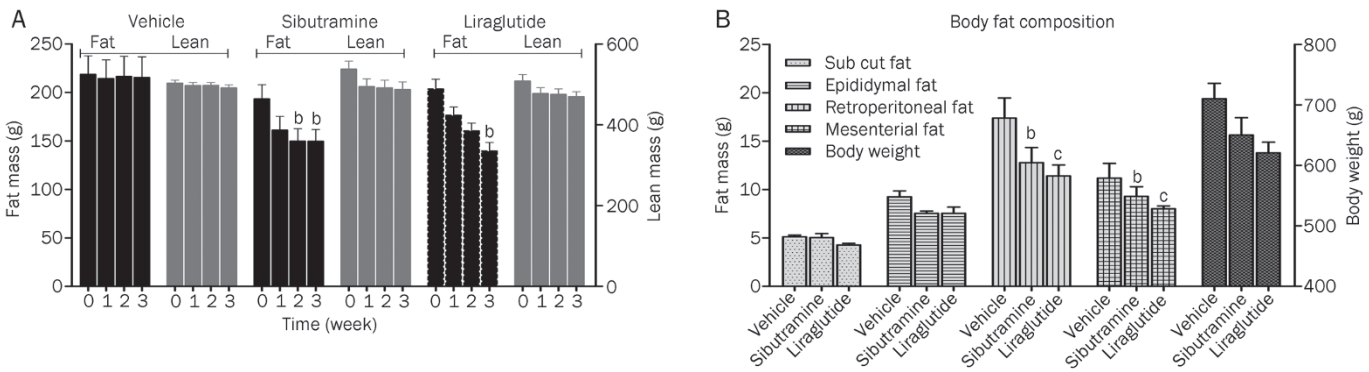

Figure 4. (A) Weekly whole body composition measuring total fat/lean body mass (g) by non-invasive EchoMRI-900 and (B) terminal fat depot analysis (g) of subcutaneous inguinal (right), epididymal (right), retroperitoneal (left), and mesenterial fat mass. $^{b}P<0.05$, $^{c}P<0.01$ *vs* vehicle group. Data are means±SEM. $n=6$.

use of a high-fat diet to induce obesity in rats was by Masek and Fabry in 1959[19]. Since then, numerous DIO models have been published using the general approach whereby diets composed of alternating components of fat and carbohydrates are administered to normal, lean rats or mice over long periods[8, 10, 20]. The so-called cafeteria diets, where animals have a choice of various palatable foods such as chocolate, peanuts etc, encourages overeating and hence provides highly relevant models for examining human diets in rodents[15, 21–23]. Although some diet-dependent differences in the phenotypes of obese animals have been reported[24], the metabolic profiles of rodents that are provided long-term access to high fat diets are reasonably uniform despite methodological variations among laboratories. In general, the currently available DIO rodent models are all based on the feeding of mice or rats diets that are high in energy, mostly from fat (*eg*, the Levin DIO, the C57BL/6J DIO) or sugar[7, 25, 26]. Once obesity has been induced, the animals exhibit stabilized body weights, marked visceral adiposity (30%–35% fat), high plasma levels of leptin, moderate insulin resistance (rather than overt diabetes) and

mild lipid abnormalities. Furthermore, the dietary-induced obesity in animals is polygenic in nature, ie, the susceptibility for weight gain is due to many different genes. In these respects, the animals display similar symptoms to those observed in common human obesity, and the factors contributing to the initiation and maintenance of obesity are similar between animals and man.

The pharmacological approaches for reducing body weight include the development of compounds which reduce food intake or absorption of fat from the gastrointestinal tract, increase energy utilization or act by a combination of these mechanisms. Modest weight loss as low as 5% of initial body weight can lead to favorable improvements in blood pressure, lipid profile, insulin sensitivity and glucose tolerance[27–29]. Thus, an anti-obesity drug[23, 30] that delivers an approximate 10% weight loss in animals with dietary-induced obesity would appear to be a sensible positive control in DIO studies.

In the current study, we aimed to validate the gubra DIO-rat as a useful animal model of human obesity. Six-week-old Sprague-Dawley rats were offered a two-choice diet consisting of standard rodent chow diet or the gubra diet, a highly palatable high-fat, high-sugar diet. After a six-month feeding period, body weight changes, ingestive responses and feeding behaviors were examined following daily administration of sibutramine and liraglutide for 23 d. Compared to the vehicle control, chronic administration of either sibutramine or liraglutide to high-fat/sugar-fed DIO-rats caused significant reductions in body weight gain, which is in agreement with previously reported data using other rodent DIO models[14, 15, 31–34]. The body weight loss observed in both groups was mainly caused by decreasing levels of total fat mass as revealed by the weekly measurements of whole body composition.

As expected, liraglutide as well as sibutramine administration was associated with a significant acute reduction in total caloric intake. Interestingly, however, liraglutide shifted the choice of food, with a significant decrease in the palatable gubra diet consumption accompanied by a relative increase in chow consumption. This shift in food preference by liraglutide is in agreement with previously published data of candy-fed obese Sprague-Dawley rats[15]. The exact mechanism for this effect of pharmacological treatment with a GLP-1 analogue is currently not known. However, it is known that not all drugs that reduce the motivation to eat do so through actions on satiety; therefore, other CNS systems associated with the pleasure or rewarding aspects of food intake, such as the opioid or dopaminergic systems, must also be considered[35]. Interestingly, altered preference for food has also been reported from patients who undergo gastric bypass surgery and exhibit altered attitudes towards and preferences for healthier food[36]. Given that gastric bypass surgery has been shown to enhance meal-induced GLP-1 secretion[37], it is tempting to speculate that the shift in food preference is related to the GLP-1 system.

Besides their weight reducing effects, and in agreement with previously published data, liraglutide was able to improve glucose tolerance[14], and sibutramine improved the insulin sensitivity index (ISI)[10]. The latter was primarily obtained via reductions in glucose-stimulated insulin secretion. The fact that the gubra DIO model rats never developed frank diabetes, which occurs in many other DIO models, is most likely attributable to our feeding regimen where the rats do not eat the high-fat diet only, but can choose freely between chow and the palatable chocolate diet.

Conclusion

Taken together, the gubra DIO model rat, which is fed a two-choice diet composed of highly palatable fat- and sugar-rich food and regular chow, seems to be a valuable polygenetic DIO model for screening compounds primarily affecting food intake and body weight. This model is therefore preferable to other DIO models where animals are force-fed high fat diets without choice, which is very unlike the human situation.

Author contribution

Niels VRANG, Gitte HANSEN, and Jacob JELSING designed research; Natascha LELLING and Gitte HANSEN performed research; Gitte HANSEN analyzed data; Niels VRANG, Gitte HANSEN, and Jacob JELSING wrote the paper.

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