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Effect of Dual Glucagon-Like Peptide 1/ Glucose-Dependent Insulinotropic Polypeptide Receptor Agonist (Tirzepatide) versus Bariatric Surgery on Weight Loss and Nonalcoholic Fatty Liver Disease

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Highlights of the Study

- This study compared bariatric surgery and weight loss drugs in a rat model for obesity and type 2 diabetes.
- Tirzepatide and surgery significantly improved metabolic parameters, with tirzepatide proving more effective for weight loss.
- Tirzepatide offers a noninvasive alternative for combating obesity and its related metabolic issues; it is particularly beneficial for those who are not eligible for surgery.

Keywords
Bariatric surgery · Tirzepatide · Type 2 diabetes · Metabolic analytes · Obesity treatment

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Abstract

Objectives: Bariatric surgery is a well-established treatment for obesity and type 2 diabetes. Tirzepatide, a dual GIP/GLP-1 receptor agonist, has emerged as a promising therapy for type 2 diabetes. This study aimed to compare the effects of bariatric surgery, semaglutide (a GLP-1 receptor agonist), and tirzepatide in Sprague-Dawley rats fed a high-fat diet. ***Methods:*** Rats were divided into surgery, semaglutide, and tirzepatide treatment groups, along with a control group (sham). Weight, oral glucose tolerance, and levels of metabolic markers were assessed, along with adipose and liver tissue analysis. ***Results:*** Surgery led to a 15.5% weight reduction, while rats treated with semaglutide exhibited a 10.7% reduction. Tirzepatide treatment at various concentrations (10, 50, and 100 nmol/kg) resulted in weight reductions of 5.0%, 14.9%, and 17.7%, respectively, compared to the sham group. Metabolic analyte levels decreased in intervention groups compared to the sham group, indicating improved metabolic health and glucose tolerance. Adipose tissue weight and hepatic liver fat droplets decreased in the intervention groups. ***Conclusion:*** Bariatric surgery and tirzepatide treatment significantly improved metabolic parameters in obese rats. Tirzepatide, particularly at higher concentrations, showed pronounced improvements compared to surgery and semaglutide. These findings suggest that high doses of tirzepatide could be explored as an alternative to bariatric surgery for the treatment of obesity.

Introduction

Obesity and its related comorbidities, such as type 2 diabetes (T2D), have become major epidemics in modern society effecting microangiopathic alterations in the eyes and kidneys and cardiovascular disease [1]. There are various approaches being implemented for the effective and long-lasting treatment of obesity and its related comorbidities [2]. Surgical intervention such as bariatric surgery is a common procedure for weight management and metabolic control [3, 4]. The increase in bariatric surgery is attributed to multiple factors, including improvements in surgical techniques, greater awareness of its benefits, and the rising prevalence of obesity and related health complications. Bariatric surgery has been shown to significantly improve metabolic health and reduce the risk of obesity-related comorbidities, such as T2D, hypertension, and sleep apnea. It has also been shown to improve the quality of life and the lifespan of individuals with severe obesity [5]. Clinical trials have reported a short-term remission of T2D in 75–90% of individuals with obesity and T2D, following bariatric surgery [6]. Although the precise mechanism behind this outcome remains unclear, studies propose a major improvement in insulin sensitivity and islet cell function, with increased glucose-stimulated insulin secretion. Furthermore, the enhanced use of glucose and increased release of gut hormones play key roles in achieving long-lasting benefits from bariatric surgery [7, 8].

Glucagon-like peptide 1 (GLP-1) receptor agonists, which simulate incretin hormone action, are currently widely used agents in the treatment of obesity and diabetes [9]. However, the idea that GLP-1 is a better pharmacological target for diabetes than glucose-dependent insulinotropic polypeptide (GIP) could be a misconception [10]. While some reports state that in T2D, there is a hyposecretion of GLP-1 and a hypersecretion of GIP during an oral glucose load, others claim that GIP improves glucose tolerance and has a therapeutic potential for managing excess body weight in patients with T2D [10]. A potential explanation for this finding is a state of dysfunctional GIP secretion or GIP resistance in T2D. Recent studies have shown that GIP analogs with agonistic properties induce a dose-dependent reduction in body weight in diet-induced obese mice. This occurs through reducing their food intake without changes in energy expenditure [11]. The benefits of GIP, independent of GLP-1, have been confirmed in vivo, and these include protection against β-cell apoptosis, increased pancreatic and β-cell area, and increased insulin content, suggesting that these two incretins may have specific and complimentary roles [12, 13]. These data suggest that a GIP/GLP-1 receptor co-agonist could be a promising therapy for T2D.

Tirzepatide, a dual GIP/GLP-1 receptor agonist has emerged as a potential therapeutic tool in the treatment of chronic metabolic diseases, such as T2D [14]. In addition to its antidiabetic functions, tirzepatide decreases lipoprotein biomarkers, such as apolipoprotein C-III, apolipoprotein B, as well as large triglyceride-rich lipoprotein particles and small low-density lipoprotein particles [15]. Tirzepatide has also been shown to increase adiponectin and decrease serum alanine aminotransferase [16]. Furthermore, the administration of a once weekly dose of tirzepatide in patients with renal impairment was well tolerated [17]. Similarly, a pharmacokinetics and tolerability study of tirzepatide in patients with hepatic impairment (with or without T2D) indicated similar pharmacokinetic results among participants with varying

degrees of hepatic impairment, thus not requiring any dose adjustments [17].

Existing literature lacks studies that directly compare the effects of bariatric surgery with currently available antiobesity and diabetes medications, such as GLP-1 agonist (semaglutide) and dual GIP/GLP-1 receptor agonist (tirzepatide). The primary objective of this study was to assess and compare the impact of bariatric surgery to various obesity drug treatments on weight loss, insulin resistance, and fatty liver in diet-induced obese Sprague-Dawley rats. The hypothesis driving this investigation was that current obesity treatment can reach an effectiveness level comparable to that of bariatric surgery.

Materials and Methods

Study Population

Male and female SD rats used in this study were maintained for 6–8 weeks prior to beginning the experiments. The rats were housed as 2 rats per cage (12 h/12 h dark/light period) keeping in mind gender similarity. To induce obesity, rats were provided high-fat cafeteria diet ad libitum as previously described [18, 19], which included foods that were high in fat and carbohydrate content, such as cookies, cupcakes, macaroni and cheese, chocolate, cream cheese, thick cream, puddings, cereals, savory snacks, flavored milk, and rat chow. Rats on this diet were referred to as high-fat diet (HFD) rats, and a total of 60 rats were used for analysis. A control group of rats ($n = 6$) were included and given ad libitum regular chow diet. The rats were continued on their respective diets for the full experimental period, a total of 28 weeks. The summary of the study design is represented in Figure 1. ARRIVE guidelines have been followed throughout this study.

Bariatric Surgery, Drug Treatment, and Euthanasia

The study was split into two phases: the first phase of the experiments was completed 4 weeks after intervention, while the second phase was completed 6 weeks following intervention. In both phases at week 21, we chose the HFD rats by comparing the weights of rats on regular chow diet to that of rats on HFD. Rats that showed an increase in weight between 100 and 200 g were considered as HFD rats and were broadly classified into two main intervention groups, namely, those that underwent surgery and those that were treated with semaglutide or tirzepatide. The rats were subdivided in the surgery group as sham and sleeve gastrectomy (SG) surgical groups (a minimum of 6 rats in each group per experimental phase). The animals were kept in a fasting state for 12–15 h prior to surgery and were housed individually in raised wire floor cages, suspended over a collection tray (Tecniplast®, Italy). SG was performed as specified in protocol (DDI/ORA/AC/039) [19], see Supplementary Figure 1 (for all online suppl. material, see https://doi.org/10.1159/000540534) for a representative image. Briefly, the surgery was performed using Coviden DST Series™ GIA™ linear cutting stapler, wherein the curvature of the stomach from the antrum to the rumen was captured between two staggered rows of the DST Series™ titanium 45 mm in length, 2.5 mm staples with 1 mm closed height/tissue thickness, and the stapler was fired to complete resection online supplementary Figure 1. Sham surgery was performed by laparotomy (DDI/ORA/AC/039). The rats were anesthetized using 5% isoflurane inhalation (Parkland Scientific® V3000PK) during the induction phase of the surgical procedure. This was followed by 1–3% isoflurane inhalation during the maintenance phase of the procedure. The rats undergoing both SG and sham surgery were given the analgesic Metacam (meloxicam 5 mg/mL) subcutaneous (SC) injection at a dose of 2 mg/kg, the antibiotic Baytril® (enrofloxacin 10%) SC injection at a dose of 5 mg/kg, and 20 mL 0.9% saline via SC injection. Some rats required administration of these drugs for a longer period up to a maximum of 5 days. No food or water was provided to these rats which were maintained on SC injection of 0.9% saline for 72 h post-surgery. The rats were maintained on a minimum quantity of a liquid diet for 48–96 h; solid food was introduced only 96 h post-surgery. The rats in the antidiabetic drug treatment group were further classified into four groups:

(1) Sema: rats treated daily with a SC injection of semaglutide (10 nmol/kg $n = 12$).
(2) Tri10: rats treated daily with a SC injection of tirzepatide (10 nmol/kg $n = 12$).
(3) Tri50: rats treated daily with a SC injection of tirzepatide (50 nmol/kg, $n = 6$).
(4) Tri100: rats treated daily with a SC injection of tirzepatide (100 nmol/kg, $n = 6$).

At the end of the second phase of the experiments at week 28, all rats were euthanized, as per the protocol (DDI/ORA/AC/003) approved by the Animal Care Committee (ACC) at Dasman Diabetes Institute (DDI). Briefly, the rats were anesthetized using 5% isoflurane inhalation and then decapitated. Different tissues were collected, such as subcutaneous adipose tissue (SAT), omental adipose tissue (OMT), brown adipose tissue (BAT), and liver. The total weight of the extracted adipose tissues was recorded. All the extracted tissues were divided into smaller sections and transferred into tubes either containing RNAlater or 10% buffered formalin. Tissues in RNAlater were stored at −20°C, while those in formalin were fixed and paraffin-embedded for future use.

Weight, Oral Glucose Tolerance Test, and Blood Sampling

The weights of the experimental rats were measured weekly to obtain a profile of their weights over time. Oral glucose tolerance test (OGTT) was performed on all rats prior to the experimental intervention. For the OGTT, the rats were in a fasting state for 12–15 h. OGTT was performed as per an ACC approved protocol # DDI/ORA/AC/030. Briefly, the rats were given 50% dextrose solution orally (Sigma-Aldrich, DX0145-3) at a volume of 2 g/kg then blood glucose level was measured using the glucometer (Freestyle Optium Neo meter) through blood drop collected from the tail tip. Glucose measurements were taken at the following time points: 0 min (T0), 15 min (T15), 30 min (T30), 60 min (T60), 90 min (T90), and 120 min (T120) post-glucose ingestion. Blood samples were also collected (up to 0.25 mL) in ethylenediamine tetra-acetic acid (EDTA) tubes at the different time points during OGTT through the tail snip. Immediately, the collected blood tubes were centrifuged at 400 *g* for 10 min. Then the plasma was transferred into new Eppendorf tubes and stored at – 80°C for future analyses. Also, OGTT was performed at 4 and 6 weeks post-experimental intervention, as described above.

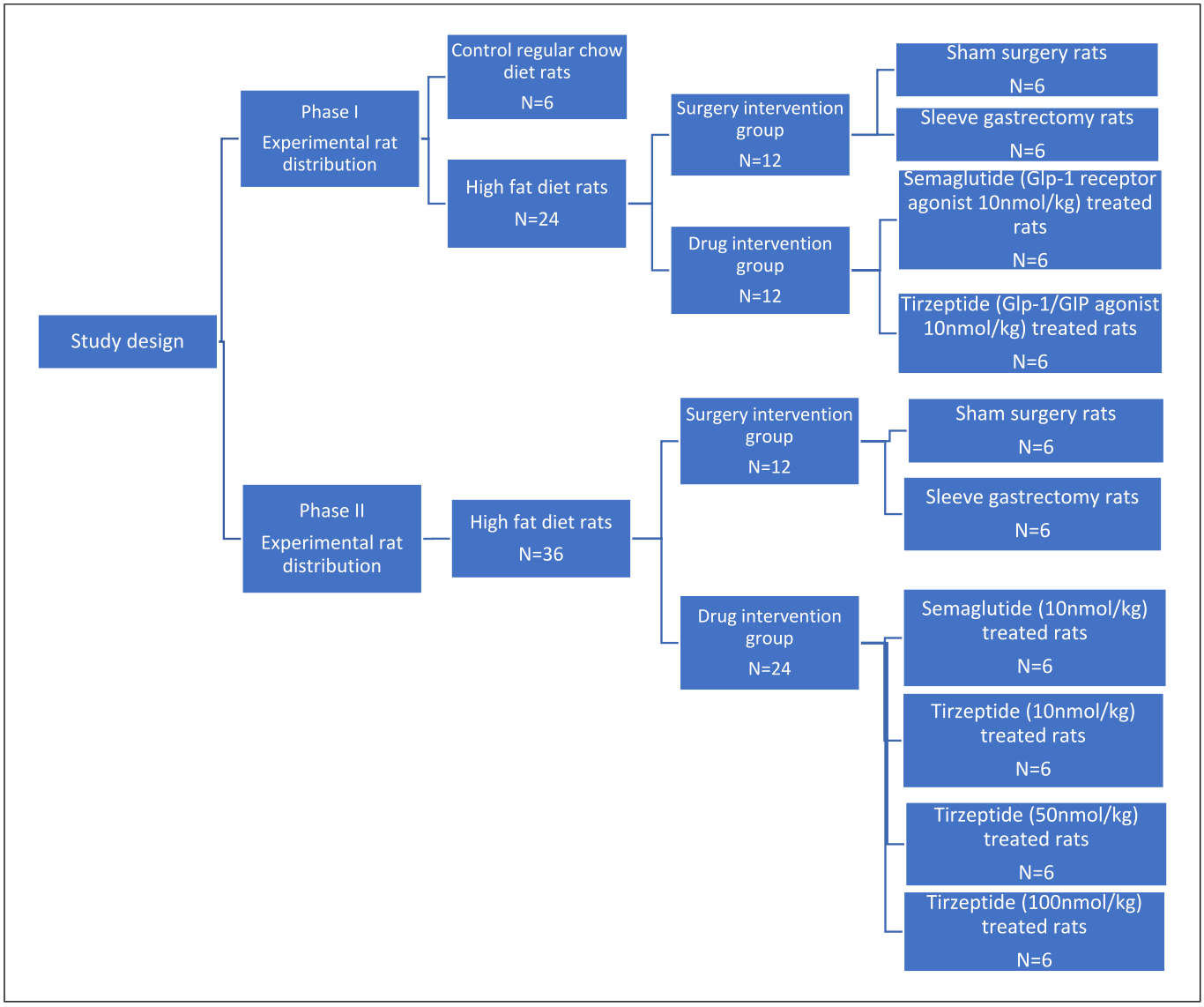

Fig. 1. Representative chart of the study design.

Hematoxylin and Eosin Staining for Morphological Analysis

Formalin-fixed and paraffin-embedded liver tissue blocks were sectioned (4–5 μm) on plain slides and used for hematoxylin and eosin staining for morphological analysis as per the protocol of Mao et al. [20].

Rat Metabolic Panel Multiplexing Assay

Plasma samples from the two experimental cohorts were extracted from blood samples collected from fasting animals and aliquoted into plates and stored at −80°C for future use. For the multiplexing analysis, plasma samples were thawed and used as per kit instruction for Luminex custom-made rat metabolic panel (Cat #RMHMAG-84K, Millipore, Darmstadt, Germany). The analytes tested were leptin, C-peptide, insulin, and amylin. Briefly, the kit standard was prepared with a 3-fold serial dilution, then standard samples and the plasma samples were added into respective wells in a 96-well plate. An equal amount of assay buffer was transferred into the sample wells, while a special serum matrix (provided in the kit) was added into the standard wells. A cocktail of antibodies complexed to magnetic beads was diluted and aliquoted into the plate and left incubated overnight at 4°C. Following the incubation, the plates were washed and incubated with detection antibody cocktail for 30 min. This was followed by a washing step then an incubation with diluted streptavidin-PE for another 30 min. The plates were washed, and the beads were resuspended with sheath fluid compatible with Bio-Plex-200 (Bio-Rad, Hercules, CA, USA) Luminex system. Data were acquired using the Bio-Plex system with parameters set as per kit specifications. The results were calculated using a 5-PL nonlinear standard curve setting in the Bio-Plex Manager software version 6.0.

Data Analyses

From the OGTT results, the area under the curve (AUC) and the incremental area under the curve were calculated using the trapezoid rule. One-way analysis of variance with Tukey's post hoc test was used to determine the significant differences in the results between the various experimental groups. GraphPad Prism 8.0 (San Diego, CA, USA) software was used for all the statistical analyses and to generate the figures. A two-way analysis of variance analysis was performed using the data acquired through the Bio-Plex Manager software. Post hoc analysis was performed using Bonferroni's multiple comparison test of means between groups. The statistical analysis was performed using GraphPad Prism version 9.0. Differences were considered statistically significant if p value <0.05.

Results

Rat Weight at Baseline and at 4 Weeks following Intervention

Initially, the experiments were performed to examine changes in body weight changes in rats that underwent surgery to those given equal doses of semaglutide or tirzepatide of 10 nmol/kg (n = 6 per group). These experiments were completed 4 weeks following intervention. A significant increase in the body weight was observed among rats in the HFD group as compared to that of the control group, as shown in Figure 2a (body weight: control [n = 7] = 492.3 ± 43.4 g, HFD [n = 24] = 610.8 ± 49.3; p value <0.0001). Four-week post-surgery, no significant change was observed in the body weight of rats in the sham and control groups as compared to their pre-surgery weight. However, a significant reduction in the weights of the rats in the SG group was seen when compared to their weight prior to surgery (Fig. 2b, $p < 0.05$). Furthermore, the rats in the SEM and TRZ1 groups given a dose of 10 nmol/kg showed a weight loss of 11% and 10%, respectively, when compared to their weight prior to intervention (Fig. 3a, $p < 0.05$).

OGTT 4 Weeks following Intervention

The AUC was determined through plotting blood glucose concentration against time (Fig. 2c). The AUC for rats in the sham group was significantly higher (Fig. 2d, $p < 0.005$) than that of control rats. However, no such significant difference in the AUC was observed when comparing the intervention groups to sham. Also, no significant differences were observed in the FBG levels between the groups. However, blood glucose levels of rats in the sham group were significantly higher than those in other groups ($p < 0.01$) at the 15-min time point of OGTT. Furthermore, at the 120-min time point of OGTT, sham rats showed significantly higher blood glucose level compared to the level seen in control rats ($p < 0.01$).

Weights of Rats and OGTT 6 Weeks following Intervention

The second phase was planned as a follow-up on the mild effect of tirzepatide (10 nmol/kg dose) on weight loss 4 weeks after intervention. It was designed to study the efficacy, safety, and tolerability of tirzepatide at three different doses of the tirzepatide (TRZ1 [10 nmol/kg], TRZ2 [50 nmol/kg], and TRZ3 [100 nmol/kg]) and to compare the outcomes to the sham, SG, and SEM groups (n = 6 in each group). Body weights were measured on a weekly basis. The results showed a consistent positive impact of tirzepatide on blood glucose control and weight loss with increasing dose, with no significant impact on tolerability. A significant increase in body weight was observed in the sham group, as compared to the intervention groups, as shown in Figure 3a (n = 6; sham group bodyweight = 510 ± 49.3, interventional group = 410–420 ± 43.4 g; $p < 0.001$).

Six-week post-surgery, no significant change was noted in the body weights of sham rats. However, the rats in the HFD group who underwent SG showed a 15.5% reduction in body weight as compared to their weight pre-surgery (Fig. 3b). On the other hand, drug intervention resulted in 10.7% body weight reduction in the rats of the SEM group, while the different doses of tirzepatide (Tri10, Tri50, and Tri100) caused 5.0%, 14.9%, and 17.7% reduction in body weight, respectively. Interestingly, a sharp loss in weight was observed at week 2 following SG (18%), which then increased at week 6, reaching an end point of 15% reduction in weight of rats in this group as compared to their pre-surgery weight.

An OGTT was performed on rats in all groups 6 weeks following intervention. The AUC was calculated from the blood glucose concentration against time (Fig. 3c). The AUC for rats across all intervention groups was significantly lower ($p < 0.05$) than that of rats in the sham group. However, no significant difference was observed in the AUC among the various intervention groups (Fig. 3d).

Effect of SG versus Drug Intervention on the Weight of Adipose Tissue Subtypes

At 6 weeks following the intervention experiments, the rats were euthanized, and various adipose tissue subtypes (SAT, OMT, and BAT) were extracted to study the impact of SG, SEM, and the different doses of TRZ on adipose tissue weight. The weight of the different adipose tissue subtypes was measured and compared to the weight of these tissues extracted from the sham group. No significant change was observed in the weight of BAT across all intervention groups as compared to sham (Fig. 4a). On

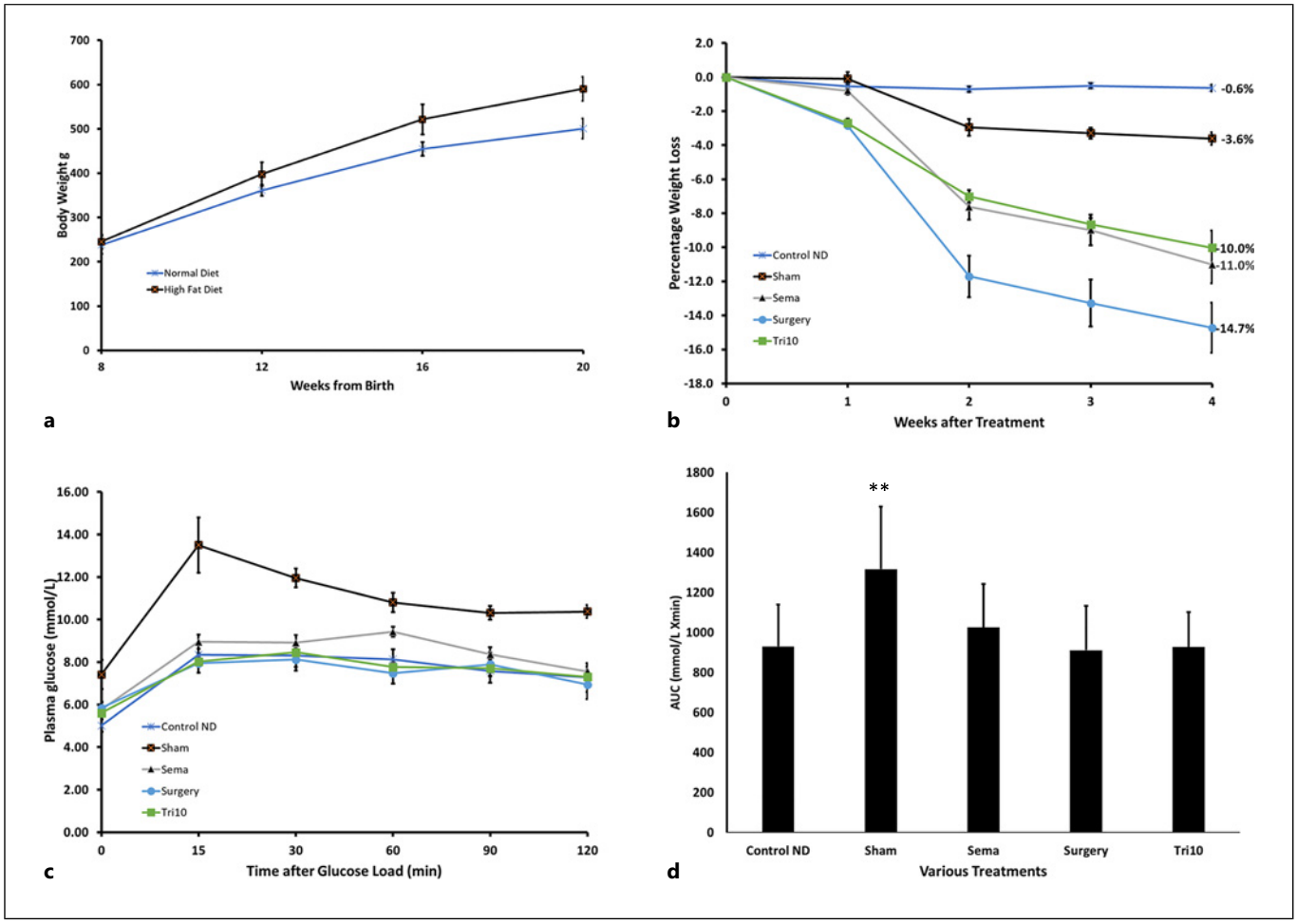

Fig. 2. Summary of results obtained from experimental phase I. **a** Representative chart of the average weight gain among rats from control and HFD groups. **b** Representative chart of the percent weight loss among rats from the different intervention groups. **c** Representative chart of the glucose load over time during the OGTT performed on the rats from the different groups after 4 weeks of intervention. **d** Representative chart of the AUC calculated from the OGTT results obtained from the different experimental groups. $^{**}p \leq 0.001$.

the other hand, a significant reduction in the weight of SAT was seen in the SG, SEM, and TRZ2/TRZ3 groups as compared to the sham group (Fig. 4b, $p < 0.05$). Interestingly, a significant reduction in OMT weight was evident in the tissues extracted only from rats in the TRZ3 group as compared to the sham group (Fig. 4c, $p < 0.05$).

Impact of SG versus Drug Intervention on Liver Fat Content

Hematoxylin and eosin-stained liver tissue samples, extracted from control (regular chow) group and those from the sham and the different intervention groups, were analyzed. Slides of liver tissues extracted from control rats were compared to those extracted from rats fed an HFD (Fig. 5). A significant increase in the intracellular lipid content in liver tissue from HFD rats was seen as compared to that of liver tissue extracted from the control rats (Fig. 5fb vs. 5a, $p < 0.0001$). This is indicative of nonalcoholic fatty liver disease (NAFLD). When the liver tissues taken from the SG group were compared to those from the sham group, a significant decrease in intracellular lipid content was observed (Fig. 5fc vs. 5b, $p < 0.0001$). Moreover, it was observed that liver tissue extracted from rats in the SEM and TRZ3 groups (Fig. 5d, e, respectively) exhibited further reduction in lipid content as compared to the sham group (Fig. 5b, $p < 0.0001$). However, the lipid content in the liver of these two groups (SEM and TRZ3) remained significantly lower than that of the control group (Fig. 5f, $p < 0.005$).

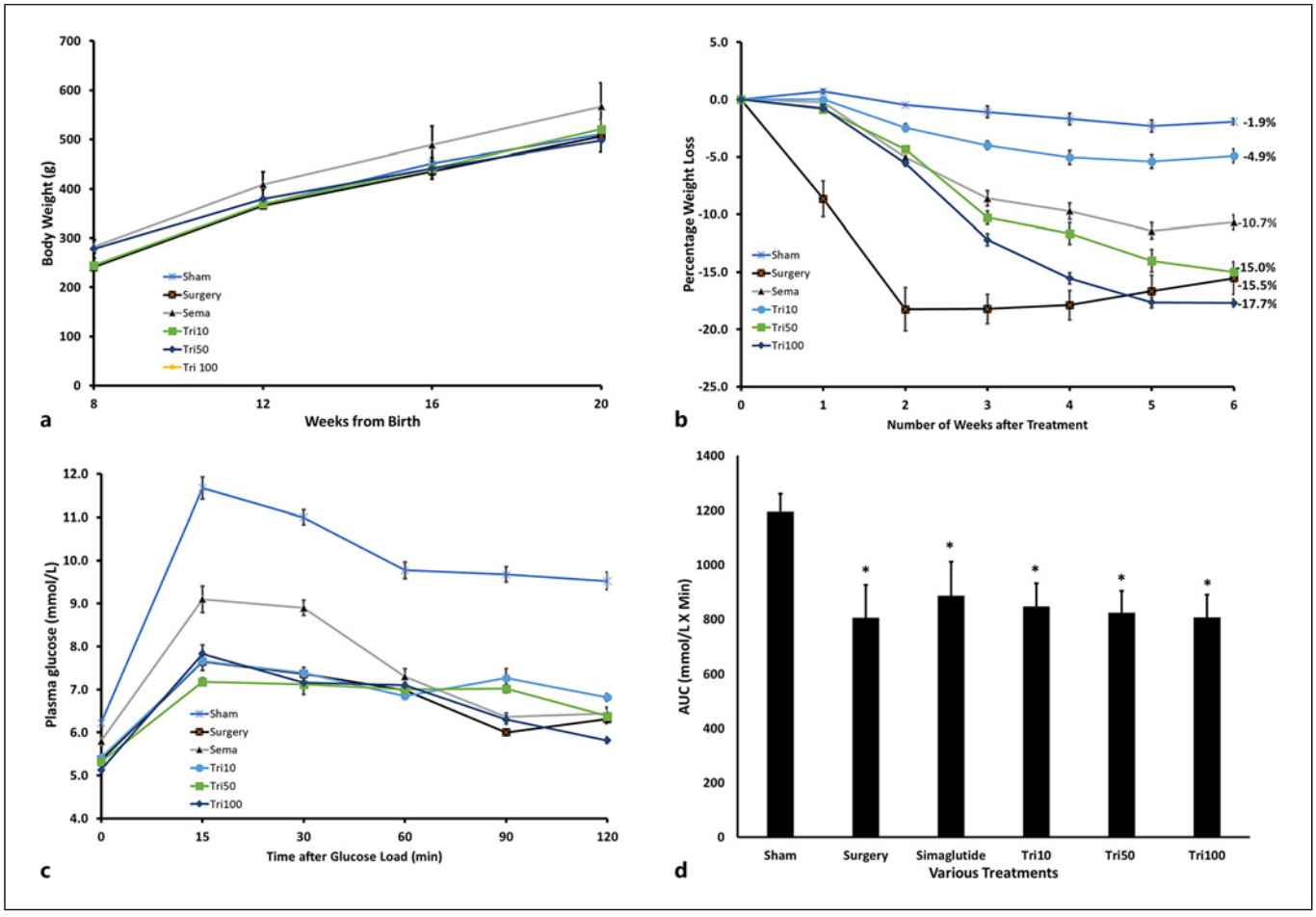

Fig. 3. Summary of results obtained from experimental phase II. **a** Representative chart of the average weight gain among rats from the HFD group that were divided into different intervention groups. **b** Representative chart of the percent weight loss among rats from the different intervention groups. **c** Representative chart of the glucose load over time during the OGTT performed on the rats from the different groups after 6 weeks of intervention. **d** Representative chart of the AUC calculated from the OGTT results obtained from the different experimental groups. *$p \leq 0.05$.

Metabolic Analyte Levels in Rats following Intervention

Plasma samples obtained from the different experimental groups of rats, at the end of the intervention period, were used to test the levels of different metabolic analytes (i.e., leptin, C-peptide, insulin, and amylin). The mean levels of the different analytes are seen in Table 1. Interestingly, it was observed that the levels of the different metabolic analytes were decreased across the intervention groups, particularly following the 100 nmol tirzepatide treatment when compared to the sham group. For example, levels of adiponectin, leptin, and BDNF were significantly reduced when compared to the sham group only in the 100 nmol tirzepatide treatment group ($p = 0.0465$ [Fig. 6d]). The expression level of these markers was not significantly different in the other intervention groups. C-peptide, insulin, and amylin had a similar expression pattern when comparing the results between the sham group and the different intervention groups. A significant increase in levels of C-peptide and insulin was observed in the sham animals when compared to lean controls. This increase returned to control levels following mild intervention such as with the 10 nmol tirzepatide treatment or was significantly reduced with the other treatments (Fig. 6a, b). Amylin level was also seen to significantly reduce following the different interventions as compared to that in the sham group (Fig. 6c).

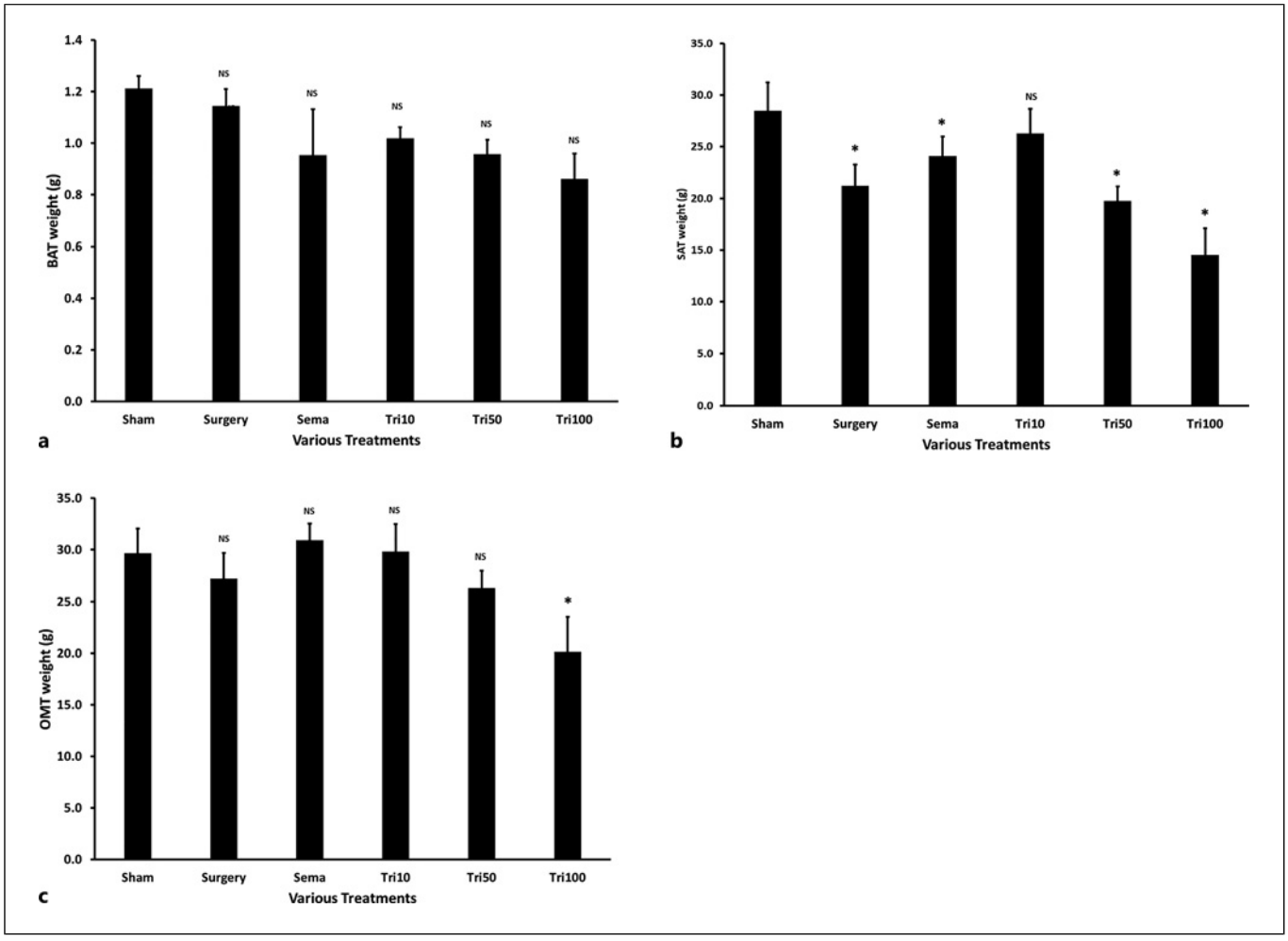

Fig. 4. Adipose tissue weight. **a** Representative chart of the average brown adipose tissue (BAT) weight extracted from the rats from the different intervention groups ($N = 6$ in each group). **b** Representative chart of the average subcutaneous adipose tissue (SAT) weight extracted from the rats from the different intervention groups. **c** of the average omental adipose tissue (OMT) weight extracted from the rats from the different intervention groups. $^{*}p \leq 0.05^{*}$.

Discussion

In this study, the effect of SG and various drug interventions on obese SD rats was investigated. The effect on weight loss, insulin resistance, and NAFLD were assessed. The intervention modalities studied, i.e., SG, treatment with semaglutide or tirzepatide lead to significant weight loss in DIO rats. Interestingly, bariatric surgery caused an average weight loss of 15.5% after 6 weeks. It is noteworthy that weight loss plateaued earlier in the surgery group compared to other intervention groups and mild weight regain was observed over time. A similar trend was reported in a study performed on human subjects following surgical intervention [21]. On the other hand, treatment with semaglutide resulted in a milder yet significant weight loss, with an average of 10.7% following treatment. Treatment with tirzepatide was found to be most effective in promoting weight loss, up to 17.7% at a dose of 100 nmol/kg, at the end of 6 weeks of treatment. Furthermore, the data showed that all the interventions impacted the levels of leptin, C-peptide, insulin, and amylin. A reduction was observed in the circulation levels of these analytes in the intervention groups when compared to the obese control group. The maximum decrease was seen with the highest dose of tirzepatide (100 nmol/kg). These findings highlight the potential of using these treatment options as effective tools for weight management in DIO rats, with

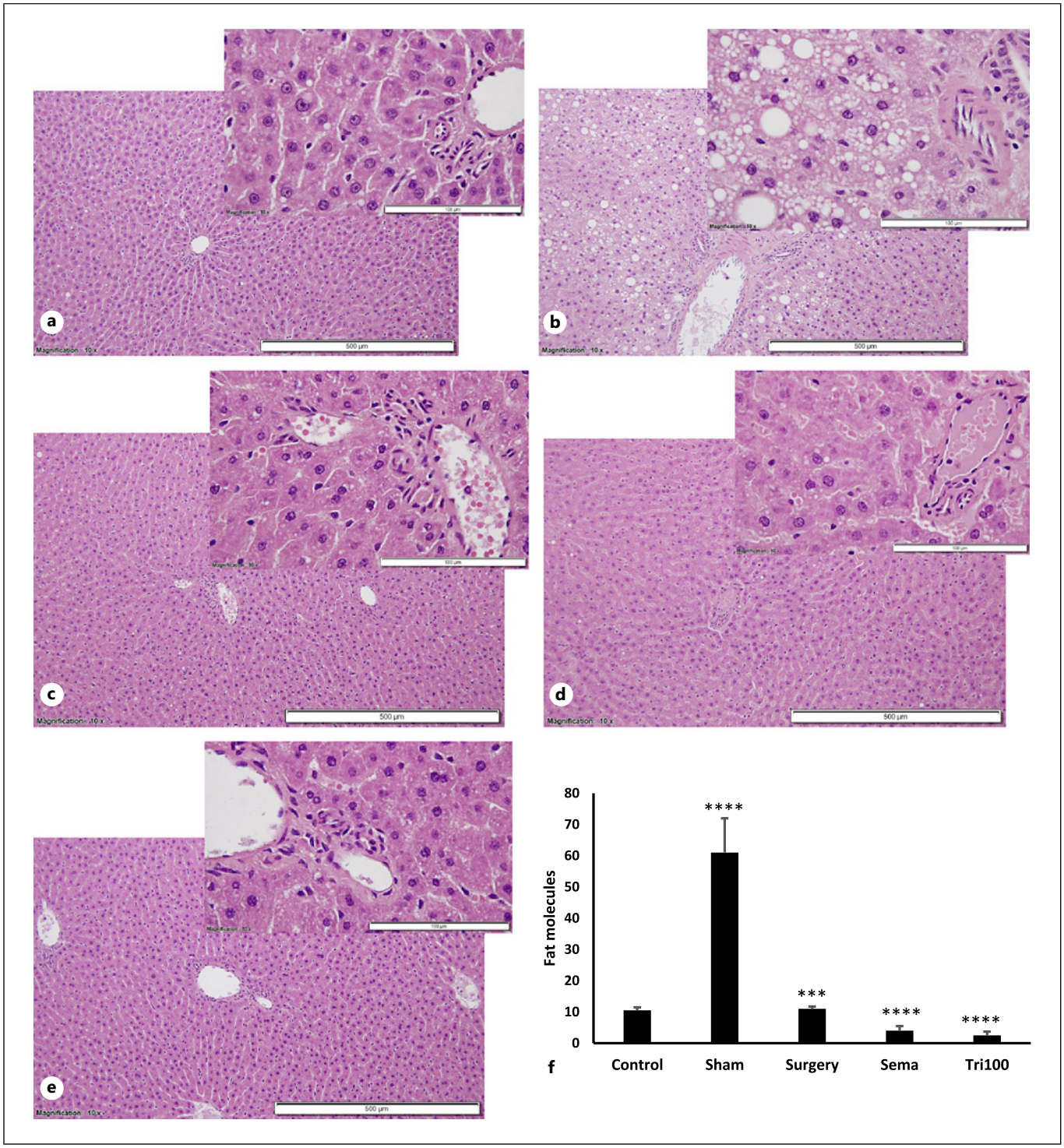

Fig. 5. Liver tissue hematoxylin and eosin staining done on tissues extracted 6 weeks following intervention. **a** Representative image of the liver tissue extracted from control (regular chow fed) rats showing normal cell shape and normal lipid distribution. **b** Representative image of the liver tissue extracted from HFD rat (high-fat diet fed) showing increased lipid content and distribution indicative of non-alcoholic fatty liver. **c** Representative image of the liver tissue extracted from HFD rats following SG showing decreased lipid content and distribution. **d** Representative image of the liver tissue extracted from HFD rats following treatment with semaglutide (10 nmol/kg) showing decreased lipid content and distribution. **e** Representative image of the liver tissue extracted from HFD rats following treatment with tirzepatide (100 nmol/kg) showing decreased lipid content and distribution. **f** Representative chart showing the average lipid molecule count performed on the liver tissues extracted from the rats following the different interventions. *$p \leq 0.05$.

Table 1. Metabolic analyte expression levels in rat plasma from the different study groups

Rat groups	Leptin, pg/mL	*p* value	C-peptide, pg/mL	*p* value	Insulin, pg/mL	*p* value	Amylin, pg/mL	*p* value
Sham (±SD)	4,902±912.5	1	674.1±33.4	1	2,140±224.2	1	29.2±2.7	1
Cont. lean (±SD)	3,000±515.4	0.209	387.7±19.8	0.006	908.7±145.2	0.003	21.4±1.8	0.151
Surgery (±SD)	3,342±332.5	0.364	454.8±60.5	0.013	834.1±102.8	0.000	17.8±1.9	0.012
Semaglutide (±SD)	3,454±495.4	0.428	543.2±29.9	0.219	1,309±116.6	0.007	20.2±2.0	0.013
Tirzepatide 10 nmol (±SD)	3,975±423	>0.99	554.4±49.8	0.182	1,960±260.3	>0.99	22.4±2.2	0.260
Tirzepatide 50 nmol (±SD)	2,429±416.8	0.108	424.6±21.3	0.025	907.7±85.3	0.002	12.3±1.8	<0.0001
Tirzepatide 100 nmol (±SD)	1,664±333.6	0.015	359.7±34.5	0.001	523.2±170.1	<0.0001	15.0±2.2	0.002

Post hoc Bonferroni test was performed adjusting multiple comparisons to the control (Sham).

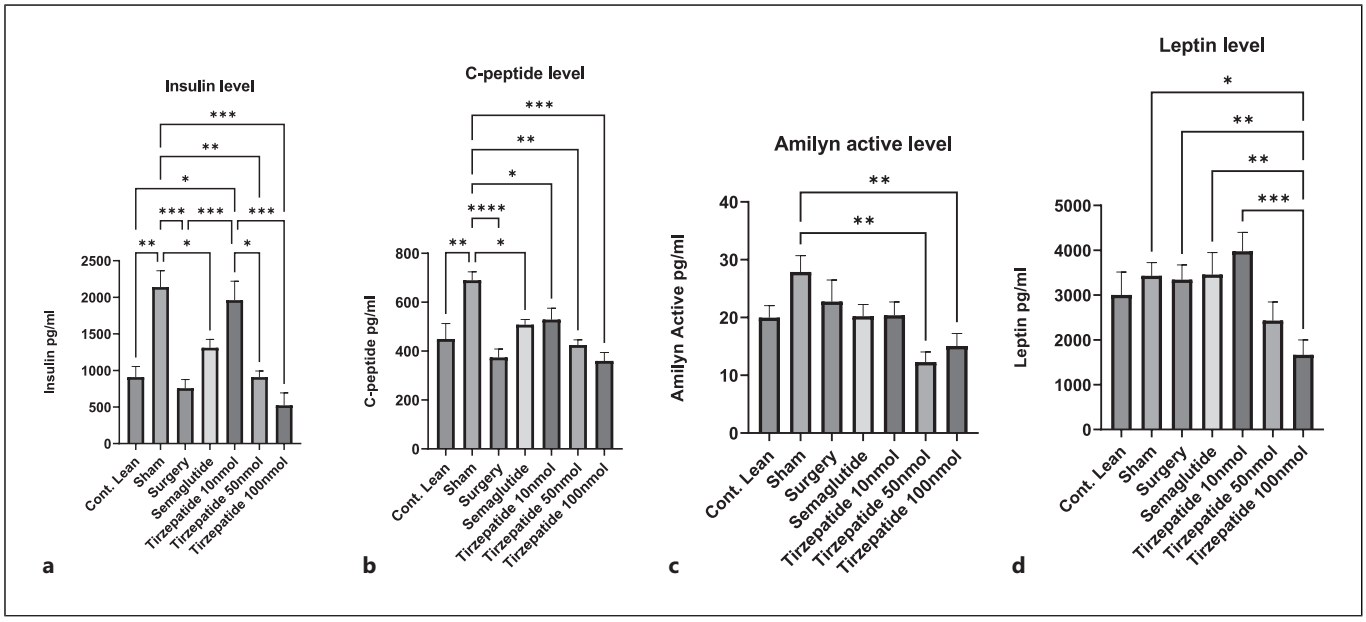

Fig. 6. The level of metabolic analytes across different treatment groups (i.e., bariatric surgery, semaglutide, Tirzepatide 10 nmol, 50 nmol, 100 nmol). **a** The level of insulin across the different treatment groups. **b** The level of C-peptide across the different treatment groups. **c** The level of amylin across the different treatment groups. **d** The level of leptin across the different treatment groups. The figures present the data as Mean ± SEM. *$p \leq 0.05$, **$p \leq 0.001$, ***$p \leq 0.0001$.

SG and tirzepatide being particularly effective in achieving rapid weight loss.

Blood sugar control across all interventions was assessed using OGTT. Rats in the SG group as well as those treated with semaglutide and tirzepatide showed significant improvements in their OGTT results. It has been widely reported that bariatric surgery has dramatic effects on reducing fasting glycemia and improving prandial glucose control almost immediately after surgery [22, 23]. It is also known that semaglutide and tirzepatide are medications that stimulate the production of insulin and reduce the production of glucose by the liver, leading to better blood sugar control [24]. The results from this study further support this notion as treatment using both surgical and drug intervention modules had a significant effect on OGTT. Rats in all groups displayed decreased

blood glucose levels and improved insulin sensitivity following treatment. Therefore, these treatments especially with drugs represent promising options for impaired glucose metabolism or diabetes. Interestingly, the data obtained from the metabolic level analysis on insulin and c-peptide levels do not reflect the anticipated increase in the production of insulin in response to glucose following drug treatment. Contrary to the expected effect of GIP/GLP treatment modules, a reduction in circulation levels of insulin and c-peptide was observed. This may be due to the increased insulin sensitivity in muscle and adipose tissue, which would lead to a quick response to glucose followed by a quick clearance of these analytes from the blood stream.

Furthermore, in this study, the effect of the different interventions on adipose tissue and liver were assessed. The results obtained add to the positive outcome of using either SG or drug treatment on the general wellbeing of the rats. White adipose tissue weight (SAT) was reduced following all treatment modules in this study. White adipose tissue is considered the main energy reservoir, and it secretes many hormones and cytokines that play a role in regulating glucose metabolism and insulin resistance [25]. Any potential changes in this tissue may play a critical role in the metabolic homeostasis of the body. It is currently well established that obesity leads to an increase in fat deposition in the white adipose tissue, mainly SAT; as SAT grows, it becomes severely dysfunctional and would not expand properly to store excess energy. This leads to deposition of the excess fat in other ectopic tissues, increasing the weight of OMT, which in turn plays a role in disrupting glucose homeostasis. This process, commonly referred to as "lipotoxicity," leads to systemic insulin resistance and an increased risk of T2D [26]. Therefore, the loss in adipose tissue weight observed following the different treatments may play an important role in improving insulin sensitivity and the general health of the rats. Notably, Tirzepatide treatment at high dose resulted not only in reduction in SAT weight but also caused a significant reduction in the more pathogenic OMT weight.

NAFLD is currently considered one of the most common liver diseases, and its prevalence continues to increase worldwide [27]. It is defined by an increased presence of fat in the liver comprising more than 5–10% of the liver weight [28]. In the present study, as expected, liver tissue of sham rats had a higher fat content, indicative of NAFLD, as compared to that of liver tissue of rats on normal diet. This increase in the fat content was reversed in all the intervention groups following treatment. The most effective result was observed with the highest dose of tirzepatide treatment. This adds further support to the beneficial effect of the tirzepatide treatment on the general wellbeing of the rats when compared to other treatment modalities. Studies on humans have also shown the beneficial effect of tirzepatide treatment on adipose tissue content and liver fat content [29]. Further studies are required to better understand the exact mechanisms involved in this process.

Bariatric surgery is a well-established treatment option for obesity [30] and can result in significant weight loss and improvement in metabolic health. However, several potential risks and complications are associated with this procedure such as bleeding, infection, blood clots, and nutritional deficiencies. In addition, surgery is not suitable for everyone, and careful consideration and evaluation is required to determine if a person is eligible for the procedure. Hence, the availability of other less invasive treatment options, such as GLP-1 receptor agonists, has revolutionized the treatment of obesity and diabetes. More recently, the dual-agonist drug tirzepatide targeting both the GLP-1 and GIP receptors has been approved for the treatment of T2D. In clinical trials, tirzepatide has shown promising results in promoting weight loss and ameliorating blood glucose control, with some patients achieving significant weight loss of up to 15–20% of their body weight [31, 32]. It has also been shown to reduce liver fat, improve cardiovascular risk, and reduce the need for insulin and other diabetes medications [29].

As compared to bariatric surgery, tirzepatide is a noninvasive treatment that can be administered subcutaneously, making it a more attractive option for patients who are not willing or able to undergo surgery. Additionally, tirzepatide is reportedly effective in patients with moderate to severe obesity [33], making it a potential alternative for patients who are not eligible for bariatric surgery due to medical or other reasons. However, the long-term efficacy and tolerability of using tirzepatide are not yet fully established. Also, the potential side effects of using this drug, such as gastrointestinal symptoms, pancreatitis, and thyroid cancer, cannot be overlooked. Additionally, tirzepatide requires regular injections and may be associated with higher costs, as compared to other diabetes medications.

Despite the promising results of our study, further research is needed to fully elucidate the long-term efficacy and tolerability of tirzepatide. Further studies are needed to compare other aspects of weight loss such as the effect on fat-free mass, change in energy expenditure, and metabolic adaptation. Moreover, while choosing a treatment for obesity, it is crucial to personalize the treatment and tailor it for each patient, as not all

individuals respond similarly to a particular treatment. The findings of our study suggest that tirzepatide treatment may offer a viable alternative to surgery in obesity management. Tirzepatide has a favorable safety profile and can be administered subcutaneously, making it a more accessible and convenient option as compared to bariatric. Additionally, the weight loss achieved with tirzepatide treatment was comparable to that achieved by bariatric surgery.

This study brings to light several novel insights in the field of obesity and T2D treatment. A key finding is the effectiveness of tirzepatide, a dual GIP/GLP-1 receptor agonist. Notably, tirzepatide demonstrated a significant weight loss of 17.7%, marking a substantial advancement in the treatment options available for these conditions. Another important insight is the comparison of the efficacy of tirzepatide with that of bariatric surgery. This comparison is crucial as it suggests that tirzepatide can offer benefits similar to surgery, including improvements in glucose tolerance, reduction in adipose tissue weight, and decrease in hepatic liver fat droplets, but as a non-invasive alternative. Furthermore, the study sheds light on the positive impact of tirzepatide on various metabolic markers, such as leptin, C-peptide, insulin, and amylin. This broadens the understanding of its metabolic benefits, extending beyond just weight loss and glucose tolerance improvements. The study reaffirms the efficacy of bariatric surgery, a well-known and effective approach for weight loss and the resolution of T2D. Additionally, the study contributes to the existing knowledge about GLP-1 receptor agonists, particularly highlighting semaglutide, a previously known agent in T2D treatment. By comparing tirzepatide with semaglutide, the study adds to our understanding of the role of GLP-1 receptor agonists in diabetes management.

One limitation of this study is that it was performed on animals, and it is essential that similar studies be performed on human subjects. The second limitation is the use of SD rats, which are a good model to study the effect of obesity, and it is challenging to induce diabetes. Consequently, while the study effectively examined the impact of various interventions on obesity and insulin resistance, the effects on diabetes were not fully explored. Our study utilized cafeteria diet (high fat/high carbohydrate) to induce obesity; thus, the precise calculation of the diet constituents during the study was not feasible. Nonetheless, this was not an outcome that we were interested in evaluating as we were mainly interested in inducing weight gain. Furthermore, the tests used to assess some of the metabolic parameters, such as glucose levels, are not the optimal option. Future investigations could employ more optimal methods, such as including assessments for fructosamine levels and fibrosis testing, to better study these parameters.

Conclusions

The study results show that though the intervention modules studied have shown promising results in the treatment of obesity and T2D, tirzepatide showed a more pronounced improvement in the various metabolic parameters studied. Hence, it may be considered the most effective and established approach for achieving significant and sustained weight loss, increased insulin sensitivity, and improvement in NAFLD. Additionally, tirzepatide is a less invasive alternative for the treatment of obesity and T2D.

Statement of Ethics

The experimental protocol was approved by the Ethical Review Committee (ERC) and Animal Care Review Committee (ACC) at DDI (RA-AM-2021-006).

Conflict of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Author Contributions

Experimental design, methodology, investigation, and writing: Mohammad Jamal, Mohammad Qaddoumi, Fahad Alajmi, Jijin Kumar, Nermeen Abukhalaf, Preethi Cherian, Dhanya Madhu, Hossein Arefanian, Carol Dsouza, Nada Alam-Eldin, Abdullah AlSabbagh, Ashraf Al Madhoun, Suleiman Al-Sabah, Fahd Al-Mulla, Mohamed Abu-Farha, and Jehad Abubaker; conceptualization, experimental design, funding acquisition, supervision, review, and editing: Salman Al-Sabah and Irina Al-Khairi.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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