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Authors: Zinah Meteab Rahi AL-shibil, Zahraa J. Kadhim

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Effect of retatrutide on body weight, lipid profile, liver function, oxidative stress, and inflammation in experimental obesity in male rats

Zinah Meteab Rahi AL-shibil [1], Zahraa J. Kadhim [2]

[1] Al-Najaf Health Directorate, Ministry of Health, Najaf, Iraq

[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

Corresponding author: Zinah M. Rahi AL-shibil, e-mail: zinahm.alshibil@student.uokufa.edu.iq

ORCID

ZMSA-S: https://orcid.org/0009-0000-8383-5502

ZJK: https://orcid.org/0000-0001-7413-7278

ABSTRACT

Introduction and aim. Obesity is a global health concern associated with an increased risk of diabetes, cardiovascular disease, hypertension, and non-alcoholic fatty liver disease, often driven by chronic low-grade inflammation. Recent evidence suggests that retatrutide, a novel GIPR/GLP-1R/GCGR tri-agonist, possesses anti-inflammatory properties in addition to its known effects on glucose metabolism, lipid profiles, and weight reduction. However, comprehensive preclinical data on retatrutide's direct impact on hepatic inflammation, oxidative stress, and FGF21 regulation in diet-induced obesity models remain limited. This study aims to investigate the potential protective effect of retatrutide on inflammatory and oxidative stress status in diet-induced obesity in male rats, thereby providing mechanistic insight into its hepatoprotective actions.

Material and methods. Twenty-eight adult male Sprague-Dawley rats were randomly assigned to four groups: normal controls (standard chow for 12 weeks), obese controls (HF/sucrose diet for 12 weeks), vehicle-treated (HF/sucrose for 8 weeks, then normal saline S.C. for 4 weeks with HF/sucrose), and retatrutide-treated (HF/sucrose for 8 weeks, then retatrutide 25 nmol/kg S.C. for 4 weeks with HF/sucrose). Serum insulin, lipid profile, liver enzymes, blood glucose, and FGF21 were measured from blood samples. Crucially, tumor necrosis factor- α (TNF-α), malondialdehyde (MDA), and glutathione (GSH) levels were measured in liver tissue samples.

Results. Modeling obesity using HF/sucrose diet significantly increased insulin levels, blood glucose, liver enzymes, lipid profile, serum FGF21, and body weight. It also considerably elevated hepatic MDA and TNF-α while reducing GSH levels. Retatrutide treatment resulted in significant improvements across most parameters compared to both the obesity and vehicle-treated groups ($p<0.0001$). Specifically, retatrutide-

treated rats showed significant reductions in body weight (e.g., approximately 25% reduction compared to obese controls), blood glucose (e.g., from 107±5.944 mg/dL to 85.714±4.785 mg/dL), and liver enzymes AST (e.g., from 89.843±4.533 U/L to 48.959±4.816U/L) and ALP (e.g., from 168.451±28.384 U/L to 97.526±13.446 U/L). Lipid profile parameters, including cholesterol (e.g., from 232.325±23.058mg/dL to 105.881±26.225 mg/dL), triglycerides (e.g., from 112.140±11.450 mg/dL to 30.355±9.479 mg/dL), and LDL (e.g., from 171.557±17.678 mg/dL to 51.341±21.858 mg/dL), were significantly improved, while HDL (e.g., from 38.339±9.670 mg/dL to 65.759±13.828 mg/dL) was significantly increased. Hepatic inflammatory (TNF-α, e.g., from 115.621±5.682 pg/mL to 92.715±5.647 pg/mL) and oxidative stress markers (MDA, e.g., from 5.409±1.078 nmol/ml to 3.120±0.401 nmol/mL) were significantly reduced, and hepatic GSH levels (e.g., from 1.220±0.545 ng/mL to 2.895±0.475 ng/mL) were significantly increased, serum FGF21 (e.g. from 115.367±6.921 pg/mL to 87.445±4.279 pg/mL) . These parameters were largely restored to near-control levels in the retatrutide-treated group.

Conclusion. This study assessed the hepatoprotective effect of retatrutide in a diet-induced obesity model. While the metabolic benefits of incretin-based therapies are well documented, data on retatrutide's direct impact on liver-specific inflammatory and oxidative stress pathways remain limited. This study is among the first to simultaneously evaluate hepatic TNF-α, oxidative stress markers (MDA and GSH), and circulating FGF21 following retatrutide treatment in obese rats, thereby providing mechanistic insight into its hepatoprotective actions beyond weight loss and glycemic control.

Keywords. inflammation, liver, obesity, retatrutide, weight loss

Introduction

Metabolic dysregulation associated with obesity arises when caloric intake consistently exceeds caloric expenditure. Adipose tissue depots expand when surplus energy is converted into triglycerides, resulting in increased body fat and weight gain.[1]

Since 1980, the proportion of individuals classified as overweight or obese has increased fourfold. According to the World Health Organization (WHO) 2024 Global Obesity Report, the prevalence of obesity has continued to escalate dramatically. As of 2023, approximately 43% of adults globally are classified as overweight or obese, representing a significant increase from previous decades. The WHO projects that by 2025, obesity will affect over 1.2 billion people worldwide.[2] In the Middle East and North Africa region, obesity prevalence has reached 28-35%, representing one of the highest rates globally.[3] This escalating epidemic is associated with increased incidence of non-communicable diseases, including type 2 diabetes mellitus, cardiovascular disease, and non-alcoholic fatty liver disease (NAFLD).[4]

Metabolic dysregulation resulting from obesity is correlated with increased concentrations of inflammatory cytokines in the bloodstream relative to individuals with lower body weight. It is proposed that these cytokines play a role in the onset of insulin resistance. In obesity, the principal source of pro-inflammatory

cytokines is adipose tissue, predominantly produced by infiltrating macrophages, but adipocytes also play a role in this process. Weight loss results in a decrease in blood levels of these cytokines.[5] The gut microbiota is associated with the pathogenesis of obesity and related metabolic disorders through various potential mechanisms. This encompasses (a) a significant prevalence of carbohydrate-fermenting bacteria, which enhances the biosynthesis of short-chain fatty acids, providing the host with an alternative energy source ultimately stored as lipids or glucose; (b) heightened intestinal permeability to bacterial lipopolysaccharides (LPS), resulting in elevated systemic LPS levels that exacerbate low-grade inflammation and insulin resistance; and (c) augmented activity of the gut endocannabinoid system.[6] The prevalent consequences of metabolic dysregulation in obesity include diabetes, cardiovascular disease, and respiratory issues.[7] Recent research suggests that the prevalence of wheeze and bronchial hyper-responsiveness, commonly associated with asthma, is elevated in overweight and obese persons. Furthermore, certain research indicates that health issues related to obesity may increase the risk of developing deep vein thrombosis, pulmonary embolism, and complications of breathing, including pulmonary hypertension and pneumonia. Weight reduction has demonstrated effectiveness in alleviating the symptoms and severity of several respiratory disorders, including obstructive sleep apnea and asthma.[8] Moreover, obesity is a contributing factor to the onset of gallbladder disease, non-alcoholic fatty liver disease (NAFLD),[9] pancreatitis, and male fertility issues, including reduced sperm count and heightened incidence of erectile dysfunction.[10] Furthermore, in females, it leads to diminished fertility, poorer outcomes following fertility treatment, and a heightened frequency of pregnancy loss, neurological disorders, immune system dysfunction,[11] musculoskeletal issues, renal illness, and psychosocial complications.[12] Dietary modifications, lifestyle alterations, physical exercise, therapeutic interventions, and surgical procedures can diminish the prevalence of health issues associated with obesity.[13]

Retatrutide (LY3437943) is an experimental medication that represents a novel class of metabolic pharmaceuticals created by Eli Lilly. Retatrutide is a novel tri-agonist peptide targeting the glucagon receptor (GCGR), glucose-dependent insulinotropic polypeptide receptor (GIPR), and glucagon-like peptide-1 receptor (GLP-1R), exhibiting 2.9-fold and 2.5-fold reduced potency at GCGR and GLP-1R, respectively, and 8.9-fold enhanced potency at the human GIP receptor. The mechanisms of action of retatrutide target the GIP, GLP-1, and glucagon receptors, resulting in enhanced glucose regulation, weight reduction, and metabolic wellness.[14] Retatrutide demonstrates decreased pro-inflammatory cytokine expression. Preclinical investigations indicate that retatrutide reduces the expression of pro-inflammatory cytokines, including TNF-α.[15] The clinical indications include obesity management,[16] type 2 diabetes,[17] non-alcoholic fatty liver disease,[18] and potentially other conditions linked to metabolic dysregulation, cardiovascular risk,[19] and cancer progression.[20]

The integrated approach – via concurrent modulation of multiple incretin receptors – offers the potential for improved weight loss outcomes, superior glycemic control, beneficial impacts on hepatic fat

accumulation relative to conventional treatments, and enhancements in essential metabolic indicators, such as lipid profiles, blood pressure, and HbA1c levels. The drug's versatility positions it as a strong candidate for obesity management and the treatment of several metabolic disorders, potentially serving as a foundational element in multi-indication therapeutic strategies.[21]

Although GLP-1 receptor agonists (e.g., liraglutide, semaglutide) and GIP/GLP-1 dual agonists (e.g., tirzepatide) reliably reduce body weight and improve metabolic outcomes in both humans and rodents, including better glucose control and improved lipid profiles. However, comprehensive preclinical data on the triple-agonist retatrutide in diet-induced obesity models are still limited.[22]

Importantly, retatrutide's effects on key liver-related mechanisms have not been well defined, including hepatic oxidative stress (malondialdehyde (MDA) and glutathione (GSH) measured together), hepatic inflammation (TNF-α), and the metabolic regulator FGF21, and the optimal rodent dosing schedule remains unclear. This study addresses these gaps by measuring MDA, GSH, hepatic TNF-α, and serum FGF21 in a diet-induced obesity model while applying a concise daily dosing regimen designed to improve peptide stability,[23] thereby clarifying retatrutide's hepatoprotective mechanisms and supporting translational relevance.

Aim

The purpose of this study is to examine the impact of retatrutide on body weight, level of inflammation, and oxidative stress in hepatic tissue by estimating the levels of MDA, GSH, TNF-α, and FGF21, and measuring serum biochemical parameters including liver function enzymes, lipid profiles, and insulin resistance levels.

Material and methods

Animal preparation

A total of 28 mature male Sprague-Dawley rats, aged 8–10 weeks and weighing between 200 and 230 grams, were sourced from the Faculty of Science, University of Kufa. They were kept in the animal department of the Faculty of Science at the University of Kufa under standard conditions. The average daily temperature was maintained at 24±2°C with 60–65% humidity, and the rats had unrestricted access to food and tap water. The animals were housed in cages with 3-4 rats per cage, with a 12-hour light/dark cycle. All experimental techniques and animal handling were conducted after clearance from the Institutional Animal Care and Use Committee (IACUC) at Kufa University, following the submission of the requisite documentation (NO. 2121) on January 23, 2025.

Compliance with ethical standards

All experimental procedures were conducted in accordance with:

- The Guide for the Care and Use of Laboratory Animals (NIH)
- ARRIVE 2.0 guidelines for reporting animal research
- Institutional policies and regulations

Study design

After a 7-day acclimatization period, a computer-generated sequence was used to randomly assign twenty-eight Sprague-Dawley rats into four experimental groups, seven rats per each group. Investigators were blinded to treatment allocation during outcome assessment. However, due to the nature of the intervention, blinding during treatment administration was not feasible.

Sample size calculation

The sample size was determined to ensure adequate statistical power while maintaining the error degrees of freedom (DFw) within the range of 10 to 20. Based on the formula Min (n) = 10/(k+2) and Max (n) = 20/(k+1), where k represents the number of groups, a minimum of 7 animals and a maximum of 11 animals per group were indicated. Consequently, 7 animals per group were selected for this study.[24.25]

Experimental groups

• Control group: The rats in this group received standard chow for 12 weeks.
• Obesity group: The rats in this group received a high-fat/sucrose diet (HF/sucrose diet) for 12 weeks.
• Vehicle-treated group: The rats in this group were fed HF/sucrose diet for eight weeks, then received subcutaneous injections of normal saline every morning for four weeks along with the HF/sucrose diet.
• Retatrutide-treated group: After eight weeks of HF/sucrose diet, the rats in this group received 25 nmol (0.118 mg/kg) of retatrutide S.C. every morning for four weeks along with the HF/sucrose diet.

Experimental diet

The 30% sucrose solution for the HF/sucrose diet group was formulated by dissolving 300 g of refined sucrose in 1000 ml of water. The fluid was filtered and administered to the rats in glass containers. This 30% sucrose concentration was chosen to effectively induce metabolic dysregulation and obesity in rodent models, consistent with established protocols.[26] To create high-fat chow, standard chow pellets containing 5% fat (LabDiet 5008, LabDiet, St. Louis, MO) were triturated, and 750 g of this powder was combined with 250 g of Beef fat to achieve a final fat content of 30%. Water was incorporated into the mixture to achieve a uniform dough; thereafter, pellets were manually formed, allowed to dry, and provided to the HF/sucrose diet group. The nutritional composition of the standard chow and high-fat/sucrose diet is detailed in Table 1.[27]

Table 1. Nutritional composition of standard chow and high-fat/sucrose diet

Nutrient	Standard chow (LabDiet 5008)	HF/sucrose diet
Protein	~20%	~15-18%
Carbohydrate	~60%	~25-30% + 30% sucrose solution
Fat	5%	30%
Fiber	~5%	~3-4%
Ash	~8%	~6-7%
Metabolizable energy (kcal/g)	~3.5	~4.8

Dose calculation

Retatrutide (cat. No. Z-peptide-24080802, China) powder with 98.89% purity was used in this study. The human clinical dose is 8 mg/week, which corresponds to 0.019 mg/kg/day for a 60 kg individual.[16] Translating dosages from humans to animals is an essential phase in preclinical research; however, it is crucial to understand the process and its constraints. The predominant and recommended approach employs Body surface area (BSA), which has a superior correlation with metabolic rate and physiological processes compared to body weight alone.[28]

Using BSA conversion based on the formula:

Animal dose (mg/kg) =human dose (mg/kg) × (human Km factor/animal Km factor)

Where Km=body weight (kg)/body surface area (m^2)

Step-by-step calculation

1. Identify the known values:
    - Human dose: 0.019 mg/kg
    - Human Km factor: For a 60 kg human, the Km factor is 37
    - Rat Km factor: For a 150-200g rat, the Km factor is 6
2. Apply the formula:
    - Rat dose (mg/kg) =0.019 mg/kg × (37 / 6)
    - Rat dose (mg/kg) =0.019 mg/kg × 6.17
    - Rat dose≈0.118 mg/kg
3. Convert to Molar Units:
    - Molecular weight for retatrutide=4731.33 g/mol
    - 0.118 mg=0.118×10^{-3} g=1.18×10^{-4} g
    - mol/kg=$1.18\times10^{-4}/4731.33=2.494\times10^{-8}$ mol/kg

- 2.494×10^{-8} mol/kg$\times10^{9}$=24.94 nmol/kg≈25 nmol/kg

Rationale for daily vs. weekly administration

Retatrutide was administered daily rather than weekly (as used clinically) for the following reasons:

4. Peptide stability: Peptides undergo rapid enzymatic degradation with short plasma half-lives. Daily dosing of GLP-1 agonists is required to achieve weight loss in rats due to species-specific pharmacokinetic and metabolic differences. Rats have higher basal metabolic rates and faster drug clearance than humans, necessitating more frequent dosing based on allometric scaling. Pharmacokinetic studies have shown that daily administration of long-acting GLP-1 agonists is necessary in rats to maintain therapeutic drug levels and achieve steady-state exposure comparable to weekly dosing in humans. (typically 2-4 hours for GLP-1 analogs).[23]
5. Bioavailability: Weekly dosing would result in suboptimal and fluctuating drug concentrations, with periods of inadequate therapeutic levels.[29]
6. Sustained effect: Daily subcutaneous administration ensures stable peptide concentrations and sustained biological effects throughout the treatment period.

Important limitation – lack of PK/PD data: However, we acknowledge that no pharmacokinetic (PK) or pharmacodynamic (PD) data specifically validating this daily dosing schedule in rats are available in the literature. This represents an empirical choice based on general peptide pharmacology principles, and actual retatrutide PK/PD in rats may differ from other GLP-1 analogs.[22] This limitation is further discussed in the study limitations section.

Blood sample collection

Male rats were anesthetized using intraperitoneal injections of ketamine hydrochloride (100 mg/kg) (Sigma-Aldrich, USA, Cat. No. K2753) combined with xylazine hydrochloride (10 mg/kg) (Sigma-Aldrich, USA, Cat. No. X1251).[30] The level of anesthesia was monitored throughout the procedure by assessing pedal withdrawal response, corneal reflex, and breathing rate. Body temperature was maintained between 36.8°C and 37.3°C using a feedback-controlled heating pad. Blood samples were collected via direct cardiac puncture using a 5 mL syringe into a gel tube (Medic-Home, China). The serum was separated by centrifugation at 3000 rpm for 15 minutes using a centrifuge (Hettich, Germany). Serum samples were then separated into Eppendorf tubes for subsequent measurement of blood glucose, serum insulin, liver enzymes, and lipid profile using a spectrophotometer (Emclab, Germany), and for identification of FGF21 using a rat-specific enzyme-linked immunosorbent assay (ELISA) kit. Investigators were blinded to treatment allocation during sample analysis. For liver enzymes aspartate transaminase (AST, Bioresearch, Jordan, Cat. No. CZ005), alanine aminotransferase (ALT, Bioresearch, Jordan, Cat. No. CZ003), alkaline

phosphatase (ALP, Bioresearch, Jordan, Cat. No. CZ001) the three kits with spectrophotometric wavelength 340).

Tissue sample preparation

A midline abdominal incision was performed to access the liver in every animal. The rat was then immediately euthanized under ethical guidelines. Afterward, clots and red blood cells were removed with ice-cold PBS (phosphate-buffered saline). The tissue was finely chopped and mixed with a volume of cold PBS buffer (w:v=1:9; 100 mg of tissue sample was mixed with 900 μL of PBS buffer) using a glass homogenizer on ice at -80°C. Next, the mixtures were centrifuged at 10,000×g for 5 minutes to obtain the supernatant. ELISA assays were employed to determine the concentration of GSH, TNF-α, and MDA. Investigators were blinded to treatment allocation during sample analysis.

Biochemical parameter measurement

Blood glucose levels were measured using the ACCU-CHEK Active glucometer (Roche, Germany). Animal body weights were obtained using an electronic balance. Serum insulin levels were measured using a Rat Insulin ELISA Kit (Ideal Medical Technology, Shanghai, Cat. No. ADL-EL-RT00483, detection range: 0, 6.25, 12.5, 25, 50, 100 μIU/ml) to assess pancreatic β-cell function and insulin secretion in response to the obesity model and retatrutide treatment. Tumor necrosis factor-alpha (TNF-α) was quantified using a Rat TNF-α ELISA Kit (Ideal Medical Technology, Shanghai, Cat. No. ADL-EL-RT00160, detection range: 0, 20, 40, 80, 160, 320 pg/ml), serving as a key marker of hepatic inflammation in obesity-related liver disease. Fibroblast growth factor 21 (FGF21), a metabolic regulator, was measured using a Rat FGF21 ELISA Kit (Ideal Medical Technology, Shanghai, Cat. No. ADL-EL-RT01162, detection range: 0, 15, 30, 60, 120, 240 pg/ml) to assess metabolic stress. Reduced glutathione (GSH), the primary intracellular antioxidant, was determined using a Rat GSH ELISA Kit (Ideal Medical Technology, Shanghai, Cat. No. ADL-EL-RT00896, detection range: 0, 0.5, 1, 2, 4, 8 ng/ml) as a marker of hepatic antioxidant defense capacity. Malondialdehyde (MDA), a marker of lipid peroxidation and oxidative stress, was quantified using a Rat MDA ELISA Kit (Ideal Medical Technology, Shanghai, Cat. No. ADL-EL-RT01047, detection range: 0, 0.3, 0.6, 1.2, 2.4, 4.8 nmol/ml). Tissue MDA and GSH levels were quantified from homogenates and normalized to total protein content using the Bradford assay method.

For liver enzymes, AST (Bioresearch, Jordan, Cat. No. CZ005), ALT (Bioresearch, Jordan, Cat. No. CZ003), and ALP (Bioresearch, Jordan, Cat. No. CZ001) were measured spectrophotometrically at a wavelength of 340 nm. The kinetic/IFCC method was applied for AST and ALT, while the kinetic/DGKC method was used for ALP.

HOMA-IR calculation

HOMA-IR was calculated using the formula (Matthews et al., 1985):

HOMA-IR= [Fasting Insulin (μU/mL) ×fasting glucose (mg/dL)]/405

This formula is a validated index of insulin resistance in rodent models and provides a non-invasive assessment of hepatic insulin sensitivity.[31]

Lipid profile analysis

A spectrophotometer (Emclab, Germany) was used to measure liver enzymes and lipid profiles. Serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically using commercial assay kits. Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) were calculated using the Friedewald formula (Friedewald et al., 1972):

LDL-C=total cholesterol−HDL-C−(triglycerides/5)

This formula was originally developed for human plasma samples and assumes a fixed relationship between triglycerides and VLDL cholesterol. Therefore, its application to rat serum should be used with caution, [32]but is still widely used in rodent studies for consistency with existing literature.

Food intake monitoring

Food intake was not quantitatively measured in this study. Therefore, any statements regarding food consumption in the Discussion section are based on observational assessments rather than systematic quantitative measurements.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 10.6 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean ± standard deviation (SD). Outcomes measured at a single time point were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Repeated measures data collected over weeks 0–4 were analyzed using mixed-model ANOVA, with treatment group, time, and their interaction as fixed effects and individual animals as a random effect. Assumptions of normality and homogeneity of variance were assessed and met. Statistical significance was set at $p < 0.05$, and results are reported with F-statistics, degrees of freedom, and exact p-values.

Results

Effect of HF/sucrose diet on body weight and fasting glucose before treatment

At baseline (Week 8, before treatment initiation), after eight weeks of diet feeding, the body weight of all groups fed HF/sucrose diet was significantly higher than that of the control diet group ($p<0.0001$ for all

comparisons). However, there were no significant differences in body weight between the groups designated for vehicle treatment and retatrutide treatment (p=0.1466–0.4953), indicating successful randomization and comparable baseline weights for assessing treatment effects. Fasting blood glucose levels were not significantly different among all groups (p=0.93–0.99), indicating that glucose dysregulation had not yet developed at this early stage of obesity induction.as shown in (Table 2).

Table 2. Body weight and fasting blood glucose before treatment (week 8) [a]

Groups	**Control diet**	**HF/sucrose diet (obesity)**	**HF/sucrose diet (before vehicle)**	**HF/sucrose diet (before treatment)**
Body weight (g)	254.857±16.847	345.571±19.251****	338.571±33.645****	368.000±27.214****
Fasting blood glucose (mg/dL)	105.143±6.229	107.714±6.601	106.571±6.779	107.000±5.944

[a] data are presented as mean±SD of seven rats in each group, **** – p<0.0001 (all HF/sucrose diet groups vs. control group), fasting blood glucose was not significantly different among all groups (p=0.93-0.99), (mixed-model ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on weight change

As mentioned earlier, at baseline, the body weight of groups fed HF/sucrose diet was higher than that of the control diet group. After week 1 of treatment with retatrutide medication, the obesity, vehicle, and retatrutide groups were significantly higher than the control diet group (p=0.0001, p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different (p=0.0902), and the vehicle and retatrutide groups were not significantly different (p=0.0619).

In week 2 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different (p=0.0936), the control and retatrutide groups were not significantly different (p=0.7685), and retatrutide was significantly lower than obesity (p=0.0001).

In week 3 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different (p=0.1466), and retatrutide was significantly lower than obesity (p=0.0001).

In week 4 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different (p=0.4953), and retatrutide was significantly lower than obesity (p=0.0001), F (12, 120) =55.54 as shown in Figure 1.

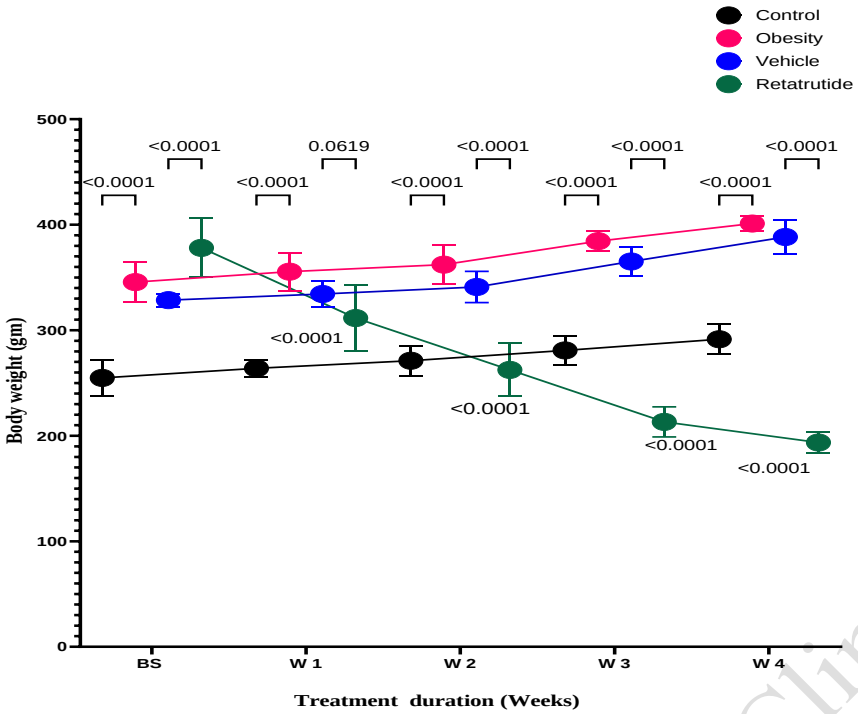

Fig. 1. Effect of retatrutide on body weight during the 4-week treatment period**,** rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for 4 weeks, in the vehicle group, rats were administered vehicle (normal saline), BS – baseline, data are presented as mean±SD of seven rats in each group, p<0.0001 retatrutide significantly decreased compared to the obesity group, p<0.0001 obesity significantly increased compared to the control group (mixed-model, Tukey's test)

Effect of HF/sucrose diet and retatrutide on fasting blood glucose

At baseline, the fasting blood glucose levels of the groups fed HF/sucrose diet and the control diet were not significantly different among all groups, as shown in Table 2.

After week 1 of treatment with retatrutide medication, the obesity and vehicle groups were significantly different than the control diet group (p=0.0073, p=0.0240, respectively). The obesity and vehicle groups were not significantly different (p=0.6033), and retatrutide was significantly lower than obesity (p=0.0004).

In week 2 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.001, p=0.001, respectively). The obesity and vehicle groups were not significantly different (p=0.9999), and retatrutide was significantly lower than obesity (p=0.0001).

In week 3 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different (p=0.8677), and retatrutide was significantly lower than obesity (p=0.0001).

In week 4 of treatment, the obesity and vehicle groups were significantly higher than the control diet group (p=0.0001, p=0.0001, respectively). The obesity and vehicle groups were not significantly different

(p=0.8525), and retatrutide was significantly lower than obesity (p=0.0001), F (12, 120)=6.782 as shown in Figure 2.

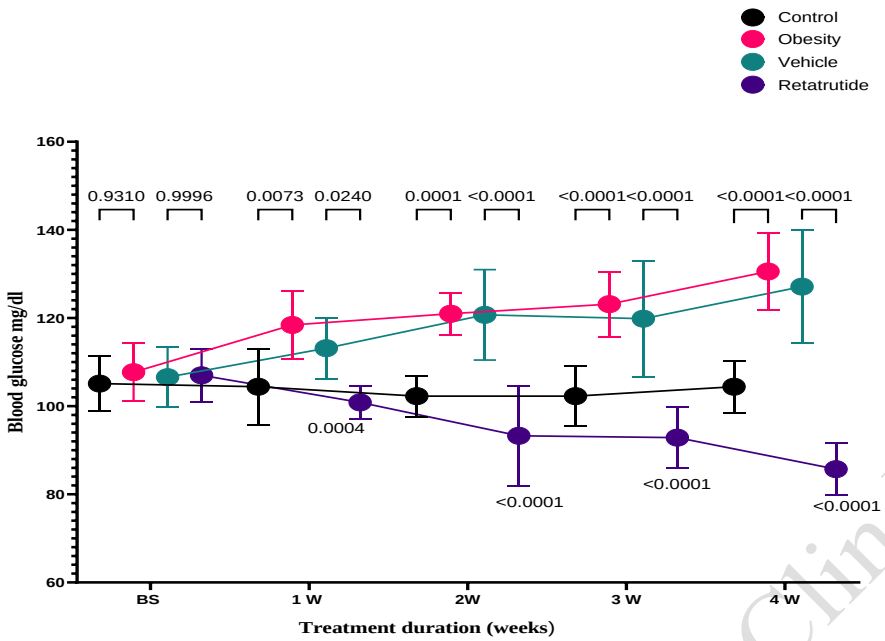

Fig. 2. Effect of retatrutide on fasting blood glucose during the 4-week treatment period**,** rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for four weeks, in the vehicle group, rats were administered vehicle (normal saline), BS – baseline, data are presented as mean±SD of seven rats in each group, p<0.0001 retatrutide significantly decreased compared to the obesity group, p<0.0001 obesity significantly increased compared to the control group (mixed-model, Tukey's test).

Effect of retatrutide on insulin resistance

The HOMA-IR levels in the obesity group were significantly increased (p=0.0001) compared to the control diet group. Conversely, the retatrutide group exhibited a significant decrease (p=0.0001) in HOMA-IR levels compared to the obesity group. There was no statistically significant difference (p=0.9057) in HOMA-IR between the vehicle and obesity groups, or between the retatrutide and control diet groups (p=0.2008), F (3, 24)=51.81.as shown in Table 3.

Table 3. Effect of retatrutide on HOMA-IR[a]

Groups	**HOMA-IR**
Control diet	2.14±0.32
HF/sucrose diet (obesity)	6.78±0.85****
Vehicle+HF/sucrose diet	6.52±0.91
Retatrutide+HF/sucrose diet	2.35±0.48††††

[a] data are presented as mean±SD of seven rats in each group, **** – p<0.0001 HF/sucrose diet (obesity) significantly increased compared to control group, †††† – p<0.0001 retatrutide+HF/sucrose diet significantly decreased compared to obesity (one-way ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on serum liver enzymes

ALT levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0366, p=0.0494, respectively). The retatrutide group showed no significant difference compared to the obesity group (p=0.1635) or the control group (p=0.8785). The obesity and vehicle groups were not significantly different (p=0.9990), F (3, 24) =4.232.

AST levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant decrease compared to the obesity group (p=0.0001) and no significant difference compared to the control group (p=0.4019). The obesity and vehicle groups were not significantly different (p=0.9504), F (3, 24) =92.74.

ALP levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant decrease compared to the obesity group (p=0.0001) and no significant difference compared to the control group (p=0.4392). The obesity and vehicle groups were not significantly different (p=0.9999), F (3, 24)=50.00.as shown in Table 4.

Table 4. Effect of retatrutide on serum liver enzymes[a]

Biochemical parameter (U/L)	Control diet	HF/sucrose diet (obesity)	Vehicle+HF/sucrose diet	Retatrutide+HF/sucrose diet
ALT	28.43±4.52	38.71±6.89*	36.85±7.23	32.14±5.67
AST	45.29±5.34	98.57±8.91****	97.14±9.45	52.43±6.78††††
ALP	52.86±6.23	112.43±10.56****	111.71±11.34	58.29±7.45††††

[a] data are presented as mean±SD of seven rats in each group, * – p<0.05 (ALT in obesity vs. control), **** – p<0.0001 (AST and ALP in HF/sucrose diet (obesity) vs. control), †††† – p<0.0001 (AST and ALP in retatrutide+HF/sucrose diet vs. HF/sucrose (obesity)), ALT was not significantly different between retatrutide and obesity groups (p=0.1635), (one-way ANOVA, Tukey's test)

The lack of statistical significance in ALT levels between the retatrutide-treated and obesity groups, while AST and ALP showed significant reductions, may be attributed to the differential sensitivity and specificity of these liver enzymes as markers of hepatocellular injury. ALT is the most sensitive marker of hepatocyte

damage but can be influenced by multiple factors and may require longer treatment periods for complete normalization. The 4-week treatment duration was sufficient to significantly reduce the more robust markers (AST and ALP), but may have been insufficient for complete ALT normalization. This pattern is consistent with previous studies of GLP-1/GIP receptor agonists, where longer treatment periods (12–24 weeks) are typically required for complete normalization of all liver enzyme markers.

Effect of HF/sucrose diet and retatrutide on serum lipid profile

Cholesterol levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant decrease compared to both the obesity group (p=0.0001) and the control group (p=0.0001). The obesity and vehicle groups were not significantly different (p=0.6919).

Triglyceride (TG) levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant decrease compared to the obesity group (p=0.0001) but no significant difference compared to the control group (p=0.9986). The obesity and vehicle groups were not significantly different (p=0.2385).

High-density lipoprotein (HDL) levels were significantly decreased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant increase compared to the obesity group (p=0.0045) but no significant difference compared to the control group (p=0.4073). The obesity and vehicle groups were not significantly different (p=0.9117).

Low-density lipoprotein (LDL) levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0001, p=0.0001, respectively). The retatrutide group showed a significant decrease compared to the obesity group (p=0.0001) but no significant difference compared to the control group (p=0.3247). The obesity and vehicle groups were not significantly different (p=0.9999).

Very-low-density lipoprotein (VLDL) levels were significantly increased in the obesity and vehicle groups compared to the control group (p=0.0488, p=0.0285, respectively). The retatrutide group showed a significant decrease compared to the obesity group (p=0.0448) but no significant difference compared to the control group (p=0.9999). The obesity and vehicle groups were not significantly different (p=0.9970), F (12, 120)=48.32 as shown in Table 5.

Table 5. Effect of retatrutide on serum lipid profile[a]

Biochemical parameter (mg/dL)	Control diet	HF/sucrose diet (obesity)	Vehicle+HF/sucrose diet	Retatrutide+HF/sucrose diet	p (vehicle vs. retatrutide)
Cholesterol	164.463±15.391	241.085±22.002****	232.325±23.058	105.881±26.225****†	<0.0001

TG	47.905±10.740	140.937±10.931****	112.140±11.450	30.356±9.479****†	<0.0001
HDL	78.185±10.125	33.069±13.949****	38.339±9.670	65.759±13.828**†	0.0045
LDL	76.696±19.774	179.827±21.412****	171.557±17.678	51.341±21.858****†	<0.0001
VLDL	9.581±2.148	28.187±2.186*	22.428±2.290	9.193±5.551*†	0.0448

[a] data are presented as mean±SD of seven rats in each group, **** – p<0.0001 (cholesterol, TG, LDL); * – p<0.05 (VLDL) in HF/sucrose diet (obesity) significantly increased compared to control group, except HDL, **** – p<0.0001 HF/sucrose diet (obesity) is significantly decreased compared to control group, **** p<0.0001 (cholesterol, TG, LDL), * p<0.05 (VLDL) in retatrutide+HF/sucrose diet significantly decrease compared to HF/sucrose diet (obesity) except** p<0.01 (HDL) significant increase compared to HF/sucrose diet (obesity), † – indicates significant difference from HF/sucrose diet (obesity) group. comparisons between vehicle+HF/sucrose diet vs. retatrutide+HF/sucrose diet show highly significant differences for all parameters (p shown in final column) (one-way ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on tumor necrosis factor-α (TNF-α)

There was a significant increase (p=0.0066) in TNF-α levels in the obesity group compared to the control group. The retatrutide group showed a significant decrease (p=0.0049) compared to the obesity group and no significant difference with the control group (p=0.9993). There was no significant difference between the obesity and vehicle groups (p=0.9998), F (3, 24)=8. 964 as shown in Figure 3.

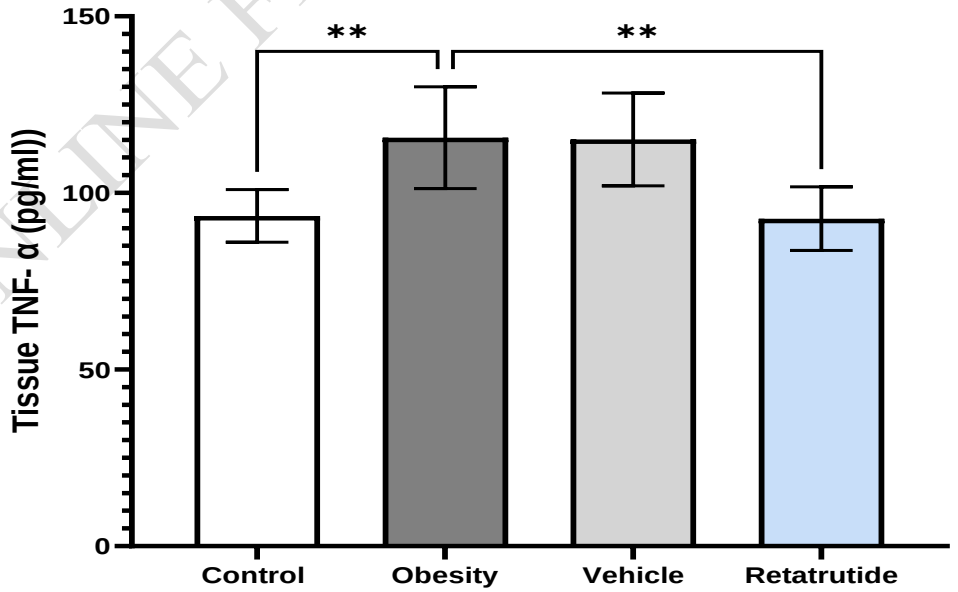

Fig. 3. Effect of retatrutide on hepatic TNF-α levels, rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for four weeks. In the vehicle group, rats were administered vehicle (normal saline), data are presented as mean±SD of seven rats in each group, obesity group significantly increased compared to control group (p=0.0066), retatrutide group significantly decreased compared to obesity group (p=0.0049) (one-way ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on MDA

The findings demonstrated a significant elevation in MDA levels (p=0.0001) in the obesity group compared to the control group. The retatrutide-treated group showed a significant reduction (p<0.0001) compared to the obesity group. There was no significant difference between the obesity and vehicle groups (p=0.9990) or between the control and retatrutide groups (p=0.9991), F (3, 24)=18.90 as shown in Figure 4.

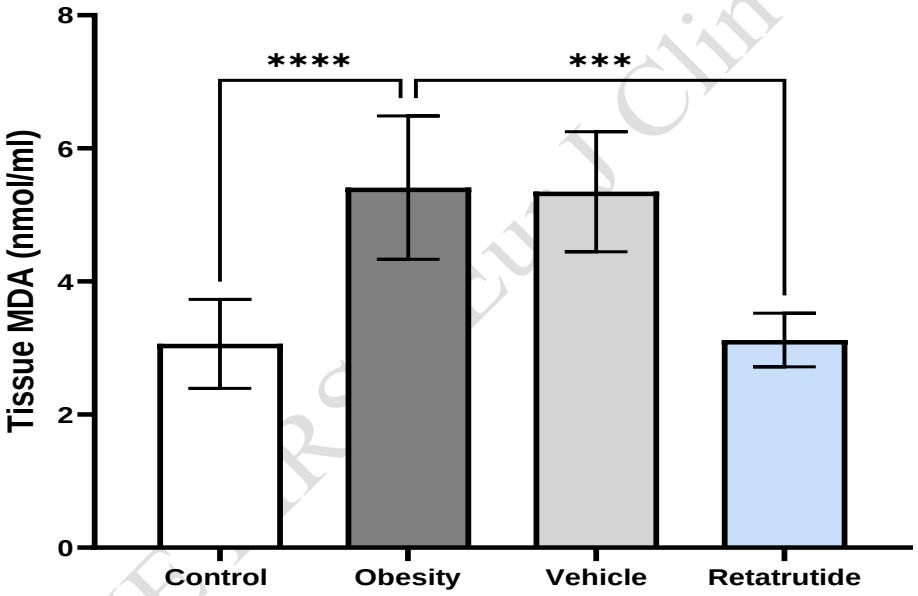

Fig. 4. Effect of retatrutide on hepatic MDA levels (lipid peroxidation marker): rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for four weeks. In the vehicle group, rats were administered vehicle (normal saline), data are presented as mean±SD of seven rats in each group. p<0.0001 obesity group significantly increased compared to control group. p<0.0001 retatrutide group significantly decreased compared to obesity group. (one-way ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on GSH

Conversely, the antioxidant GSH demonstrated a significant reduction (p=0.0001) in the obesity group compared to the control group. The retatrutide-treated group showed a significant elevation (p=0.0001) compared to the obesity group. There was no significant difference between the obesity and vehicle groups

(p=0.9971) or between the control and retatrutide groups (p=0.9998), F (3, 24)=28.18 as shown in Figure 5.

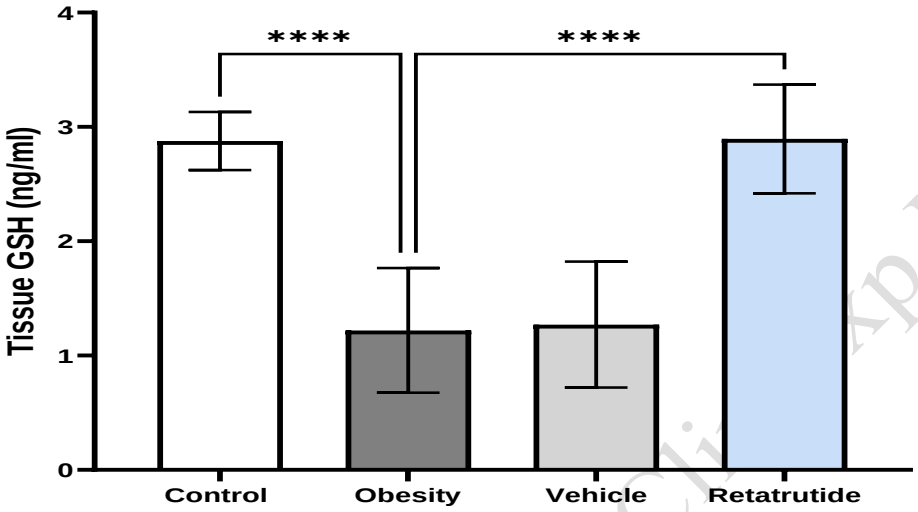

Fig. 5. Effect of retatrutide on hepatic GSH levels (antioxidant marker)**,** rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for four weeks. In the vehicle group, rats were administered vehicle (normal saline), data are presented as mean±SD of seven rats in each group, $p<0.0001$ obesity group significantly decreased compared to control group, $p<0.0001$ retatrutide group significantly increased compared to obesity group (one-way ANOVA, Tukey's test)

Effect of HF/sucrose diet and retatrutide on FGF21

There was a significant increase (p=0.0015) in FGF21 levels in the obesity group compared to the control group. After treatment with retatrutide medication, there was a significant decrease (p=0.0063) compared to the obesity group and no significant difference with the control group (p=0.9361). There was no significant difference between the obese and vehicle groups (p=0.9999), F(3, 24)=10.53 as shown in Figure 6.

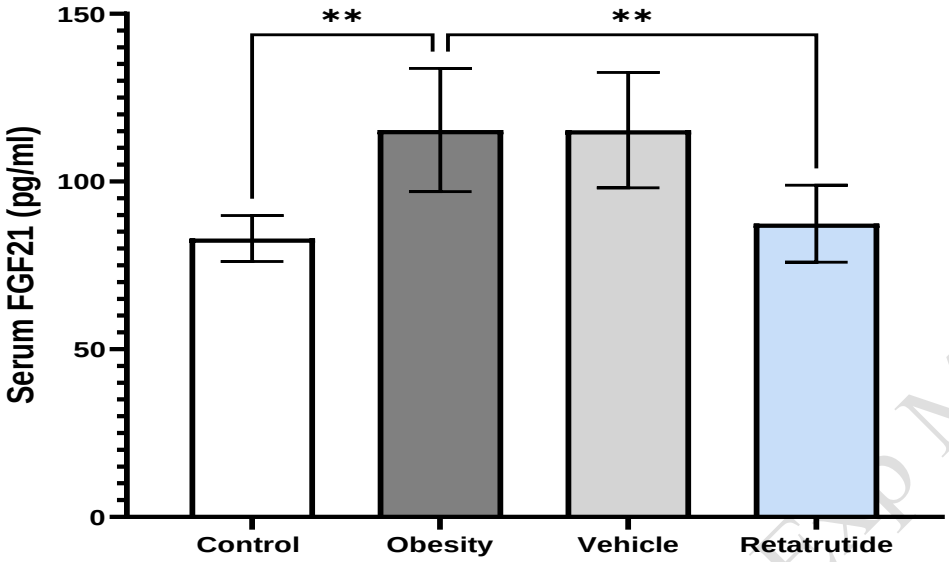

Fig. 6. Effect of retatrutide on serum FGF21 levels, rats fed HF/sucrose diet were treated with retatrutide 25 nmol/kg daily for four weeks, in the vehicle group, rats were administered vehicle (normal saline), data are presented as mean±SD of seven rats in each group, p=0.0015 obesity group significantly increased compared to control group, p=0.0063 retatrutide group showed significant decrease compared to obesity group, (one-way ANOVA, Tukey's test)

Discussion

Metabolic dysregulation linked to obesity and overweight, stemming from excessive food intake, is now acknowledged as a distinct chronic illness that significantly contributes to the global epidemic of chronic, non-communicable diseases.[33] Palatable foods, particularly those rich in sugar and fat, enhance appetite and inhibit satiety signals. The rats induced with obesity were verified as obese after exhibiting significantly higher body weight compared to the negative control rats, following their respective diets. Both animals and humans experience obesity due to a diet rich in fats. Previous studies have demonstrated a positive link between body weight or weight gain and the fat and sugar content of the diet, as evidenced in Table 2 showing a high increase in weight after 8 weeks of feeding HF/sucrose.[34]

In our experience, we used pure peptide powder that was reconstituted in sterile water and administered daily instead of weekly to ensure constant bioactivity. Peptides undergo rapid enzymatic breakdown and possess brief plasma half-lives, resulting in diminished effectiveness over time.[23] For GLP-1 agonists to achieve weight loss in rats a daily dosing is required. This can be justified firstly by allometric scaling of rat metabolic rates to human as rats have much higher basal metabolic rates and faster renal clearance. Secondly, according to the pharmacokinetic studies, to achieve a therapeutic level of a drug, the frequency of dosing must be changed according to the drug half-life in certain species. In a previous pharmacokinetic study, it was found that daily dosing of long-acting GLP-1 agonist is necessary in rat studies. Another

pharmacological study demonstrated that to mimic the human weekly dosing of GLP1 agonist in rats, a daily administration of the drug is required to obtain a steady-state concentration. Daily administration guarantees consistent peptide concentration and biological effects,[29]

though daily dosing was empirical and not validated in the literature. The findings indicated that after 12 weeks of weekly glucometer assessments, the control group exhibited significantly lower blood glucose levels compared to the obesity and obesity plus vehicle groups. Subsequent to the introduction of obesity, an elevation in glucose levels is noted. Animals subjected to a high-fat/sucrose diet exhibited elevated blood glucose levels; through many mechanisms, sustained exposure to high-fat conditions enhances fatty acid oxidation while diminishing glucose oxidation.[35] Obese rats and obese rats administered vehicle had significantly higher insulin resistance (HOMA-IR) levels than rats on a control diet. Blood glucose levels and insulin resistance were considerably reduced when retatrutide was administered subcutaneously for four weeks, because it acts on triple hormone receptors (GLP-1, GIP, and glucagon). These receptors are highly distributed, especially in the small intestine and brain, which delays gastric emptying and increases satiety, suppresses appetite, slows digestion, increases fat metabolism, and increases insulin sensitivity, leading to weight loss and glycemic control.[17] Consequently, being overweight is a major contributor to the onset and progression of insulin resistance.[36] With regards to weight after twelve weeks of HF/sucrose diet and measuring weekly, animals exhibited significantly elevated weight compared to control diet. There was a significant reduction in weight in the retatrutide group that was administered S.C. for four weeks, compared to the obesity and obesity plus vehicle groups. Retatrutide facilitated weight reduction by diminishing food consumption, as we observed in our experiment where male rats reduced HF/sucrose diet intake.[21]

The study found that obesity significantly increased AST, ALP, and ALT levels compared to the control diet group. When using retatrutide medication, there was a significant decrease in hepatic ALP and AST compared to the obesity group, while ALT was not significant; this is somewhat consistent with what was found in some studies, taking into account the short research period of only 12 weeks.[18] The results may be different if the study duration were longer.[16] The results showed a highly significant increase in cholesterol, triglycerides, LDL, and VLDL levels in the obesity group compared to the control group, while HDL showed a significant decrease.[37] When using retatrutide, there was a significant decrease in cholesterol, LDL, triglycerides, and VLDL levels, and a significant increase in HDL compared to the obesity group. The safety profile aligns with GLP-1 receptor agonists and GIP with GLP-1 receptor agonists.[16] It is worth mentioning that we used the Friedewald formula to calculate LDL; this formula was originally developed for human plasma samples and assumes a fixed relationship between triglycerides and VLDL cholesterol. Therefore, its application to rat serum should be used with caution, but it is still widely used in rodent studies.[38]

The group treated with retatrutide showed a significant decrease in the hepatic inflammatory marker TNF-α compared to the obesity group that did not receive treatment. These results support earlier studies in this field.[39,40] Retatrutide also reduces inflammation. Indeed, substantial data indicate that retatrutide can potentially modify or decrease inflammatory processes.[15] Our understanding suggests it is not exactly clear by which mechanism, but may be explained by two main mechanisms via which retatrutide reduces inflammation: altering immune system activation and decreasing levels of inflammatory cytokines.[41] Retatrutide induces immune reprogramming systemically.[20]

The significant reduction in hepatic TNF-α following retatrutide treatment ($p=0.0049$) supports a direct anti-inflammatory action of this triple-agonist. Mechanistically, this effect may involve GLP-1 receptor signaling, as GLP-1 activation can suppress NF-κB activity in hepatocytes and macrophages,[15] thereby lowering pro-inflammatory cytokine production. In addition, the marked weight loss observed with retatrutide likely contributes indirectly by reducing adipose tissue mass and adipose inflammation, a major systemic source of TNF-α driven by macrophage infiltration. Retatrutide may also act directly on hepatic immune cells (e.g., Kupffer cells/macrophages), attenuating cytokine output independent of weight reduction.

Consistent with this interpretation, hepatic TNF-α (inflammation) and MDA (oxidative stress) showed a positive correlation with the atherogenic index,[42] indicating that greater inflammatory and oxidative burden aligns with a more atherogenic lipid pattern. This relationship is biologically plausible because high-fat, high-cholesterol dietary exposure promotes weight gain and worsens circulating lipid and cholesterol levels, which can amplify both oxidative stress and inflammatory signaling and thereby increase cardiovascular risk profiles.[37]

The study's data suggested that obesity resulted in heightened lipid peroxidation, as evidenced by increased MDA levels and decreased GSH levels. Mitochondrial glutathione depletion leads to heightened mitochondrial reactive oxygen species exposure, which disrupts bioenergetics and facilitates the opening of the mitochondrial permeability transition pore, a crucial event in cell death.[43]

Retatrutide appears to mitigate hepatic oxidative stress by improving both sides of the redox balance – reducing oxidative damage while restoring antioxidant capacity.[44] In this study, hepatic MDA, a lipid peroxidation marker, was markedly reduced versus the obese group ($p<0.0001$), indicating less membrane lipid damage and overall oxidative injury in hepatocytes. In parallel, GSH, a key intracellular antioxidant, was significantly depleted in the obese and obese+vehicle groups, but retatrutide substantially increased hepatic GSH compared with obesity ($p<0.0001$). The combined pattern – lower MDA alongside higher GSH – strongly suggests that retatrutide restores hepatic antioxidant defenses rather than merely masking oxidative injury.[45]

Mechanistically, GSH restoration may occur through several complementary routes: (1) reduced reactive oxygen species (ROS) generation due to dampened inflammatory signaling and improved mitochondrial

function, (2) enhanced GSH synthesis supported by better cellular energetics and substrate availability, and (3) reduced GSH consumption because the oxidative burden is lower. Prior work with GLP-1–based therapies report similar antioxidant shifts (decreased MDA[46] and increased GSH[47]) in liver and adipose tissue, supporting the plausibility of these pathways; importantly, retatrutide's triple-agonist profile may further amplify mitochondrial and metabolic recovery via additional glucagon receptor signaling, potentially strengthening antioxidant outcomes beyond GLP-1/GIP agonism alone.[14]

Elevated levels of FGF21 have been correlated with many metabolic disorders, including obesity and type 2 diabetes mellitus (T2DM).[48] Serum FGF21 concentrations are typically elevated in cases of obesity and fatty liver disease (non-alcoholic fatty liver disease or NAFLD).[49] This elevation is frequently regarded as a compensatory mechanism to metabolic stress and insulin resistance, although it may also signify a state of FGF21 resistance. The study found that obesity significantly increased FGF21 in both the obese and obese+vehicle groups. Serum FGF21 circulation decreased in the retatrutide group, indicating improved FGF21 receptor sensitivity.[50]

The observed reduction in FGF21 after retatrutide treatment likely reflects relief of the metabolic strain imposed by diet-induced obesity and a shift toward more normalized metabolic regulation. In obesity, FGF21 is often elevated as a compensatory "stress hormone" response to insulin resistance, dyslipidemia, and hepatic lipid overload. By promoting weight loss and improving glycemic control, retatrutide may lower this systemic and hepatic metabolic stress, thereby reducing the need for compensatory FGF21 upregulation.[51]

In addition, declining circulating FGF21 may indicate improved responsiveness of the FGF21 pathway (i.e., reduced "FGF21 resistance") and restoration of downstream receptor signaling, consistent with improved metabolic homeostasis. This is clinically meaningful because effective FGF21 signaling supports lipid handling, glucose regulation, mitochondrial function, and hepatic metabolic recovery; thus, the reduction in FGF21 with retatrutide aligns with a broader normalization of cardiometabolic and liver-related physiology rather than a loss of a protective factor.[52]

Study limitations

This study has several limitations. Liver histopathology was not performed, limiting direct assessment of steatosis, inflammation, and fibrosis. The 4-week treatment duration may have been insufficient to capture maximal hepatic improvement, and inclusion of only male rats restricts generalizability across sexes. The dosing regimen was empirical and not supported by rat-specific PK/PD data, and only a single dose was evaluated without comprehensive safety assessment. Methodological constraints include the use of the Friedewald formula for lipid calculations, lack of quantitative food-intake monitoring, and limited existing

rodent data on retatrutide. Future studies should incorporate histological analyses, extended treatment duration, both sexes, PK/PD-guided dosing, dose–response evaluation, and broader toxicity assessments.

Conclusion

This study provided evidence that retatrutide effectively improves weight loss, decreases blood glucose, improves lipid profile, and protects hepatic tissues through decreased inflammation and oxidative stress marker levels.

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Declarations

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Author contributions

Conceptualization: Z.M.R.A. and Z.J.K.; Methodology: Z.M.R.A.; Software: Z.M.R.A.; Validation: Z.M.R.A. and Z.J.K.; Formal Analysis: Z.J.K.; Investigation: Z.M.R.A.; Resources: Z.J.K.; Data Curation: Z.M.R.A.; Writing – Original Draft Preparation: Z.M.R.A.; Writing – Review & Editing: Z.J.K.; Visualization: Z.M.R.A.; Supervision: Z.J.K.; Project Administration: Z.M.R.A.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability

All clinical and statistical data and materials are available upon reasonable request from the corresponding author.

Ethics approval

The study followed the ARRIVE 2.0 guidelines for reporting animal experiments and complied with ethical standards. Approval was obtained from the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa, Iraq, following the submission of the required documentation (NO. 2121) on January 23, 2025.

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