Liraglutide Ameliorates Metabolic Dysfunction Targeting the NRF2 Pathway in Obese Rats Induced by a High-Fat Diet.

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Abstract:

Background: Obesity, driven largely by high-fat diets, is strongly linked to insulin resistance, dyslipidemia, and oxidative stress. The nuclear factor erythroid 2-related factor 2 (NRF2) pathway is very important for keeping the redox balance in cells and guarding against liver damage caused by obesity.

Liraglutide, a Glucagon like peptide-1 receptor agonist (GLP-1RA), has demonstrated potential in metabolic control and antioxidant defense.

Objective: This study investigated the dose-dependent effects of liraglutide on weight control, glycemic status, lipid profile, and hepatic NRF2 expression in high-fat diet–induced obese rats.

Methods: Thirty-five male Wistar rats were divided randomly into five groups: control, high-fat diet (HFD), and three liraglutide-treated groups (100, 200, and 400 µg/kg/day, subcutaneously for 28 days following HFD induction). Body weight, Lee index, fasting glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), lipid parameters, and NRF2 (immunohistochemistry) were assessed. Statistical significance was evaluated by ANOVA with Tukey's post hoc test.

Results: High-fat diet induction caused marked obesity, dyslipidemia, hyperglycemia, hyperinsulinemia, and a sharp reduction in hepatic NRF2 expression. Liraglutide produced clear dose-dependent improvements across all parameters. Body weight and Lee index declined significantly after treatment, with the 400 µg/kg dose nearly restoring normal adiposity. Glycemic indices (glucose, insulin, HOMA-IR) improved progressively, and the highest dose achieved near-normal insulin sensitivity. Lipid abnormalities were corrected, showing increased HDL and reduced LDL, cholesterol, and triglycerides. NRF2 immuno-expression increased markedly in a dose-dependent manner, with the high dose showing the strongest restoration of antioxidant defense.

Conclusion: Liraglutide exerts a strong dose-dependent protective effect against HFD-induced obesity and metabolic dysfunction. Its dual action on glycemic control and NRF2-mediated antioxidant defense highlights its therapeutic potential for managing obesity, insulin resistance, and related hepatic complications.

Keywords: Obesity, Liraglutide, GLP-1RA, NRF2, Oxidative Stress.

الليراغلوتايد يخفف الاضطراب الأيضي باستهداف مسار NRF2 في الجرذان المصابة بالسمنة المستحثّة بنظام غذائي عالي الدهون.

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الخلاصة:

الخلفية: تعود السمنة الناتجة بدرجة كبيرة الى الانظمة الغذائية الغنية بالدهون مرتبطة ارتباطا وثيقا بمقاومة الانسولين واضطراب دهون الدم والاجهاد التأكسدي. ويعد NRF2 بالغ الاهمية في الحفاظ على التوازن الاختزالي داخل الخلايا وحماية الكبد من الضرر الناجم عن السمنة. لقد اظهر الليراغلوتايد وهو ناهض لمستقبل GLP-1 قدرة واعدة في التحكم الايضي والدفاع المضاد للاكسدة.

الهدف: هدفت هذه الدراسة الى تقصي التأثيرات المعتمدة على جرع الليراغلوتايد في ضبط الوزن والحالة السكرية وبروفايل الدهون والتعبير الكبدي لـ NRF2 في الجرذان المصابة بالسمنة المستحثة بنظام غذائي عالي الدهون.

الطرائق: جرى توزيع خمسة وثلاثين جرذا من نوع ويستر ذكور بصورة عشوائية الى خمس مجموعات: مجموعة ضابطة ومجموعة خضعت لنظام غذائي عالي الدهون HFD وثلاث مجموعات عولجت بالليراغلوتايد بجرعات 100 و200 و400 ميكروغرام/كغ/يوم تحت الجلد لمدة 28 يوما بعد التعرض لنظام عالي الدهون. جرى تقييم وزن الجسم ومعامل لي وغلوكوز الصيام والانسولين ومؤشر مقاومة الانسولين HOMA-IR ومعايير الدهون اضافة الى التعبير عن NRF2 بتقنية الكيمياء المناعية النسيجية. وقد قيمت الدلالة الاحصائية باستخدام تحليل التباين ANOVA واختبار توكي البعدي.

النتائج: أدى النظام الغذائي عالي الدهون إلى سمنة واضحة واضطراب دهون الدم وفرط سكر الدم وفرط أنسولين الدم، مع انخفاض حاد في التعبير الكبدي لـ NRF2. أما الليراغلوتايد فقد أحدث تحسناً معتمداً على الجرعة في جميع المقاييس؛ إذ انخفض وزن الجسم ومعامل لي بشكل ملحوظ، وحققت جرعة 400 µg/kg استعادة شبه كاملة لمؤشرات الانسجة الدهنية الطبيعية. كما تحسنت المؤشرات السكرية تدريجياً (الغلوكوز، الإنسولين، HOMA-IR)، لتقترب أعلى جرعة من القيم الطبيعية. وصحّحت اضطرابات الدهون بارتفاع HDL وانخفاض LDL والكوليسترول والدهون الثلاثية. وازداد التعبير المناعي لـ NRF2 بصورة واضحة، وكانت أعلى جرعة هي الأكثر فعالية في تعزيز الدفاع المضاد للأكسدة.

الاستنتاج: يظهر الليراغلوتايد تأثيرا وقائيا قويا معتمدا على الجرعة ضد السمنة واضطرابات الايض المستحثة بالنظام الغذائي عالي الدهون. ويبرز فعله المزدوج على ضبط سكر الدم وتنشيط الدفاع المضاد للأكسدة عبر NRF2 كخيار علاجي واعد لادارة السمنة ومقاومة الانسولين والمضاعفات الكبدية المرتبطة بها.

الكلمات المفتاحية: السمنة، الليراغلوتايد، GLP-1RA، NRF2، الاجهاد التأكسدي.

Introduction

Obesity is the worldwide epidemic of the 21st century(1). Obesity rates are increasing at an alarming rate, having nearly tripled globally since 1975(2). The World Health Organization reported that more than 66% of the adult population in the United States are classified as overweight or obese, with an anticipated rise by 2030(2). Besides that, the incidence of diverse cardiometabolic disorders escalated along with the swift rise in obesity rates, resulting in elevated morbidity and death (2).Obesity, defined by the excessive accumulation of fatty acids in adipose tissue, is rapidly escalating, exacerbating the worldwide obesity crisis (3,4) . Obesity is significantly associated with diabetes mellitus, stroke, hyperlipidemia, malignancies, cardiovascular disease, and osteoarthritis (1) .

Nuclear related factor 2 (Nrf2) is an important controller of how cells respond to oxidative stress. (5). When oxidative and electrophilic stress happens, Nrf2 accumulates and translocates into the nucleus. It then heterodimerizes with

other proteins and binds to antioxidant response elements (AREs) in the promoters of a number of detoxifying and antioxidant genes, therefore improving their transcription of cytoprotective enzymes and supporting the cellular defense system (6,7) .

The treatment of obesity is a comprehensive strategy that includes behavioral therapies, pharmacological therapy, and bariatric surgery. Nonetheless, sustaining long-term weight loss becomes difficult due to the variability in weight reduction outcomes. Obesity treatments exhibit restricted effectiveness and raise safety concerns. Recent revelations on the pathophysiology of obesity have facilitated the creation of innovative and effective medicines, including liraglutide which has received approval for obesity management (8). Liraglutide is a synthetic glucagon-like peptide-1 (GLP-1) receptor agonist(9), principally indicated for the management of type 2 diabetes and obesity(10). This injectable drug simulates the effects of the natural incretin hormone GLP-1, supporting glucose regulation and appetite management. The medication, which is a long-acting analog of human GLP-1RA, has been structurally changed to prolong its half-life, enabling once-daily dosing (11). Dosing in clinical practice begins at 0.6 mg per day and is incrementally raised weekly until the maximum dosage of 3.0 mg is attained (12). Liraglutide binds to and activates the GLP-1 receptor, a G protein-coupled receptor located in several organs, including pancreatic β-cells, the central nervous system, and the gastrointestinal tract (13). Such activation initiates a number of intracellular signaling pathways that mediate its effects by enhancing glucose-dependent insulin production and suppressing glucagon release, hence improving glycemic control. It also postpones stomach emptying, thereby moderating postprandial glucose fluctuations, and has a central effect on the hypothalamus diminishing hunger, and resulting in weight reduction (14). These integrated systems provide dual advantages in the management of type 2 diabetes and the treatment of obesity**.**

High-fat diet (HFD) feeding is a widely used experimental approach for inducing obesity-associated metabolic disturbances, particularly dyslipidemia, in rodent models, thereby mimicking key features of human obesity. Excessive lipid intake promotes abnormal lipid profiles and adipose tissue expansion, making this model suitable for evaluating obesity-related pathophysiology and therapeutic interventions. In this context, obesity assessment commonly relies on morphometric parameters such as body weight, naso-anal length, and the Lee index, which adjusts body mass relative to linear body length and provides a reliable estimate of adiposity in experimental animals.

This study aims to investigate the dose-dependent effects of liraglutide on body weight, glycemic control, and lipid metabolism in high-fat diet-induced obese rats, with a particular focus on the modulatory role of the Nrf2 signaling pathway in mediating its metabolic and antioxidant benefits.

Methodology

High-Fat Diet Obesity Model

Obesity was induced by feeding rats an HFD consisting of 72.8% standard chow, 25% lard, 2% cholesterol, and 0.2% bile salts(15). Obesity confirmation was based on the Lee index(16).

Experimental Design

Thirty-five male Wistar rats (150–200 g) were randomly assigned to five groups (n = 7). Group 1 served as the control (distilled water). Group 2 received a high-fat diet (HFD) to induce obesity. Groups 3–5 received liraglutide (100,

200, and 400 µg/kg/day, respectively) subcutaneously for 28 days following a 4-week HFD induction (17–19). All procedures followed the approved ethical guidelines of the College of Pharmacy (File No. 53, 18/10/2024). Rats were housed under controlled environmental conditions (24 ± 5°C; 40–60% humidity), with free access to water and food, and acclimatized for 1 week prior to experimentation.

Experimental Duration and Animal Handling

The study was conducted from 18 October to 25 December 2024, including acclimatization (10 days), HFD induction (4 weeks), and liraglutide treatment (4 weeks). At the end of the study, rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg), followed by cardiac puncture for blood collection(20) .

Lee index assessment

The Lee index is a widely used as indicator for the assessment of obesity and adiposity in experimental rodent models. It is based on body weight and the naso-anal length, defined as the linear distance from the tip of the nose to the anal orifice. Naso-anal length was measured using a flexible ruler and recorded in centimeters (cm). The Lee index provides a normalized estimate of body fat accumulation by relating body weight to linear body length, thereby minimizing the influence of overall body size. The Lee index was calculated using the following formula:

$$\textbf{Lee index} = \sqrt[3]{\textbf{Body weight (g)}} \div \textbf{Naso} - \textbf{anal length (cm)} \times \textbf{1000}$$

Lee considered values greater than 310 as an indicator of obesity(16).

Sample Collection and Tissue Preparation

Blood samples were centrifuged at 2500 rpm for 15 minutes, and serum was stored at −20°C for biochemical assessments. Livers were excised, rinsed, and fixed in 10% neutral-buffered formalin for immunohistochemical examination.

Measurement of Lipid Profile and blood Glucose levels:

Serum total cholesterol, triglycerides, HDL and blood glucose were determined using enzymatic colorimetric kits according to manufacturer instructions (21–24). LDL was calculated using Friedewald's formula (25).

Measurement of serum insulin:

Insulin levels were quantified using a commercially available sandwich ELISA kit, following the manufacturer's protocol(26).

Immunohistochemistry (IHC)

Formalin-fixed paraffin-embedded liver sections (4 µm) underwent standard IHC processing, including deparaffinization, rehydration, antigen retrieval (Tris-EDTA, pH 9), and incubation with anti-NRF2 primary antibody (1:500) overnight at 4°C. Detection was performed using secondary antibody and DAB chromogen, followed by hematoxylin counterstaining. NRF2 expression was evaluated semi-quantitatively based on staining intensity (0–3) and percentage of positive cells (0–4).

Statistical Analysis

Data were analyzed using SPSS v26 and GraphPad Prism v9. One-way ANOVA followed by Tukey's post hoc test was applied. Statistical significance was set at $p < 0.05$. Variables analyzed included body weight, naso-anal length, Lee index, insulin, glucose, HOMA-IR, lipid profile, and NRF2 expression.

Results

Body Weight, Naso-Anal Length, and Lee Index Measurements:

- **Body Weight**

Table 1 below shows the analysis of weight changes for each group, including initial, peak, and final weights, and the effect of high fat induction and treatment. The longitudinal assessment of body weight demonstrated considerable and statistically significant disparities across the experimental groups. During the induction phase (weeks 0–4), all groups except the negative control (G1) showed marked weight gain. The high-fat diet group (G2) exhibited the greatest absolute and percentage increase, rising from 179.0 ± 8.49 g at week 0 to 288.1 ± 13.26 g at week 4, corresponding to a 109.1 g gain. This robust increase persisted throughout the study, culminating in a peak weight of 348.1 ± 19.24 g at week 8, which remained significantly higher than all other groups. In contrast, the control group (G1) maintained the lowest and most stable weights, increasing only slightly from 183.1 ± 4.85 g at baseline to 213.1 ± 9.91 g by week 8, an overall rise.

The intervention groups (G3–G5) initially gained weight during induction at levels comparable to G2 (week 4: 296.9 ± 9.34 g, 290.0 ± 14.54 g, and 301.1 ± 9.14 g, respectively). However, following the introduction of treatment at week 4, these groups exhibited reversals in weight trajectory. By week 8, body weights had decreased significantly: G3: 296.9 → 260.1 g (−36.7 g), G4: 290.0 → 242.0 g (−48.0 g), G5: 301.1 → 232.1 g (−69.0 g). Notably, the greatest attenuation occurred in the high-dose treatment group (G5), which achieved a near-normalization of weight by week 8, approaching the trajectory of the negative control group (G1).

Table 1: body Weight Change Analysis

Group	Initial Weight (g) (week 0)	End of Induction (g) (week 4)	Final Weight (g) (week 8)	Peak Week	Effect of Induction (week 4–week 0)	Effect of Treatment (week 8–week 4)
G1	183.14 ± 4.85	195.0 ± 16.03	213.14 ± 9.91	8	11.86	18.14
G2	179.0 ± 8.49	288.14 ± 13.26	348.14 ± 19.24	8	109.14	60
G3	188.0 ± 7.07	296.86 ± 9.34	260.14 ± 16.35	4	108.86	-36.71
G4	186.0 ± 9.33	290.0 ± 14.54	242.0 ± 8.25	4	104	-48
G5	191.86 ± 9.37	301.14 ± 9.14	232.14 ± 13.28	4	109.29	-69

Notes:** **Mean ± SD presented for each group.** ***Effect of induction = (Week 4) – (Week 0). Effect of treatment = (Week 8) – (Week 4)

- **Naso-anal Length**

The analysis of naso-anal length measurement in table 2, indicating a linear growth, revealed no statistically significant differences across groups at any week. All groups displayed gradual increases in length across the study duration, consistent with normal postnatal development. The final measurements at week 8 ranged from 21.8 to 22.3 cm across groups, with minimal variation. The lack of significant group-wise differences indicates that neither high-fat diet induction nor

subsequent therapeutic intervention exerted a measurable effect on overall skeletal growth or body length in this model.

Table 2 : mean of naso anal length over the experimental time intervals.

Group	Initial Length (cm) (week 0)	End of Induction (cm) (week 4)	Final Length (cm) (week 8)	Peak Week	Effect of Induction (week 4–week 0)	Effect of Treatment (week 8–week 4)
G1	18.7	20.4	21.8	8	2.3	1.4
G2	18.3	20.7	22.1	8	2.4	1.4
G3	18.3	20.8	22.0	8	2.5	1.2
G4	18.8	21.0	22.1	8	2.2	1.1
G5	18.7	21.1	22.3	8	2.4	1.2

***p-values compare the effect magnitude and group means using one-way ANOVA, with post hoc (Tukey) indicating significant pairwise differences (for weight, G2 is always significantly different from other groups; for length, no significant differences found).**

- **Lee Index**

The Lee index, an established indicator of adiposity in rodent models, demonstrated dynamic changes in response to both HFD induction and therapeutic intervention. Table 3 below shows the calculated Lee Index for each group at various weeks (week 0,4,8). Values greater than 310 (indicating obesity) are displayed **pink color**. Throughout the induction phase, all experimental groups except the negative control (G1) exhibited increasing Lee index values, with the high-fat diet (G2), low-dose (G3), and high-dose (G5) groups reaching the highest values by week 4 (319.08, 320.71, and 317.67, respectively). This trajectory reflects the accumulation of adipose tissue resulting from excess caloric intake. Following the initiation of treatment, a divergence emerged: G2 maintained an elevated Lee index through week 8 (318.32), while all intervention groups demonstrated substantial reductions (G3: from 320.71 at week 4 to 290.17 at week 8; G4: from 315.2 to 281.98), and G5 showing the most pronounced decline (G5: from 317.67 to 275.6). The negative control (G1) consistently showed the lowest Lee index values across all weeks. Statistical analysis validated substantial disparities across groups during both the induction and treatment phases, highlighting the influence of both food and intervention.

Table Error! No text of specified style in document. **: Calculated Lee Index for each group at various time points**

Group	Week 0	Week 4	Week 8
G1	303.7	284.26	274.01
G2	307.96	319.08	318.32
G3	313.04	320.71	290.17
G4	303.63	315.2	281.98
G5	308.43	317.67	275.6

***Note:Body Weight was taken in grams (g). Naso-anal Length was taken in centimeters (cm). Lee Index values were calculated using the formula: (cube root of body weighting / naso-anal length in cm) × 1000. Values > 310 indicate obesity according to Lee's criteria (16).**

Glycemic Indices

A significant difference in serum insulin levels was detected across the experimental groups, as shown in table 4. The high-fat diet group (G2) exhibited the highest mean insulin concentration (34.69 ± 4.33 µIU/mL), which was significantly greater than the levels observed in all other groups. In contrast, the negative control group (G1) showed the lowest insulin value (13.80 ± 3.34 µIU/mL). The low- and medium-dose treatment groups (G3 and G4) demonstrated partial reductions (25.56 ± 2.30 and 24.09 ± 3.51 µIU/mL, respectively), while the high-dose group (G5) achieved a more pronounced improvement (20.26 ± 2.35 µIU/mL).

Similarly, fasting blood glucose levels were significantly affected by different group. The highest mean glucose concentration was observed in the high-fat diet group (G2, 101.71 ± 2.56 mg/dl), which was significantly elevated compared with the negative control (G1, 90.86 ± 2.79 mg/dl) and the high-dose treatment group (G5, 80.43 ± 6.27 mg/dl). The low- and medium-dose groups (G3, 98.71 ± 5.65; G4, 95.00 ± 2.65 mg/dl) showed intermediate improvements, however, did not fully normalize their glucose levels.

Regarding insulin resistance, there were also highly significant differences in HOMA-IR values across the groups, closely paralleling the trends observed for serum insulin. The high-fat diet group (G2) demonstrated the highest mean HOMA-IR (8.71 ± 1.11), which was significantly greater than all other groups. Both the low- and medium-dose treatment groups exhibited intermediate reductions (G3, 6.21 ± 0.45; G4, 5.63 ± 0.71), which were significantly lower than G2 but remained elevated compared with the negative control (G1, 3.09 ± 0.70). The high-dose group (G5, 4.04 ± 0.70) achieved a level of insulin resistance that was not significantly different from the control, indicating near-normalization of insulin sensitivity

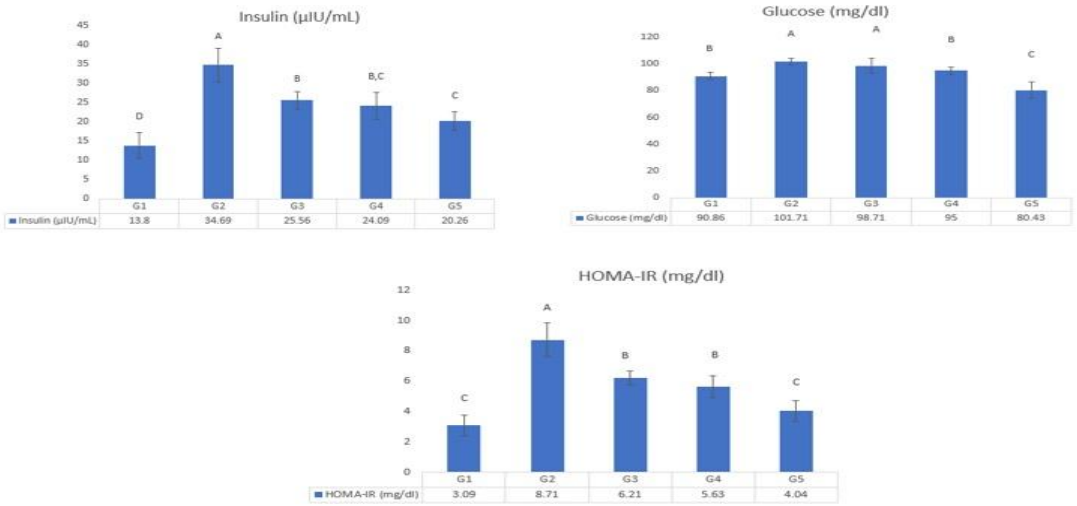

Figure 1: Insulin and Serum Glucose Levels Across Different Experimental Groups
***The data are shown as the Mean ± Standard Deviation (SD). Different capital letters (A, B, C) above the bars show that there are statistically significant differences between groups ($p < 0.05$), as shown by post-hoc analysis (e.g., ANOVA followed by Tukey's HSD). Groups that have the same letter are not considered significantly different.**

Table 4: Effect of Different Doses of Liraglutide on Blood Glucose, Insulin Levels and HOMA-IR in High-Fat Diet-Induced Obese Rats.

Group	Insulin (µIU/mL)	Glucose (mg/dl)	HOMA-IR (mg/dl)
G1	13.80 ± 3.34^{D}	90.86 ± 2.79^{B}	3.09 ± 0.70^{C}
G2	34.69 ± 4.33^{A}	101.71 ± 2.56^{A}	8.71 ± 1.11^{A}
G3	25.56 ± 2.30^{B}	98.71 ± 5.65^{A}	6.21 ± 0.45^{B}
G4	$24.09 \pm 3.51^{B, C}$	95.00 ± 2.65^{B}	5.63 ± 0.71^{B}
G5	20.26 ± 2.35^{C}	80.43 ± 6.27^{C}	4.04 ± 0.70^{C}
ANOVA p	<0.001	0.024	<0.001

***Mean ± SD presented for each group. Tukey = group letters from Tukey's post hoc analysis (groups sharing a letter are not significantly different at p < 0.05).**

Lipid Profile

Table 5 presents the effects of liraglutide treatment on lipid profile parameters in high-fat diet–induced obese rats. Statistical analysis revealed significant alterations in serum lipids among the groups. The high-fat diet group (G2) exhibited the most pronounced dyslipidemia, with markedly reduced HDL (22.00 ± 4.16 mg/dl) and elevated LDL (58.57 ± 5.38 mg/dl), total cholesterol (179.7 ± 7.1 mg/dl), and triglycerides (159.20 ± 8.90 mg/dl). These values were significantly different from those of the negative control group (G1), which demonstrated the most favorable lipid profile, characterized by the highest HDL (39.86 ± 4.88 mg/dl) and the lowest LDL (29.57 ± 3.74 mg/dl), total cholesterol (99.3 ± 6.6 mg/dl), and triglycerides (92.10 ± 6.80 mg/dl). The low- and medium-dose treatment groups (G3 and G4) showed partial improvements in lipid levels compared with G2. G3 (25.00 ± 4.51; 43.86 ± 3.18; 161.9 ± 6.0; 145.30 ± 8.00 for HDL, LDL, total cholesterol, and TG), respectively, demonstrated modest amelioration, while G4 (29.86 ± 2.79; 42.43 ± 3.64; 141.7 ± 5.7; 118.20 ± 7.10) exhibited further reductions, particularly in total cholesterol and triglycerides. Notably, the high-dose treatment group (G5) achieved the most substantial lipid normalization among treated groups, with HDL (34.29 ± 3.90 mg/dl) approaching the control level, and LDL (33.00 ± 2.16 mg/dl), total cholesterol (121.4 ± 5.5 mg/dl), and triglycerides (101.60 ± 6.50 mg/dl) significantly being reduced compared with G2.

Table 5: Lipid Profile Parameters

Group	HDL (mg/dl)	LDL (mg/dl)	T.Cholesterol (mg/dl)	TG (mg/dl)
G1	39.86 ± 4.88^{A}	29.57 ± 3.74^{C}	99.3 ± 6.6^{E}	92.10 ± 6.80^{D}
G2	22.00 ± 4.16^{C}	58.57 ± 5.38^{A}	179.7 ± 7.1^{A}	159.20 ± 8.90^{A}
G3	25.00 ± 4.51^{C}	43.86 ± 3.18^{B}	161.9 ± 6.0^{B}	145.30 ± 8.00^{B}
G4	29.86 ± 2.79^{B}	42.43 ± 3.64^{B}	141.7 ± 5.7^{C}	118.20 ± 7.10^{C}
G5	34.29 ± 3.90^{A}	33.00 ± 2.16^{C}	121.4 ± 5.5^{D}	101.60 ± 6.50^{D}

***Mean ± SD presented for each group. Tukey = group letters from Tukey's post hoc analysis (groups sharing a letter are not significantly different at p < 0.05).**

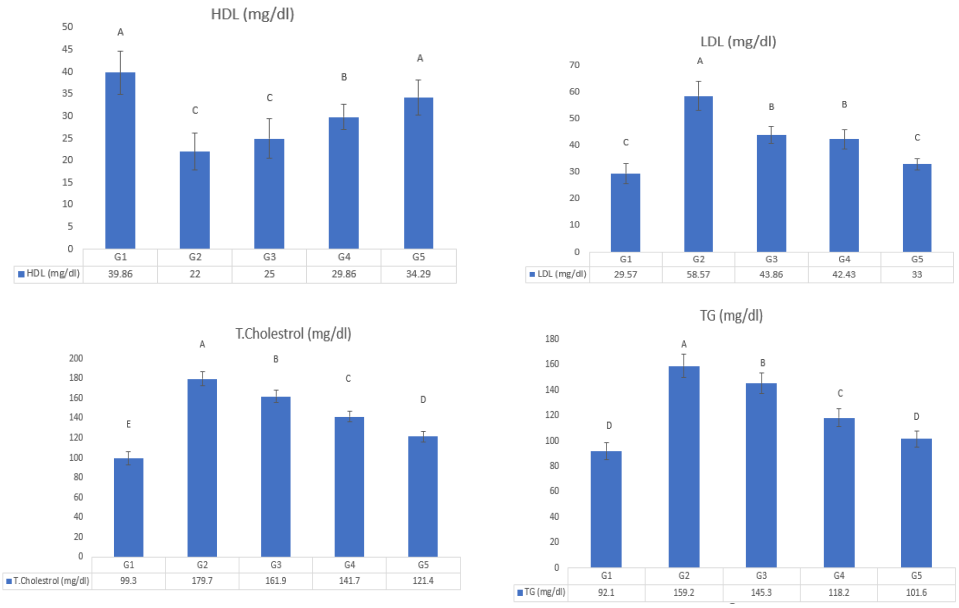

Figure 1 Lipid Profile Analysis Across Different Experimental Groups

***Data are presented as the Mean ± Standard Deviation (SD). Statistically significant differences between groups ($p < 0.05$), as determined by post-hoc analysis (e.g., ANOVA followed by Tukey's HSD), are indicated by different uppercase letters (A, B, C) above the bars. Groups that share the same letter are not considered significantly different.**

Assessment of NRF2 expression levels measured by IHC.

Table 6 presents the effects of liraglutide treatment on the Immunohistochemical expression of NRF-2 in high-fat diet (HFD)-induced obese rats. The expression of NRF-2 was significantly altered across the experimental groups. The high-fat diet group (G2) exhibited the lowest NRF-2 expression level (0.1076 ± 0.0069), which was significantly reduced compared to the negative control group (G1: 0.1903 ± 0.0096). Treatment with liraglutide resulted in a dose-dependent restoration of NRF-2 expression. The low-dose group (G3: 0.2107 ± 0.0011) showed a modest increase, while the medium-dose group (G4: 0.3234 ± 0.0193) demonstrated a more pronounced elevation. Notably, the high-dose treatment group (G5: 0.4142 ± 0.0036) achieved the highest NRF-2 expression, significantly exceeding all other groups (Tukey A). These findings indicate that liraglutide effectively counteracted the HFD-induced suppression of NRF-2, with the strongest reactivation observed at the highest dose, thereby supporting its role in restoring the antioxidant response pathway.

Table 6: NRF-2 protein expression in rat liver tissue.

Group	NRF-2 (mean ± SD)
G1	0.1903 ± 0.0096^{D}
G2	0.1076 ± 0.0069^{E}
G3	0.2107 ± 0.0011^{C}
G4	0.3234 ± 0.0193^{B}
G5	0.4142 ± 0.0036^{A}

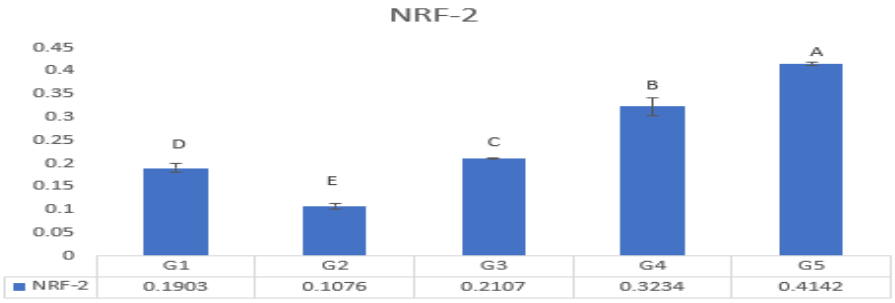

Figure 3: NRF2 Expression Across Different Experimental Groups

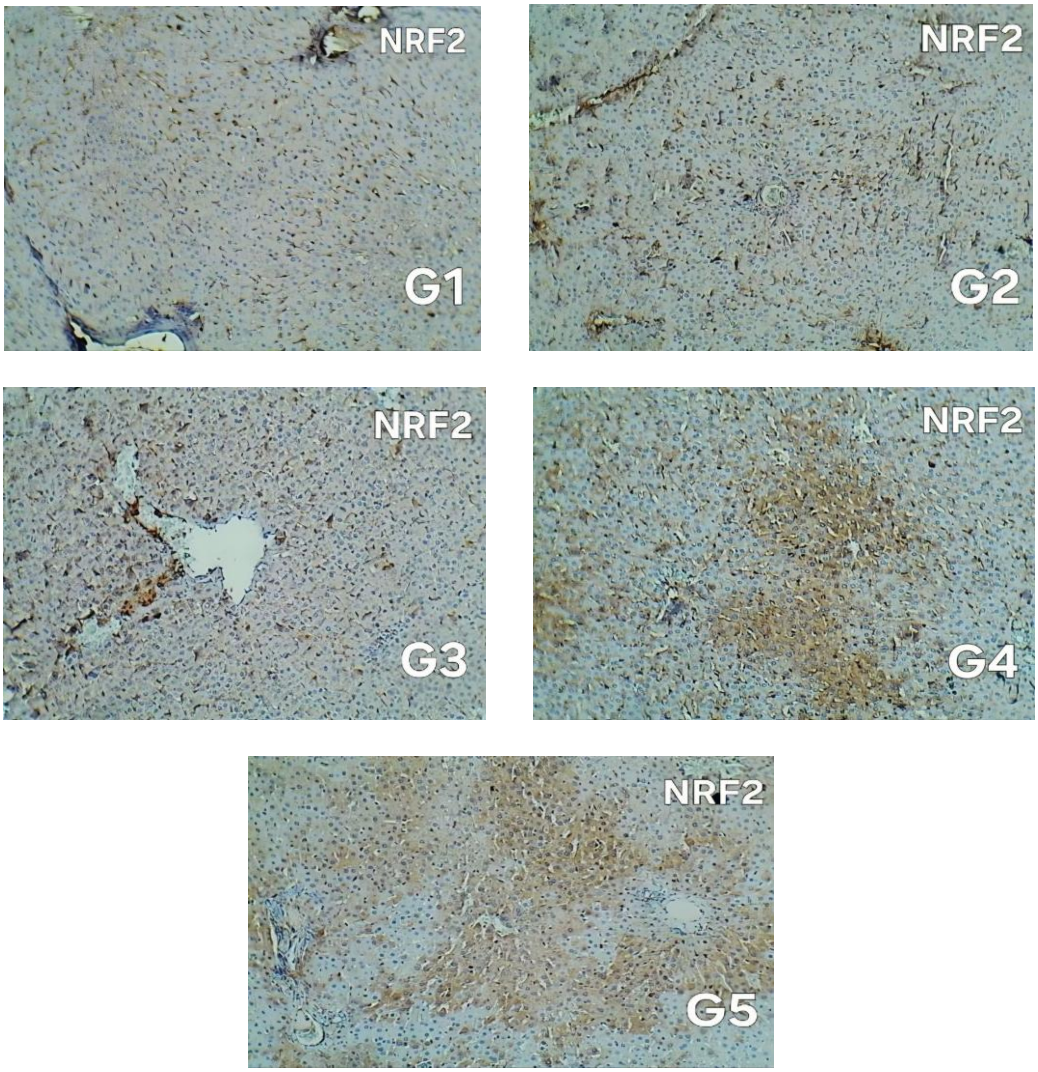

Figure 4: NRF-2 immuno-expression of liver tissue.

G1: grade zero (less than 10%) positive reaction of reactive cells.
G2: grade zero (less than 10%) positive reaction of reactive cells with weak intensity.
G3: grade 1 (+) positive reaction of reactive cells with moderate intensity.
G4: grade 3 (+++) positive reaction of reactive cells with Moderate intensity
G5: grade 3 (+++) positive reaction of reactive cells with severe intensity.

Discussion

The combined assessment of body weight, naso-anal length, and Lee index provides a detailed assessment of somatic and metabolic responses to high-fat diet and medication use. The substantial weight and Lee index increases noted throughout the induction period, together with the lack of significant alterations in linear growth, indicate that diet-induced obesity in this animal is predominantly marked by heightened adiposity rather than overall somatic enlargement. The notable alteration of these indicators post-treatment, particularly at elevated dosages, confirms the effectiveness of the therapy regimen in mitigating obesity-related characteristics. These results align with existing evidence on high-fat diet models and endorse the intervention's translational potential for managing metabolic syndrome and obesity. Prior research and the FDA acknowledged the impact of liraglutide in reducing body weight (27,28). The current study demonstrated that all liraglutide dosage groups (G3, G4, G5) had a reduction in the Lee index from pre-treatment to post-treatment, hence underscoring liraglutide's efficacy in reducing fat mass. The drop in the Lee index corresponds with decreases in body weight, further substantiating the anti-obesity effects of liraglutide, especially at elevated dosages (400 µg/kg).

The present data indicates that liraglutide significantly regulates lipid profile abnormalities caused by a high-fat diet (HFD). The adoption of a high-fat diet (G2) led to pronounced dyslipidemia, evidenced by substantial increases in total cholesterol, low-density lipoprotein (LDL), and triglycerides (TG), with a decrease in high-density lipoprotein (HDL). These findings correspond with existing data that high-fat diets (HFD) facilitate obesity-related metabolic disturbances through enhanced lipid buildup and diminished lipid clearance (29). In the context of liraglutide therapy, especially at medium and high dosages (200mg/kg, 400mg/kg), a significant improvement in dyslipidemia was noted. Substantial decreases in LDL, TG, and total cholesterol were observed in the G4 and G5 groups, with G5 exhibiting the most pronounced enhancement close to normal values. Nonetheless, HDL levels persisted at relatively lower levels, indicating a partial restoration of lipid balance**.**

Obesity, especially when caused by a high-fat diet (HFD), correlates with systemic oxidative stress resulting from food overload, mitochondrial dysfunction, and chronic inflammation (30). This redox imbalance in the liver causes lipid peroxidation, damage to cells, and the progression of metabolic illnesses including non-alcoholic fatty liver disease (NAFLD). The NRF2 signaling pathway is an important way for cells to protect themselves. It controls how antioxidant genes like heme oxygenase-1 (HO-1) and glutathione peroxidase 4 (GPX4) are expressed (31). NRF2 activation protects against oxidative damage and lipid peroxidation (32). The present study noted that rats on a high-fat diet (G2) exhibited a vast drop in hepatic NRF2 expression compared to control rats (G1), suggesting that their antioxidant defense was weaker. These results corroborate prior studies demonstrating that a high-fat diet (HFD) reduces NRF2 activity, lessens the transcription of antioxidant genes, and heightens vulnerability to oxidative liver damage (33) . Liraglutide treatment resulted in a distinct dose-dependent increase in NRF-2 expression. At the modest dosage (G3), NRF-2 was partly recovered, surpassing even the baseline control levels, indicating early activation of NRF-2 signaling. NRF-2 expression increased steadily and dramatically with

medium (G4) and high dosage (G5) treatment; surpassing values observed in the control group. This improvement underscores liraglutide's capacity to activate the NRF-2/ HO-1 antioxidant pathway, thus mitigating oxidative damage.

The results regarding Insulin, Glucose, and HOMA-IR indicate that the introduction of a high-fat meal significantly impaired glucose homeostasis, evidenced by increased levels of circulating insulin, fasting glucose, and HOMA-IR, therefore confirming the onset of insulin resistance. Conversely, the normal control group displayed stable glycemic readings, underscoring the detrimental effect of excessive dietary fat on metabolic regulation. Treatment with liraglutide resulted in a dose-dependent enhancement in all measures. Lower dosages resulted in a modest alleviation of hyperinsulinemia and insulin resistance, suggesting a limited therapeutic impact. Intermediate dose further improved glucose control and insulin sensitivity, demonstrating liraglutide's capacity to mitigate the metabolic effects of obesity. The high-dose group had the most significant improvement in glycemic control, nearing values akin to the healthy control group, highlighting the effectiveness of liraglutide in mitigating diet-induced insulin resistance. The results can be attributed to the different modes of action of liraglutide. Liraglutide stimulates the GLP-1 receptor, enhancing glucose-dependent insulin production while concurrently decreasing inappropriate glucagon release. Furthermore, liraglutide facilitates weight loss, enhances lipid metabolism, and diminishes oxidative stress, all of which enhance insulin sensitivity. These outcomes collectively emphasize liraglutide's therapeutic promise in alleviating obesity-related metabolic disturbances and averting the advancement of type 2 diabetes.

The high-fat diet model successfully induced obesity and dyslipidemia, as demonstrated by elevated LDL, cholesterol, and triglycerides, together with increased body weight and Lee index, while naso-anal length remained unchanged, confirming true adiposity rather than normal growth. This metabolic disruption was accompanied by a marked reduction in hepatic NRF2 expression, indicating impaired antioxidant defense under HFD conditions.

Liraglutide treatment produced clear dose-dependent improvements across all obesity-related parameters. The reduction in Lee index, together with stable naso-anal length, confirmed a true loss of fat mass, paralleled by significant correction of dyslipidemia and improvement in glycemic markers. Importantly, restoration of NRF2 expression after liraglutide administration showed a direct relationship between reduced adiposity and enhanced antioxidant capacity. The highest dose produced the greatest improvement in Lee index and lipid profile, which corresponded to the strongest reactivation of NRF2 signaling, highlighting its role in mediating the metabolic benefits of GLP-1 agonist therapy.

Conclusion

This study demonstrates that liraglutide exerts a potent, dose-dependent modulatory effect on high-fat diet–induced obesity in rats. Liraglutide significantly reduced body weight and Lee index, improved glycemic control, corrected dyslipidemia, and enhanced hepatic antioxidant defense through activation of the NRF2 pathway. These integrated effects highlight liraglutide’s multifaceted role in alleviating

metabolic dysfunction, reducing oxidative stress, and restoring insulin sensitivity. Collectively, the findings support liraglutide as a promising therapeutic strategy for obesity and its associated metabolic and hepatic complications.

Study Limitations

- **Single species and sex**: Only male Wistar rats were used. Responses may differ in females or other strains, given hormonal and genetic influences on adiposity and antioxidant pathways.
- **Lack of pharmacokinetic data:** Serum liraglutide levels were not measured, so dose-response interpretation is based on administered dose rather than systemic exposure.
- **HFD model only:** The obesity phenotype was induced exclusively with a high-fat diet. Including other models (genetic obesity) would clarify whether the benefits of liraglutide are broadly applicable.
- **Sample size and single-center design:** The experiment included 35 rats from one facility, which limits the breadth of biological variability. Larger, multicenter studies would strengthen generalizability.

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