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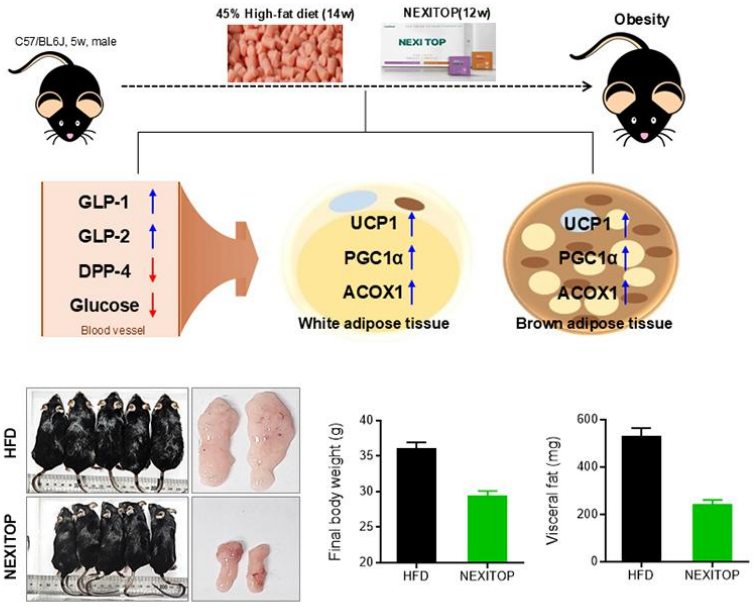
Original article

Anti-obesity effects of the herbal formula NEXITOP through modulation of lipolysis mediated by glucagon like peptide-1 in a high fat diet-fed mouse

Graphical Abstract



Highlights

- NEXITOP treatment enhanced circulating GLP-1 and GLP-2 levels while suppressing DPP-4 activity in high-fat diet-induced obese mice.
- Upregulation of thermogenic genes (UCP1, PGC-1α, ACOX1) was observed in both white and brown adipose tissue.
- Enhanced incretin signaling through GLP-1 activation contributed to improved energy expenditure and adipose tissue remodeling.

Authors

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In Brief

NEXITOP enhanced GLP-1/GLP-2 signaling and reduced DPP-4 activity in obese mice, improving glucose tolerance and decreasing visceral fat. It also upregulated UCP1, PGC1α, and ACOX1, promoting thermogenic and oxidative metabolism in adipose tissue.

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Anti-obesity effects of the herbal formula NEXITOP through modulation of lipolysis mediated by glucagon like peptide-1 in a high fat diet-fed mouse

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ABSTRACT

Objective: GLP-1 is a key incretin hormone that not only regulates glucose metabolism but also contributes to energy expenditure and adipose tissue remodeling. However, the integrative role of GLP-1 in coordinating lipid oxidation and thermogenic gene expression in adipose tissues remains incompletely understood. Thus, we investigated anti-obesity effects of NEXITOP including regulation of incretin hormones and thermogenic genes related to fat metabolism.

Materials and Methods: Male C57BL/6J mice were fed a 45% high-fat diet (HFD) for 14 weeks to induce obesity, followed by oral administration of NEXITOP, a novel GLP-1 regulator for 12 weeks. Serum incretin hormones markers (GLP-1, GLP-2, DPP-4) and glucose levels were analyzed, and expression of genes encoding thermogenic and fat oxidation markers (UCP1, PGC1α, ACOX1) was examined in white and brown adipose tissue.

Results: NEXITOP administration significantly increased circulating GLP-1 and GLP-2 levels while reducing DPP-4 activity and fasting glucose. These hormonal changes were accompanied by a significant reduction in final body weight and visceral fat mass compared with the HFD group. At the tissue level, both white and brown adipose tissue exhibited upregulated expression of UCP1, PGC1α, and ACOX1, suggesting enhanced mitochondrial and peroxisomal fatty acid oxidation.

Conclusion: NEXITOP ameliorates HFD-induced obesity by enhancing GLP-1-mediated signaling and reprogramming adipose tissue metabolism toward an oxidative and thermogenic phenotype. These findings highlight a potential therapeutic strategy combining incretin modulation and adipose metabolic remodeling for obesity and metabolic disorders.

Keywords NEXITOP, obesity, incretin hormones, thermogenesis, adipose tissue

INTRODUCTION

Obesity has emerged as one of the most pressing global public-health challenges of the twenty-first century. Its prevalence has risen sharply over recent decades and now affects a large fraction of the world's population, driving a major burden of fatty liver diseases, cardiometabolic disease, metabolic disability, and mortality.[1] According to global surveillance, approximately 43% of adults were overweight and 16% lived with obesity, as of 2022.[2]

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A fundamental strategy for obesity management is the restoration of energy balance through reduced caloric intake and increased physical activity.[3,4] However, such substantial lifestyle modifications are very difficult to achieve and maintain which the need for adjunctive therapeutic approaches. In recent years, pharmacotherapy has emerged as a valuable complement to lifestyle intervention, with growing attention directed toward the glucagon-like peptide-1 (GLP-1) signaling pathway. GLP-1, an incretin hormone secreted from intestinal L-cells in response to nutrient ingestion, promotes glucose-dependent insulin secretion, delays gastric emptying, and suppresses appetite through central and peripheral mechanisms.[5]

GLP-1 receptor agonists (GLP-1RAs) has proven highly effective in promoting weight loss and improving glycemic control in individuals with obesity and type 2 diabetes.[6,7] In addition to synthetic GLP-1RAs, certain bioactive food-derived compounds including berberine, curcumin, and resveratrol have

been shown to enhance endogenous GLP-1 secretion or expression through modulation of intestinal L-cell function and nutrient-sensing pathways.[8–10] Such findings have sparked growing interest in developing nutraceutical or pharmacological strategies that harness GLP-1 biology to achieve sustained metabolic benefits with improved tolerability and accessibility.

NEXITOP is the herbal formula including Berberis asiatica, Momordica charantia, Cinnamomum tamala, Camellia sinensis, Curcuma longa etc. In a previous study, we demonstrated anti-obesity effects of NEXITOP in mice fed 60% high-fat diet (HFD) for 10 weeks.[11] While 60% HFD-fed mouse models are widely used due to their ability to induce rapid and consistent obesity phenotypes, such extreme formulations can lead to non-physiological disease progression including, hepatic inflammation, severe steatosis and pancreatic islet stress.[12–14] Moreover, reduced palatability and feeding aversion can introduce variability in energy-intake measurements, thereby confounding metabolic analyses.[15] The discrepancy between the fat content of 60% HFD and typical human diets (approximately 30–35% kcal from fat) limits their translational relevance for modeling human obesity.

In contrast to 60% HFD, 45% HFD induces gradual metabolic alterations, including sustained weight gain, appropriate adipocyte hypertrophy, and the slow onset of insulin resistance, allowing precise evaluation of disease progression in long-term studies. Moreover, the latter diets may impose less physiological stress, resulting in appropriate hepatic steatosis, renal burden, and inflammatory responses that better mimic the chronic metabolic dysfunction observed in humans. Additionally, their higher palatability advantageous for assessing appetite regulation, energy intake, and nutrient metabolism under controlled laboratory conditions.[12,13] These prompted us to investigate the anti-obesity effects of NEXITOP and molecular mechanism in mice fed 45% HFD for 14 weeks, with particular emphasis on the upregulation of GLP-1 signaling and enhancement of fat oxidation. Provide the background, rationale, and objectives of the study.

MATERIALS AND METHODS

Preparation of NEXITOP and semaglutide

NEXITOP was provided by JBKLAB Co., Ltd (Gyeonggi-do, South Korea. Semaglutide, a GLP-1 receptor agonist, was used as a positive control (Med Chem Express, NJ, USA).

Cell culture

Both 3T3-L1 (mouse fibroblast pre-adipocyte) and NCI-H716 (human colorectal adenocarcinoma) cells were obtained from Korea Cell Line Bank (Seoul, South Korea) and cultured using complete medium at 37°C and 5% CO2 chamber. The composition of each complete medium is as follows: for 3T3-L1 cells, DMEM (Cytiva, MA, USA) with 10% bovine calf serum (BCS, Thermo Fisher Scientific, MA, USA) and 1% anti-biotics solution (ABS, Cytiva, MA, USA); for NCI-H716 cells, RPMI1640 with 10% fetal bovine serum (FBS, Cytiva, MA, USA) and 1% ABS.

Differentiation of 3T3-L1 cells and Oil red O staining

3T3-L1 pre-adipocytes were seeded in a 24-well plate (2×104 cells/mL) and cultured using complete medium for 2 days. These cells were differentiated by treatment with medium containing 10% FBS, 1% ABS, 1 μM dexamethasone (DEX; Abcam, Cambridge, UK), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, MO, USA) and 1 μg/mL insulin (Sigma-Aldrich, MO, USA) for 2 days, and maintained in maintain medium with 10% FBS, 1% ABS and 1 μg/mL insulin for 5 days. To evaluate the inhibitory effect of NEXITOP on lipid accumulation, NEXITOP (5, 10 and 25 μg/mL) every other day for total 7 days of differentiation duration.

On the final day, cells were washed in PBS and fixed in 10% formalin for 1 h. After washing the fixed cells, 60% isopropanol was added for 5 min at room temperature. And then, cells were stained with Oil Red O working solution for 10 min and visualized under an optical microscope (×100 magnification, Leica, Wetzlar, Germany) after washing. The residual Oil red O solution was eluted and subjected to measurement of an O.D value using a microplate reader (Thermo Fisher Scientific, MA, USA).

Measurement of GLP-1 secretion in NCI-H716 cells

NCI-H716 cells were seeded in 48-well plates (5x105 cells/mL) and cultured in Krebs-Ringer Bicarbonate buffer (Biosolution, Seoul, South Korea) for 30 min. After additional 2 h incubation with NEXITOP (5, 10 and 25 μg/mL), supernatant was collected and the amount of GLP-1 was measured using the common ELISA kit.

Animal and diet

Male C57BL/6J mice (5-weeks-old, 21–23 g) were obtained from DBL (Chung-Buk, South Korea). A total of 42 mice were housed (4-6 mice in one cage) with free access to food and water in a room maintained at 22 ± 2 °C under a 12 h light: 12 h dark cycle. The normal diet (3.0 kcal/g) and 45% high-fat diet (5.1 kcal/g) were provided by DBL and Research Diets (NJ, USA), respectively.

Experiment design

After a one-week acclimatization period, 38 mice excluding the normal with 50% glycol 400 polyethylene (PEG) in distilled water (DW) groups (n = 4) were fed 45% HFD for two weeks. In the third week, the HFD-fed mice were randomly divided into four groups based on their average body weight: HFD with DW (n=11), 200 μg/kg semaglutide (n=8), 1,300 mg/kg NEXITOP (n = 10) and 2,600 mg/kg NEXITOP (n = 9). All five groups received daily oral administration of the respective formulation dissolved in 50% PEG (10mL/kg) for 12 weeks. Control mice were treated with the vehicle alone. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of JBKLAB Co., Ltd.

(Approval No. JBK-25-06-001), and the experimental design is summarized in Fig. 1. At the end of the experimental period, mice were anesthetized and euthanized in an isoflurane chamber. Blood samples were collected from the abdominal vein, and three types of abdominal fat tissues (epididymal, retroperitoneal, and visceral) were carefully dissected and weighed.

Food intake and body weight

Food intake and body weight were measured in the morning on Monday and Thursday for 8 weeks. Food intake was calculated based on caloric content and expressed as cumulative, weekly change, and average values over the 8-week period. Simultaneously with measurement of food intake, body weight was recorded and presented as weekly change.

Hematoxylin and eosin staining

For histological analysis, epididymal fat tissues were fixed in 10% formalin, embedded in paraffin, and sectioned at a thickness of 6 μm. The sections were stained with Mayer's hematoxylin and eosin (H&E; Sigma-Aldrich, MO, USA) and mounted on slides using mounting medium (Sigma-Aldrich, MO, USA). The stained sections were examined under a microscope at ×200 magnification.

Measurement of protein levels of GLP-1, GLP-2 and DPP-4 in serum

In the present study, orbital blood samples were collected at weeks 3, 6, and 9 of drug administration. After allowing the blood to clot at room temperature for 40 min, the samples were centrifuged at 8,000 rpm for 15 min to separate the serum. The serum samples were used to measure protein levels of GLP-1 (Thermo Fisher Scientific, MA, USA), GLP-2 (Abcam, Cambridge, UK) and DPP-4 (R&D System, MN, USA) using a common ELISA kit.

Oral glucose tolerance test

Oral glucose tolerance test was conducted after 4 and 8 weeks of drug administration. All mice were fasted for 20 h with free access to water prior to testing. Baseline blood glucose levels were measured from the tail vein using a glucometer (i-SENSE, Seoul, South Korea), and then glucose (2 g/kg body weight) was orally administered. Blood glucose levels were measured at 30, 60, 90, and 120 min after glucose administration.

Blood cell counting and serum biochemistry

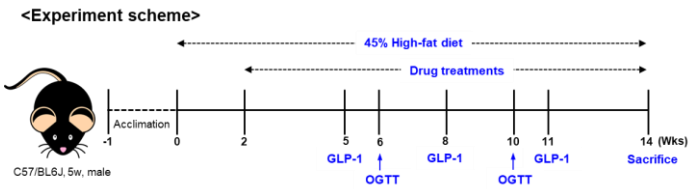
On the final day, blood was respectively collected in EDTA-tube and serum-separated tube from inferior vena cava and divided into whole blood and serum. Total white blood cells (WBC), neutrophils and lymphocytes were analyzed in whole blood using the BC-5000 vet analyzer (Mindray, Shenzhen, China). Serum levels of triacyl glycerol (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glucose were determined in serum using the Hitachi 7180 analyzer (Hitachi, Tokyo, Japan).

Real-time PCR in white and brown adipose tissues

Total RNA was isolated from white and brown adipose tissues using TRIzol™ reagent (Invitrogen; CA, USA) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using the PrimeScript™ RT Reagent Kit (Takara Bio Inc., Shiga, Japan). Quantitative real-time PCR (qRT-PCR) was performed on genes of uncoupling protein 1 (UCP1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), acyl-CoA oxidase 1 (ACOX1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the QuantStudio™ 3 Real-Time PCR System (Applied Biosystems, MA, USA). Relative gene expression was calculated using the ΔCt method, with GAPDH serving as the internal reference. The primer sequences used for amplification are listed in Table 1.

Statistical analysis

All results are expressed as the mean ± standard deviation (SD) or dot plot. Group differences were assessed using one-way analysis of variance (ANOVA). Post-hoc multiple comparisons for each group using Tukey's Honestly Significant Difference (HSD) test, performed with Prism version 8.0. (GraphPad; CA, USA). Statistical significance is expressed as follows: $^{\#}p$ <0.05, $^{\#\#}p$ <0.01 and $^{\#\#\#}p$ <0.001 for normal vs. control groups; p < 0.01



Groups	45% High fat diet	Drug treatment	Number of mouse
Normal	X	D.W, p.o, daily	4
Control	O	D.W, p.o, daily	11
Semaglutide	O	Semaglutide 200 μg/kg, i.p, once a week	8
NEXITOP Low	O	NEXITOP 1,300 mg/kg, p.o, daily	10
NEXITOP High	O	NEXITOP 2,600 mg/kg, p.o daily	9

Fig. 1. Summary of experiment schedule.

and $^{***}p < 0.001$ for control vs. each group or vs. semaglutide group; $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ for control vs. NEXITOP low group; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$ and $^{\&\&\&}p < 0.001$ for control vs. NEXITOP high group.

RESULTS

NEXITOP regulated lipid accumulation and GLP-1 expression in 3T3-L1 and NCI-H716 cells

Differentiated 3T3-L1 cells showed abundant lipid droplet accumulation as assessed by using Oil Red O staining, while NEXITOP treatment markedly reduced intracellular lipid accumulation in a concentration-dependent manner (Fig. 2A). Quantitative analysis of Oil Red O staining revealed that lipid accumulation was significantly decreased by NEXITOP at concentrations of 10 and 25 μg/mL compared with the differentiated control group (Fig. 2B). Also, NEXITOP significantly increased GLP-1 secretion in a dose-dependent manner, with the highest effect observed at 25 μg/mL (Fig. 2C).

NEXITOP reduced body weight and food intake

After 12 weeks of HFD feeding, the control group exhibited markedly higher body weight and food intake than the normal diet group, with approximately 27% and 18% increases, respectively. Representative images illustrating these effects are shown in Fig. 3A. Administration of NEXITOP at both low and high doses for one week significantly repressed increases in the body weight, which subsequently remained comparable to normal levels throughout the 12-week treatment period (Fig. 3B). Consequently, NEXITOP markedly decreased the final body weight compared with the control group ($p < 0.001$ for both doses; Fig. 3C). In addition, NEXITOP treatment maintained lower caloric consumption and significantly reduced the average caloric intake ($p < 0.001$; Fig. 3D and E). Semaglutide treatment exerted similar effects to NEXITOP on body weight reduction, although no significant changes were observed in caloric intake.

NEXITOP improved fat contents in tissue and serum

HFD feeding dramatically increased the accumulation of abdominal fat depots, including visceral, retroperitoneal, and epididymal fats, in the control group compared with the normal group (1.7-, 5.6-, and 3.7-fold increases, respectively). These elevations were significantly attenuated by NEXITOP administration at both low- and high- doses (Fig. 4A to C). The effects achieved with semaglutide treatment are comparable to those of NEXITOP. Consistent with these findings, representative images revealed a clear reduction in abdominal fat mass in NEXITOP-treated mice compared with the control group (Fig. 4D upper panels). Furthermore, histological examination of epididymal adipose tissue by H&E staining confirmed a marked decrease in adipocyte size and lipid droplet accumulation following NEXITOP treatment (Fig. 4D upper panels). Compared to normal groups, the control group exhibited considerably increases in serum levels of TG, TC, HDL and

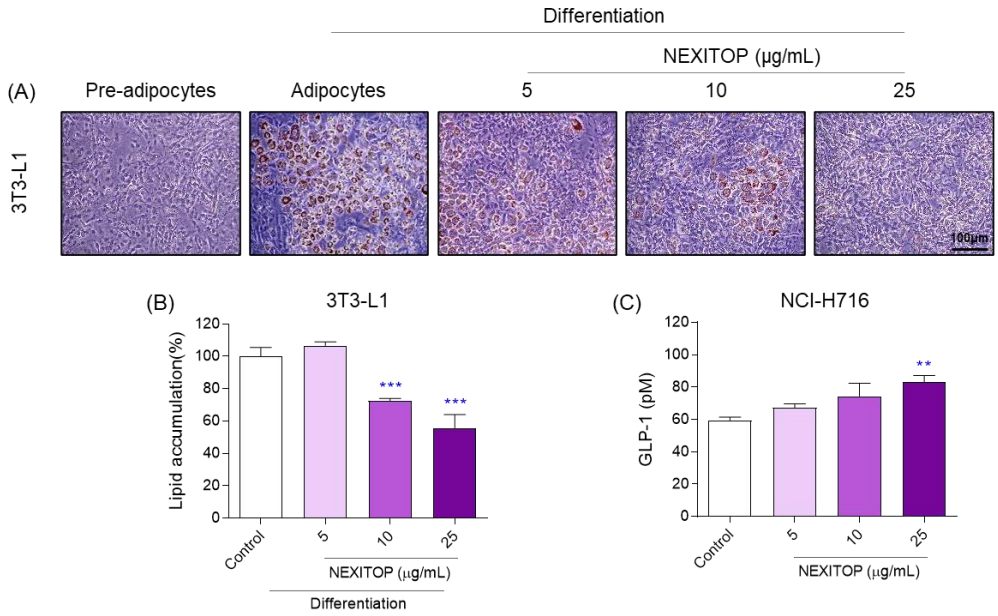


Fig. 2. Effects of NEXITOP on lipid accumulation and GLP-1 expression in cell-based models. 3T3-L1 pre-adipocytes were induced to differentiate for 7 days in differentiation medium with or without NEXITOP (5, 10, and 25 μg/mL). (A) Lipid droplet accumulation was observed at microscope (×100 magnification) and (B) quantified using microplate reader after elution of Oil Red O dye. (C) GLP-1 secretion in NCI-H716 enteroendocrine cells following 2 h treatment with NEXITOP (5, 10, and 25 μg/mL). Supernatant GLP-1 concentrations were determined using an ELISA kit. Data was presented as mean ± SD. Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{**}p < 0.01$ or $^{***}p < 0.001$ for control vs. all of treatment group.

glucose (1.5-, 1.3-, 1.3, and 1.6-fold increases, respectively. Notably, both low- and high-dose NEXITOP treatments significantly attenuated serum TG, TC, HDL and glucose levels compared with the control group (Fig. 5A to D) In complete blood counting, total number of white blood cells, neutrophils, and lymphocytes were not significantly changed with the HFD

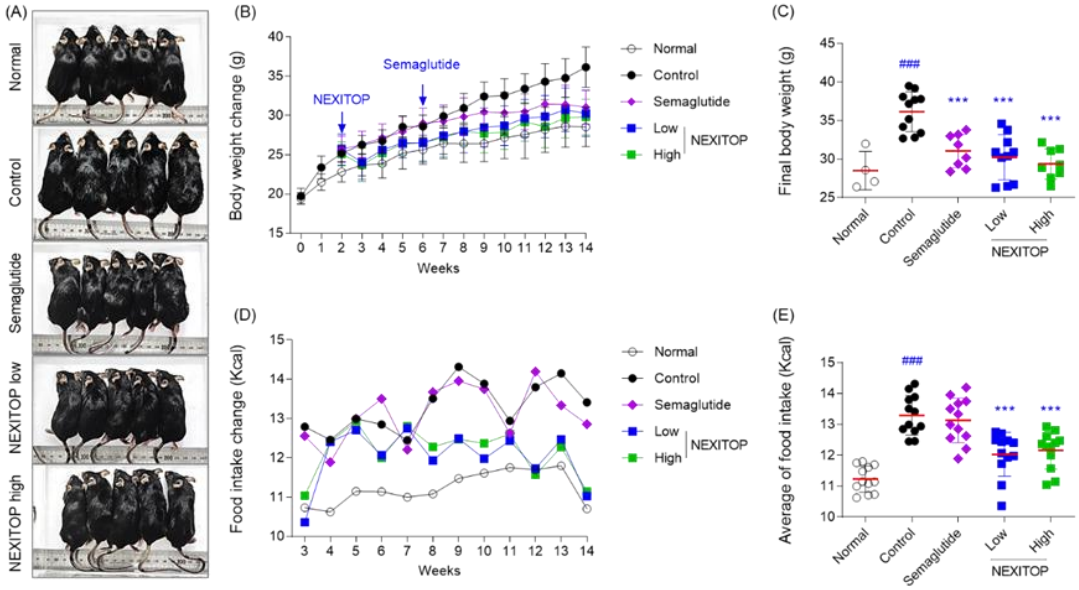


Fig. 3. Effects of NEXITOP on changes of body weight and calorie intake. Body weight and food intake were measured on every Monday and Thursday for 14 weeks. Body weight data were presented as (B) body weight change for 14 weeks and (C) final body weight after 14 weeks. Food intake data were presented as (D) change and (E) average food intake for 12 weeks after NEXITOP administration. On the last day, (A) representative pictures of the mice in each group were taken. Data was presented as mean ± dot plot. Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{###}p$ <0.001 for normal vs. control group; $^{***}p$ < 0.001 for control vs. each treatment group.

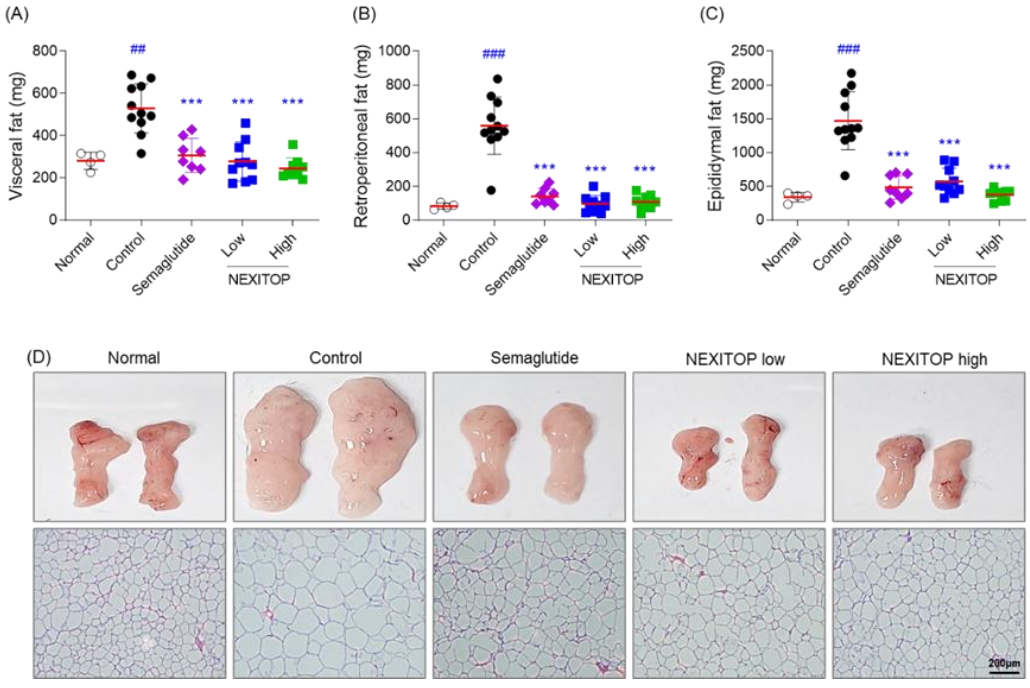


Fig. 4. Effects of NEXITOP on three abdominal fat tissues. The (A) visceral, (B) epididymal and (C) retroperitoneal fats weighed after sacrifice on the final day. Data was presented as mean ± dot plot. The (D) representative epididymal fat tissues were stained with H&E and examined under a microscope (×200 magnification). Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{##}p$ <0.01 or $^{###}p$ <0.001 for normal vs. control group; $^{***}p$ < 0.001 for control vs. each treatment group.

(Fig. 5E to G).

NEXITOP modulated serum GLP-1, GLP-2, and DPP-4, and blood glucose levels

To investigate the influence of NEXITOP on incretin-related metabolic regulation, serum levels of GLP-1, GLP-2 and DPP-4 were measured after 3, 6, and 9 weeks of NEXITOP administration. In the control group, GLP-1 and GLP-2 concentrations remained consistently low throughout the experimental period, whereas NEXITOP administration resulted in a marked elevation of both GLP-1 and GLP-2 (Fig. 6A and B). Both low- and high-dose NEXITOP groups showed a considerable increase in serum GLP-1 (both doses at 6 and 9 weeks) and GLP-2 (high-dose at 6 weeks). As illustrated in Fig.6C, serum DPP-4 levels elevated by HFD significantly decreased at 3, 6 and 9 weeks (both doses). Under the same experimental conditions, semaglutide showed low serum levels of GLP-1 and GLP-2 and high serum levels of DPP-4. To assess the functional impact of these hormonal changes on glucose metabolism, oral glucose tolerance test was performed after 4 and 8 weeks of treatment. The control group dramatically elevated blood glucose levels following glucose challenge, whereas both low- and high-dose NEXITOP groups demonstrated significantly reduced blood glucose concentration at all-time post-glucose administration compared to control group (Fig. 6D and E), comparable to the semaglutide-treated group.

NEXITOP increased lipolysis in white and brown adipose tissues

To further elucidate the mechanisms underlying the anti-obesity effects of NEXITOP, we analyzed the expression of three representative lipolysis-related genes in white and brown adipose tissues. In white adipose tissue, NEXITOP treatment significantly upregulated UCP1 (low dose) and PGC1α (high dose) mRNA expression, while ACOX1 expression was not significantly changed (Fig. 7A). These effects were more pronounced in brown adipose tissue in which NEXITOP markedly elevated UCP1 (low dose), PGC1α (both doses), and ACOX1 (both doses) expression compared with the control group (Fig. 7B). In contrast, semaglutide significantly increased the expression of these genes only in brown adipose tissue.

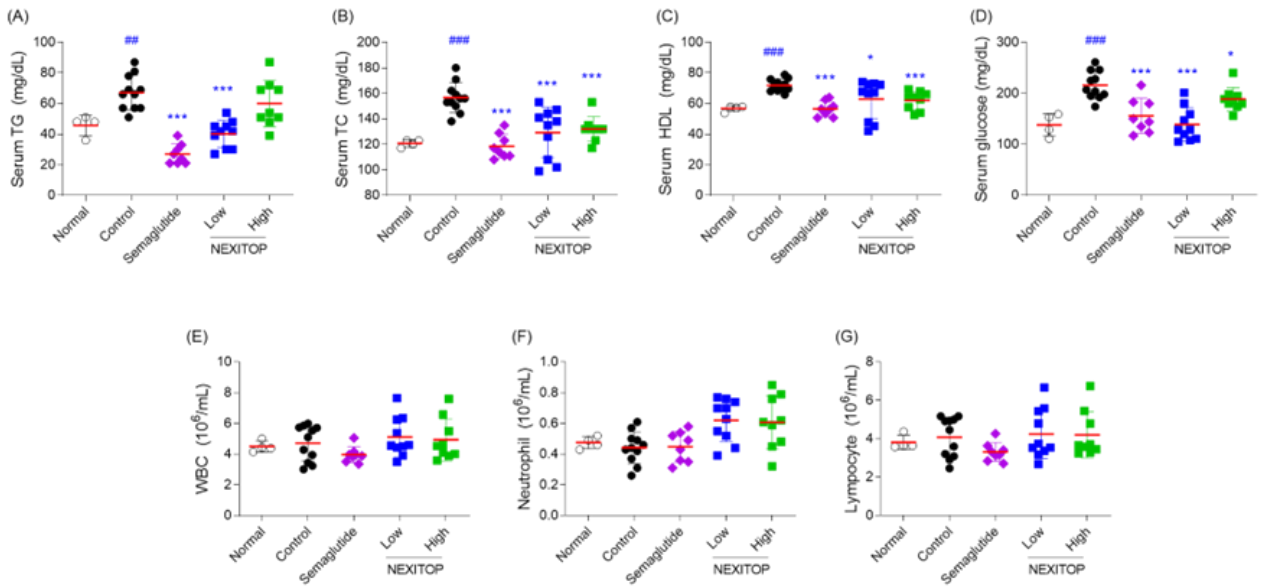


Fig. 5. Effects of NEXITOP on fat contents. On the final day, blood was respectively collected in EDTA-tube and serum-separated tube from the inferior vena cava. Some blood samples were separated into serum and analyzed with serum levels of (A) TG, (B) TC, (C) HDL and (D) glucose. Other blood samples were analyzed with number of (E) Total white blood cell, (F) neutrophil and (G) lymphocyte using a hematology analyzer. Data was presented as mean ± dot plot. Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{\#\#}p<0.01$ or $^{\#\#\#}p<0.001$ for normal vs. control group; $^{*}p<0.05$ or $^{***}p<0.001$ for control vs. each treatment group.

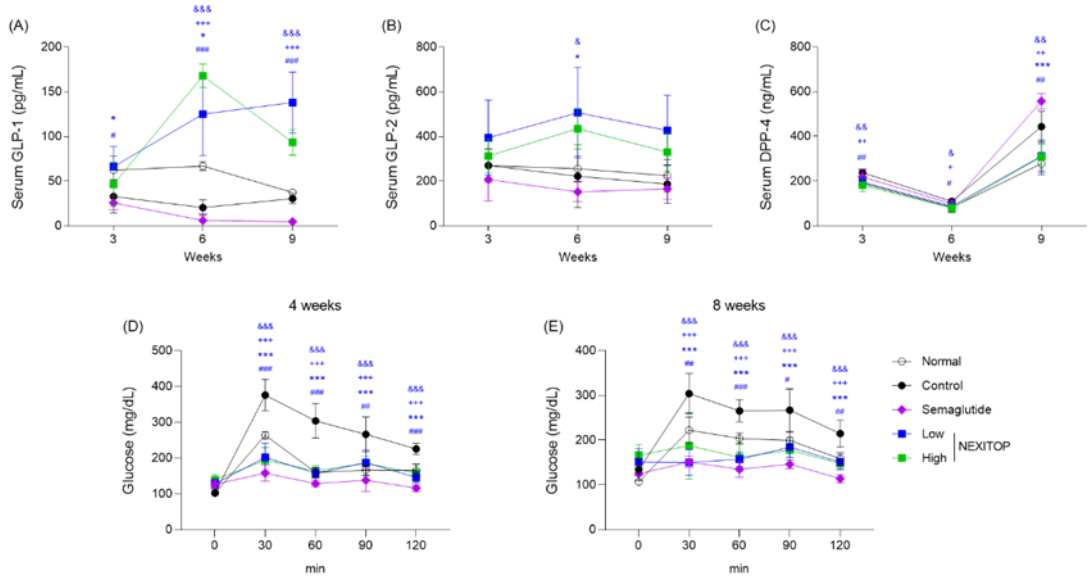


Fig. 6. Effects of NEXITOP on change of GLP-1, GLP-2 and DPP-4 and OGTT. Orbital blood collection was conducted in the 3, 6 and 9 weeks of drug administration and separated into serum. The serum samples were measured with (A) GLP-1, (B) GLP-2 and (C) DPP-4. Also, OGTT was conducted in the 4 and 8 weeks of drug administration. Data was presented as mean ± SD. Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{\#}p<0.01$, $^{\#\#}p<0.01$ or $^{\#\#\#}p<0.001$ for normal vs. control group; $^{*}p<0.05$, or $^{***}p<0.001$ for control vs. semaglutide group; $^{+}p<0.01$, $^{++}p<0.01$ or $^{+++}p<0.001$ for control vs. NEXITOP low group; $^{\&}p<0.01$, $^{\&\&}p<0.01$ or $^{\&\&\&}p<0.001$ for control vs. NEXITOP high group.

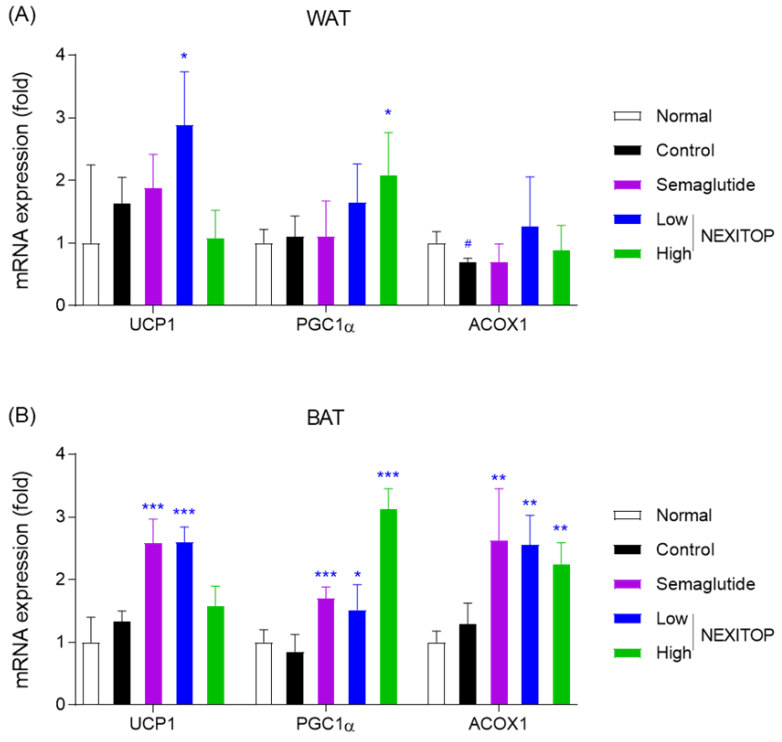


Fig. 7. Effects of NEXITOP on gene expression of UCP1, PGC1α and ACOX1 in white and brown adipose tissues. Analyses of mRNA expression levels of UCP1, PGC1α and ACOX1 in (A) white adipose tissue (WAT) and (B) brown adipose tissue (BAT) were performed using real-time PCR. Data was presented as fold change of mean ± SD after normalization to GAPDH. Statistical significance is expressed using one-way ANOVA followed by Tukey's post hoc test as follows by $^{\#}p<0.05$ or for normal vs. control group; $^{*}p<0.05$, $^{**}p<0.01$ or $^{***}p<0.001$ for control vs. each treatment group.

DISCUSSION

In the present study, oral administration of NEXITOP in 45% HFD-induced obese mice resulted in marked increases in circulating GLP-1 and GLP-2 levels and a concomitant decrease in DPP-4 activity. These hormonal changes were associated with upregulation of UCP1, PGC-1α, and ACOX1 in both white and brown adipose tissues. Consequently, NEXITOP showed significant reduction in abnormal weight gain and hypertrophic fat accumulation by HFD feeding.

GLP-1 receptor activation has recently been recognized beyond the pancreatic β-cell function to include direct and indirect effects on energy metabolism and thermogenesis. Several studies have demonstrated that GLP-1RAs such as semaglutide enhance activation of brown adipose tissue and promote the browning of white adipose tissue, resulting in increased energy expenditure and improved metabolic homeostasis.[16–18] The elevated GLP-1 and GLP-2 levels and reduced DPP-4 activity observed in the present study imply sustained incretin signaling that may contribute to adipose tissue remodeling through both peripheral and central pathways.

Importantly, the concurrent upregulation of UCP1 and PGC-1α in white and brown adipose tissues indicates that NEXITOP facilitates the conversion of white adipose tissue from a storage-oriented phenotype to a metabolically active, oxidative state. PGC-1α acts as a master regulator of mitochondrial biogenesis and oxidative metabolism, while UCP1 uncouples oxidative phosphorylation to produce heat rather than ATP, increasing energy expenditure.[19–21] Previous reports have shown that GLP-1RAs can induce UCP1 and PGC-1α expression via SIRT1-dependent pathways, supporting a mechanistic link between incretin signaling and adipocyte thermogenesis.[22,23] Consistent with these observations, our results demonstrate that NEXITOP simultaneously upregulates these genes in both white and brown adipose tissues.

In addition to mitochondrial oxidation, the upregulation of ACOX1, an essential enzyme catalyzing the first step of peroxisomal β-oxidation suggests activation of complementary fatty acid oxidation pathways.[24] The PPARα-PGC-1α axis is known to regulate both mitochondrial and peroxisomal fatty acid metabolism. Thus, increased ACOX1 expression may reflect broader stimulation of lipid catabolism beyond mitochondrial processes,[25,26] and this expanded oxidative capacity could contribute to the observed reduction in visceral fat mass and overall body weight.

In the present study, the reduction in body weight and visceral fat mass following NEXITOP administration appears to result from a coordinated interplay of several metabolic processes: (1) enhancement of incretin activity through upregulation of GLP-1 and GLP-2 with concomitant DPP-4 suppression, (2) upregulation of UCP1, PGC-1α, and ACOX1 in both white and brown adipose tissues, and (3) a subsequent increase in fatty acid oxidation and energy expenditure. This integrated mechanism supports the notion that GLP-1 receptor activation exerts beneficial effects on lipid metabolism and adipose tissue inflammation, consistent with recent findings that GLP-1R signaling promotes thermogenic remodeling and metabolic flexibility.[16]

Nevertheless, several limitations should be noted in this study. First, although transcriptional changes in thermogenic and oxidative genes were observed, functional assessments such as oxygen consumption rate, energy expenditure, or thermographic imaging were not performed. Confirming these parameters would further support the metabolic activation implied by gene expression data. Second, whether GLP-1 signaling acts directly on adipose tissue or indirectly through central nervous system–mediated sympathetic activation remains to be clarified. Indeed, previous reports have shown that GLP-1 can regulate brown adipose tissue thermogenesis via vagal neurons.[17] Third, histological confirmation of adipocyte browning and quantification of beige adipocytes would strengthen the evidence of white adipose tissue remodeling. Finally, as this study was conducted in mice, further translational research is required to determine whether similar mechanisms occur in humans.

In conclusion, the current findings demonstrate that NEXITOP effectively counteracts HFD-induced obesity by enhancing incretin-mediated signaling and inducing a metabolic shift in adipose tissues toward oxidative and thermogenic activity. The simultaneous induction of UCP1, PGC-1α, and ACOX1 in both white and brown adipose tissues represents a comprehensive activation of mitochondrial and peroxisomal lipid oxidation pathways. This dual modulation combining incretin enhancement with adipose metabolic remodeling offers a promising therapeutic avenue for obesity and related metabolic disorders.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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