A novel anti-obesity mechanism for liraglutide by improving adipose tissue leptin resistance in high-fat diet-fed obese mice


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Abstract. Liraglutide has been approved for the treatment of obesity in the past few years. Both oxidative stress and leptin resistance are the critical drivers of obesity. The present study investigated the mechanism of liraglutide protection against obesity by ameliorating leptin resistance and oxidative stress. Male C57BL/6J mice were fed a high-fat diet (HFD) and subcutaneously injected with 200 μg/kg/d liraglutide for 20 weeks. Body weight, fat mass, serum levels of leptin, insulin, and superoxide dismutase (SOD) activities were measured. In addition, glucose and insulin tolerance tests were performed. The expressions of leptin, its signaling genes, and antioxidant enzymes were detected using RT-qPCR and western blot methods in liver and white adipose tissue (WAT) of mice. The results depicted that liraglutide treatment significantly slowed weight gain of body, reduced the fat mass, ameliorated glucose and lipid metabolism, and hepatic steatosis in HFD-fed obese mice. Further study demonstrated that liraglutide treatment resulted in decreased serum levels and the transcript levels of leptin as well as leptin signaling inhibitory regulators. However, it increased leptin receptor expression and the phosphorylation of signal transducer and activator of transcription 3 (p-STAT3) in WAT (p < 0.05). In addition, the antioxidant enzyme expression was elevated in both liver and WAT of liraglutide-treated mice (p < 0.05). In conclusion, liraglutide conspicuously prevented obesity and ameliorated glucose and lipid metabolism in obese mice through a novel mechanism that improves peripheral leptin resistance in WAT and enhance the antioxidant enzyme expression in both liver and WAT.

Key words: Liraglutide, Anti-obesity, Leptin resistance, Signal transducer and activator of transcription 3 (STAT3), Antioxidant enzymes

WITH THE INCREASED PREVALENCE in the past 50 years, obesity has currently become a global health challenge [1]. It confers significant risks in type 2 diabetes (T2DM), cardiovascular disease, non-alcoholic fatty liver disease (NAFLD), and cancer, leading to a considerable decline in the quality of life and life expectancy [2, 3].

Leptin is an adipokine secreted by white adipose tissue (WAT) [4]. It can inhibit appetite, promote energy consumption and heat production, reducing body weight [5, 6]. However, it was observed that serum leptin levels were increased in most obese patients, which was defined as hyperleptinemia [7, 8]. Excessive leptin would lose its normal physical functions like appetite suppression and weight loss, which was described as leptin resistance, leading to the occurrence and development of obesity [9, 10]. Leptin resistance could occur in both central and peripheral tissues; the latter included adipose tissue, liver, skeletal muscle, among others. Furthermore, peripheral leptin resistance could impair the homeostasis of lipid and glucose metabolism [9]. Therefore, to prevent the development of obesity, it was imperative to explore novel therapeutic strategies for ameliorating leptin resistance.

Oxidative stress was defined as an imbalance between oxidative and anti-oxidative systems, causing an impairment of redox signaling [11, 12], which was closely related to obesity and its associated disorders [13]. Both obese patients and mice possessed lower antioxidant capacity than normal control [14, 15]. Besides, the expression of antioxidant enzymes in adipose tissue was also reduced in obese mice, leading to adipose tissue dysfunction and obesity development [16]. However,
antioxidant supplementation could restore the redox balance within obese and diabetic subjects, alleviating the glucose and lipid metabolism [17, 18]. Consistently, elevated expression of antioxidant enzymes could relieve oxidative stress and adipose tissue dysfunction. Therefore, improving antioxidant capability could be a suitable strategy to prevent against obesity.

Liraglutide was a long-acting glucagon-like peptide-1 receptor agonist (GLP-1RA) approved by the United States Food and Drug Administration to treat obesity in 2014 [19]. The mainly anti-obesity mechanisms of liraglutide were considered to be associated with appetite suppression, energy intake reduction, delay in gastric emptying, and intestinal peristalsis inhibition [20-23]. Interestingly, liraglutide was revealed to improve palmitate-induced endothelial dysfunction by improving leptin resistance in endothelial cells of the human umbilical vein [24]. Moreover, liraglutide ameliorated glucose and lipid metabolism, liver lesions, and insulin resistance by restoring the redox balance in the T2DM/NAFLD rats [25]. However, no study explored the relation of the anti-obesity effects of liraglutide in improving leptin resistance and restoring redox balance in WAT and liver. Therefore, in the present study, we firstly investigated the effects of liraglutide on preventing obesity and its association with the improvement of leptin resistance and oxidative stress in the liver and adipose tissue.

Materials and Methods

Animal experiments

Male C57BL/6J mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The mice were housed in a 12 h dark/ light cycle environment at the age of seven weeks and had free access to food and water. After one week of acclimation, mice were randomly placed on a standard food (SF; 10% kcal fat, n = 10; H10010, Beijing HFK Bioscience Co., Ltd., Beijing, China) or a HFD (45% kcal fat, n = 20; H10045, Beijing HFK Bioscience Co., Ltd., Beijing, China), and maintained on the same diet for 20 weeks. The mice fed with HFD were randomly divided into the HFD and the HFD-Liraglutide groups. Then, the mice in the HFD-Liraglutide group were subcutaneously injected with 200 μg/kg/d liraglutide (15676, Novo Nordisk, Denmark) for 20 weeks, while the SF group and the HFD group were intraperitoneally injected with equal volumes of saline. The body weight of mice was recorded twice a week and food intake was recorded weekly. All the experimental animal procedures were approved by the ethics committee of Peking Union Medical College Hospital (XHDW-2017-001).

Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT)

IPGTT and IPITT were conducted after the liraglutide intervention for 20 weeks. For IPGTT, the mice had fasted overnight, and 50% dextrose (2 g/kg) was administrated intraperitoneally. For IPITT, mice had fasted in the morning for 5 h, and were intraperitoneally injected with insulin (0.72 IU/kg, Novolin R, Novo Nordisk, Denmark). Blood glucose levels were measured from the tail before dextrose or insulin was administered as well as at 30, 60, 90, and 120 min after administration.

Samples collection and assay of biochemical parameters, leptin, superoxide dismutase (SOD) activities, and malondialdehyde (MDA)

The mice were starved overnight and anesthetized two days after IPITT. Blood samples were collected and centrifuged at 3,000 rpm for 10min at 4°C to collect serum. Besides, subcutaneous white adipose tissue (WAT), perirenal WAT, epididymal WAT and liver tissue were also collected and stored at –80°C until further analysis.

Serum biochemical parameters were measured using routine automated laboratory methods. Serum levels of insulin and leptin were measured with corresponding ELISA kits (MEA448Mu, MEA084Mu, Wuhan USCN Business Co., Ltd., Wuhan, China) according to the instruction manual. Malondialdehyde (MDA) levels and Superoxide dismutase (SOD) activities in serum and tissues were detected by the MDA (A003-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and the SOD (A001-3, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) assay kit, respectively. In addition, the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as described in a previous study [26].

Liver histologic analysis and triglyceride contents measurements

A part of liver tissues was fixed in 10% formalin. Paraffin-embedding, sectioning (3 μm) and Hematoxylin-Eosin (H&E) staining were undergone using standard procedures. Histological staining of liver tissues was visualized with a digital camera (Nikon DS-U3, Japan) at 200× magnification (scale bars, 100 μm). Liver triglyceride (TG) contents were measured with a corresponding assay kit (A110-1-1, Nanjing Jincheng Bioengineering Institute, Nanjing, China) as described in our previous studies [27].

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The total RNA for RT-qPCR was extracted from the subcutaneous WAT, epididymal WAT and liver tissue
with the Total RNA Kit II (R6934, Omega Biotek, USA) following the manufacturer’s protocol. Complementary DNA (cDNA) was synthesized with 1 μg of total RNA using the PrimeScript™ RT reagent Kit with the gDNA Eraser (RR047A, TaKaRa, Japan) under the following conditions: 42°C for 2 min, 37°C for 15 min, 85°C for 5 s. The expression of target genes was measured by using the TB Green® Premix Ex Taq™ II (RR820A, TaKaRa, Japan) in the ABI7500 PCR system (Applied Biosystems, San Francisco, CA, USA), including leptin, leptin receptor, protein tyrosine phosphatase1B (PTP1B), suppressor of cytokine signaling 3 (SOCS3), matrix metalloproteinase-2 (MMP2), nuclear factor erythroid 2-related factor 2 (Nrf2), SOD1, glutamate-cysteine ligase catalytic subunit (GCLC) and NAD(P)H dehydrogenase (quinone 1) (Nqo1). In addition, peptidylprolyl isomerase A (PPIA) was used as endogenous control. The relative expression of target genes was calculated by the \(2^{-\Delta\Delta C_{t}}\) method [28]. The primers used in RT-qPCR are listed in the Table S1.

**Total protein extraction and western blot**

Total protein samples from epididymal WAT and subcutaneous WAT were isolated by treating the tissues with RIPA lysis buffer supplemented with phosphatase inhibitor and protease inhibitor cocktail (Applygen, Beijing, China). The BCA Protein Assay Reagent Kit (Beyotime Biotechnology, China) quantified the protein concentrations. Proteins (10 μg) were separated using 4%–12% SDS-PAGE gels, and then transferred onto PVDF membranes (MilliporeImmobilon-PSQ, ISEQ00010, USA). After being blocked in blocking reagent (Beyotime, China), the membranes were incubated overnight at 4°C with primary antibodies, including Rabbit anti-β actin antibody (CST, 4970, USA), anti-signal transducer and activator of transcription 3 (STAT3) antibody (CST, 4904, USA), anti-phospho-STAT3 (Tyr705) (CST, 9145, USA) antibody. Then the membranes were incubated with the corresponding secondary antibodies (CST, 7074, USA) for 1 h at room temperature. The Tanon-5200 Chemiluminescent Imaging System (Tanon Science & Technology, Beijing, China) was used for signal detection. The protein expression levels were quantified by the Image J software (version 1.48, National Institutes of Health, Bethesda, MD, USA).

**Statistical analysis**

All the data were presented as mean ± standard error (SE). The statistical analysis was conducted using the Prism version 8 software (GraphPad, La Jolla, CA) through the one-way ANOVA, two-way ANOVA or the Kruskal-Wallit’s test. \(P\) values less than 0.05 were considered statistically significant.

**Results**

**Administration of liraglutide protected against obesity in HFD-fed mice**

As shown in Fig. 1A, the body weight of mice in the HFD group was continuously higher than the SF group from the 2\(^{nd}\) week to the 20\(^{th}\) week (\(P < 0.05\)). Both body weight gain and body weight gain percentage of the HFD-fed mice were significantly increased to 2.7-folds of the SF-fed mice (Fig. 1B, \(P < 0.05\)), while the WAT mass and WAT percentage of the HFD group also substantially enhanced to 3.8- and 2.9-folds of the SF group (Fig. 1D, \(P < 0.05\)), respectively. However, liraglutide treatment led to a significant lower body weight than the HFD group from 3\(^{rd}\) week to the 20\(^{th}\) week except for the 6\(^{th}\) week (\(P < 0.05\)). Liraglutide also notably slowed the body weight gain and body weight gain percentage, equivalent to 34.3% and 33.2% of the HFD group, respectively (Fig. 1B, \(P < 0.05\)). Similarly, the WAT mass and WAT percentage of liraglutide-treated mice also conspicuously decreased to 39.6% and 50.3% of HFD group, respectively (Fig. 1D, \(P < 0.05\)). In addition, there was a decreasing trend in food intake of HFD-fed mice treated with liraglutide, but there was no statistical significance (Fig. 1C).

**Liraglutide ameliorated glucose and lipid metabolism in HFD-fed mice**

After 20 weeks of HFD feeding, the mice showed abnormal glucose and lipid metabolism. HFD-fed mice displayed slower glucose disposal in IPGTT and a worse responsiveness to insulin in IPITT compared to the SF group, respectively (Fig. 1E, 1F, \(P < 0.05\)). Accordingly, the AUC of IPGTT and IPITT of HFD-fed obese mice significantly increased to 1.3- and 1.4-folds in the SF group, respectively (Fig. 1H, 1I, \(P < 0.05\)). Besides, the serum levels of high-sensitivity C-reactive protein (hsCRP), fasting blood glucose (FBG), HOMA-IR, TC, and low-density lipoprotein cholesterol (LDL-c) were all conspicuously increased as detailed in Fig. 2 (\(P < 0.05\)). However, the treatment of HFD-fed mice with liraglutide significantly improved their glucose and lipid metabolism. Faster disposal of glucose in IPGTT and a greater responsiveness to insulin in IPITT were mainly observed in the liraglutide-treated mice than the HFD-fed control mice (Fig. 1E, 1F, \(P < 0.05\)). Thus, the AUC of IPGTT and IPITT of mice in the HFD-Liraglutide group decreased to 67.2% and 57.2% of the HFD group, respectively (Fig. 1H, 1I, \(P < 0.05\)). Meanwhile, liraglutide administration also decreased serum hsCRP, FBG, HOMA-IR, TC, TG and LDL-c levels in mice to 50.1%, 80.1%, 63.4%, 81.9%, 58.7% and 74.2% of the HFD-fed control mice (Fig. 2, \(P < 0.05\)).
In addition, the serum leptin levels of HFD-fed obese mice were conspicuously elevated to 12.8-fold of mice in the SF group \((p < 0.05)\). However, treatment of HFD-fed mice with liraglutide significantly decreased the serum leptin levels to 28.2% of the HFD group (Fig. 2M, \(p < 0.05)\).

**Liraglutide improved hepatic steatosis and liver function in HFD-fed mice**

As shown in liver histology by the H&E staining, distinct hepatic lobules and normal hepatocytes were observed in livers of mice in the SF group. At the same time, there were destroyed hepatic lobules and disordered hepatocyte with plenty of lipid droplets in livers of mice in the HFD group, indicating hepatic steatosis in the liver (Fig. 3A, 3B). Moreover, the liver TG contents and serum ALT levels of HFD-fed obese mice were elevated to 1.5- and 2.2-folds of the SF group, respectively (Figs. 2A, 3D, \(p < 0.05)\). However, after liraglutide treatment for 20 weeks, the livers of mice depicted restored hepatic lobular and fewer cellular lipid droplets than the HFD-fed control mice, indicating improved hepatic steatosis in the liver (Fig. 3C). Consistently, the liver mass, TG contents and serum ALT levels of liraglutide-treated mice also decreased to 79.4%, 59.3% and 58.2% of the HFD group, respectively (Figs. 1G, 2A, 3D, \(p < 0.05)\).

**Liraglutide mitigated the leptin resistance in adipose tissues of HFD-fed mice**

HFD-fed obese mice showed higher serum leptin levels, while administering liraglutide significantly decreased the leptin levels in HFD-fed mice. To further investigate the reasons for the decrease in serum leptin levels, we observed leptin expression in WAT after liraglutide intervention, owing to leptin mainly secreted by WAT. Moreover, treatment of HFD-fed mice with
Liraglutide decreased the leptin mRNA levels in both subcutaneous and epididymal WAT to 23.3% and 51.3% of HFD-fed mice, respectively (Fig. 4A, 4D, \( p < 0.05 \)).

We analyzed expressions of leptin receptor and leptin cellular signaling regulators in WAT after liraglutide treatment since it caused lower leptin expression in WAT. The results showed that the treatment of HFD-fed mice with liraglutide significantly elevated the leptin receptor mRNA levels in both subcutaneous and epididymal WAT to 7.4- and 2.3-folds of mice in the HFD group, respectively (Fig. 4B, 4E, \( p < 0.05 \)). Meanwhile, the mRNA levels of PTP1B, SOCS3 and MMP2 in epididymal WAT of liraglutide-treated mice were decreased to 60.2%, 35.9% and 33.5% when compared with the HFD control mice, respectively (Fig. 4F, \( p < 0.05 \)). However, no significant change in the mRNA levels of PTP1B, SOCS3 and MMP2 in subcutaneous WAT was observed after liraglutide treatment (Fig. 4C).

In addition, the phosphorylation of STAT3 in subcutaneous WAT and epididymal WAT of HFD-fed obese mice were significantly decreased in comparison with the SF mice, presenting as the ratio of p-STAT3/STAT3 was notably decreased as shown in Fig. 4G–4L (\( p < 0.05 \)). After treatment with liraglutide, the phosphorylation of STAT3 in both subcutaneous and epididymal WAT significantly increased, and the ratio of p-STAT3/STAT3 was significantly increased to 1.5- and 3.7-folds of the control mice, respectively, (Fig. 4J–4L, \( p < 0.05 \)).

**Liraglutide enhanced SOD activities in adipose tissues of HFD-fed mice**

The SOD activities and MDA levels of serum, WAT,
and liver tissue were measured to assess whether liraglutide could improve oxidative stress. In both subcutaneous and epididymal WAT, SOD activities of HFD-fed obese mice were reduced to 50.9% and 68.5% of the SF mice, respectively (Fig. 5B, 5C, \( p < 0.05 \)). After liraglutide treatment, SOD activities in the subcutaneous and epididymal WAT of the HFD-fed mice were increased to 1.9- and 1.3-folds of the HFD group, respectively (Fig. 5B, 5C, \( p < 0.05 \)). In contrary, liraglutide had no significant effect on SOD activities in the serum and liver tissues (Fig. 5A, 5D). However, liraglutide treatment led to increased MDA levels in subcutaneous WAT, which elevated to 2.4-folds of HFD group (Fig. 5F). No significant change was observed in MDA levels in serum, epididymal WAT, and liver after liraglutide treatment (Fig. 5E, 5G, 5H).

**Liraglutide increased the expression of antioxidant enzymes in adipose tissues and liver tissue of HFD-fed mice**

We investigated whether liraglutide treatment could increase the expression of antioxidant enzymes in adipose tissues and liver tissue since liraglutide significantly enhanced SOD activities in adipose tissues of HFD-fed mice. As presented in Fig. 6, liraglutide treatment obviously promoted the expression of Nrf2, SOD1, and GCLC in the subcutaneous WAT to 1.4-, 1.5- and 1.4-folds of the HFD group, respectively (\( p < 0.05 \)). Meanwhile, the mRNA levels of SOD1 and GCLG in the epididymal WAT of liraglutide-treated mice were also increased to 1.6- and 2.0-folds of the HFD group, respectively (Fig. 6F, 6H, \( p < 0.05 \)). Moreover, liraglutide treatment enhanced the mRNA levels of Nqo1 and GCLC to 1.9- and 1.5-folds in the liver tissue of mice when compared with HFD group, respectively (Fig. 6K, 6L, \( p < 0.05 \)).

**Discussion**

Liraglutide is a long-acting GLP-1RA, which has been approved to treat obesity for seven years. The present study is the first to demonstrate that liraglutide prevented HFD-induced obesity by attenuating leptin resistance via lowering leptin expression, increasing leptin receptor expression, enhancing leptin signaling pathway in adipose tissue, and restoring redox balance by increasing the expression of antioxidant enzymes in the liver and adipose tissue.
Leptin resistance could occur in the hypothalamus, which was named central leptin resistance, while it could also happen in the adipose tissue, liver, skeletal muscle, which was called peripheral leptin resistance [9]. Although leptin mainly acted in the central system, peripheral leptin resistance could also impair the lipid and glucose metabolism [9]. For instance, leptin has a directly lipolytic effect by activating hormone-sensitive lipase (HSL) in addition to its impact on appetite inhibition [29, 30]. Frühbeck revealed that the leptin-deficient ob/ob mice were more sensitive to exogenous leptin actin than its lean normal littermates. In addition, the lipolytic effect of leptin in adipocytes from leptin-deficient ob/ob mice was significantly higher than those from lean normal mice [31]. Besides, the lipolytic effect of leptin was dose-dependent in adipocytes from leptin-deficient ob/ob mice, but not in a dose-dependent manner in adipocyte from lean normal mice, indicating an early saturation of leptin response in lean normal mice [31]. More importantly, a supraphysiological dose of leptin would significantly attenuate its lipolytic effect in adipocytes, even if it was from leptin-deficient ob/ob mice, suggesting the occurrence of leptin resistance [31, 32]. Thus, peripheral leptin resistance might increase adipocyte size and number in adipose tissue, leading to excessive fat mass accumulation [9]. Therefore, improving peripheral leptin
resistance in adipose tissue could be a suitable strategy to alleviate obesity. We demonstrated that liraglutide could ameliorate peripheral leptin resistance in WAT of HFD-fed mice through following ways. First, hyperleptinemia was a driver of leptin resistance [6], and partial leptin reduction could make leptin more effective in reducing food intake and lowering body weight [33]. In our current study, liraglutide treatment significantly decreased serum leptin levels and the leptin mRNA level in WAT of HFD-fed mice. Second, reduced leptin receptor expression could also result in leptin resistance [34], and MMP2 activation would cleave the extracellular domain of the leptin receptor, contributing to the reduced leptin receptor and impaired post receptor signal transduction pathway [35]. For the first time, our study demonstrated that liraglutide could significantly increase leptin receptor mRNA levels and decrease MMP2 mRNA levels in WAT of HFD-fed mice. Third, the leptin cellular signaling impairment was also a critical factor in leptin resistance [34]. The JAK2-STAT3 signaling pathway mainly

Fig. 5 Effects of liraglutide on superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels in mice. Mice were treated in the same way as mentioned in Fig. 1. Blood samples were collected and centrifuged for serum collection. Liver tissue, subcutaneous WAT and epididymal WAT were also collected. SOD activities in serum (A), subcutaneous WAT (B), epididymal WAT (C) and liver (D) were measured by SOD assay kit. MDA levels in serum (E) and epididymal WAT (F) were measured by MDA assay kit. SF: standard food group; HFD: high fat diet group; HFD-Lira: high fat diet with liraglutide treatment group. The data were presented as mean ± SE. * p < 0.05 vs. SF group, # p < 0.05 vs. HFD group (n = 10 in each group).
modulated the leptin function. After leptin binds to its receptor, the latter will be phosphorylated by JAK2. Next, STAT3 will be activated by phosphorylation, then p-STAT3 protein translocate into the nucleus, modulating the transcription of downstream signaling molecules [5]. Thus, the decreased expression of p-STAT3 would contribute to leptin resistance [34]. In our study, liraglutide treatment conspicuously elevated the ratio of p-STAT3/STAT3 in both epididymal and subcutaneous WAT of HFD-fed mice. Besides, PTP1B and SOCS3 were reported to be leptin signaling inhibitory regulators [36]. The significantly reduced mRNA levels of PTP1B and SOCS3 were observed in epididymal WAT of liraglutide-treated mice. Therefore, our study firstly elucidated a novel antiobesity mechanism of liraglutide that prevented obesity by ameliorating peripheral leptin resistance in adipose tissue. The process included decreased serum leptin levels and leptin expression, increased leptin receptor expression, enhanced leptin signaling pathway and reduced leptin signaling inhibitory regulators in WAT.

Whether improving leptin resistance played an essential role in the anti-obesity effect of liraglutide since liraglutide could improve glucose and lipid metabolism and attenuate non-alcoholic fatty liver in both ob/ob and db/db mice? In studies by Moreira and Porter, no significant weight loss was observed in liraglutide-treated leptin-deficient ob/ob mice [37, 38]. Similarly, liraglutide did not affect the body weight gain of leptin receptor-deficient db/db mice in Liu’s study [39], while the body weight of liraglutide-treated db/db mice was lower than HFD control group only at 10 and 14 weeks in Wu’s study [40]. Moreover, liraglutide reduced fat mass in the HFD mice compared to ob/ob mice in Moreira’s study [35]. All these results indicated that the weight-loss and fat reduction effect of liraglutide might, if not all, at least partially depend on leptin and its signaling pathway.

Hyperleptinemia was reported to have a critical role in obesity-induced oxidative stress [41]. Leptin could increase the production of H2O2 and radical hydroxyl production while attenuate antioxidant enzyme paraxonase-1, inducing the development of oxidative stress [42, 43]. Both human and animal studies depicted that obese subjects had lower antioxidant capacity [14, 27, 44], while antioxidant supplementation could ameliorate obesity and associated comorbidities [45]. Nrf2 was an important transcription factor regulating the expression of downstream antioxidant enzymes such as SOD1, Nqo1, GCLC, among others [46]. The elevated
expression of antioxidant enzymes in WAT could improve oxidative stress, restoring adipose tissue dys-function [41]. In this study, the administration of liraglutide significantly increased SOD1 activities and mRNA levels of Nrf2 and antioxidant enzymes in WAT of HFD-fed mice. The results could be associated with the effects of liraglutide in protecting against obesity and improving glucose and lipid metabolism. Matic et al. indicated that liraglutide could prevent body weight gain in obese SD rats through increasing the mRNA level of antioxidant enzymes, consistent with our findings [47]. Thus, liraglutide could prevent HFD-fed obesity in mice through increasing expression of antioxidant enzymes via improving peripheral leptin resistance in WAT. However, Yagishita reported that increased Nrf2 signaling inhibited oxidative stress inside hypothalamus of mice and then improved leptin resistance [48]. Therefore, the causal relationship between oxidative stress development and leptin resistance is still not completely clear. Further studies should investigate the causal relationship of liraglutide improving oxidative stress and leptin resistance.

Oxidative stress was also a critical factor in developing NAFLD, while antioxidants were recommended as a novel therapeutic modality for NAFLD [49, 50]. In the present study, liraglutide treatment conspicuously reduced the fat accumulation in the liver and improved the liver function, accompanied by increased antioxidant enzyme expression. The above results indicate that the hepatoprotective effects of liraglutide could also be related to its antioxidant capacity in the liver tissue. Consistently, Xu et al. also showed that liraglutide treatment improved hepatic steatosis and liver function by elevating the antioxidant enzyme SOD [25].

In our study, no statistically significant differences were observed in the food intake of HFD-fed obese mice after liraglutide treatment. Consistently, liraglutide did not affect the food intake of HFD-induced obese mice and ob/ob mice in the studies of Moreira and Zhou [35, 51]. Interestingly, inhibiting appetite was the prominent role of leptin in the central nervous system. Therefore, the above results suggested that the anti-obesity effect of liraglutide might not rely on ameliorating central leptin resistance, at least, in our study. However, more studies were needed to verify further whether liraglutide could improve the central leptin resistance or not.

Another interestingly finding was that the mRNA levels of SOCS3 and PTP1B were elevated in visceral WAT of HFD-fed mice, while they were reduced in subcutaneous WAT compared to the SF-fed mice. Indeed, subcutaneous WAT and visceral WAT play different roles in the occurrence and development of obesity and its associated diseases [52]. Since there are more glucocorticoid and androgen receptors in visceral WAT than the subcutaneous WAT, adipocytes in visceral WAT are more metabolically active and more sensitive to lipolysis than adipocytes in subcutaneous WAT. Thus, they are more likely to cause metabolic abnormalities and insulin resistant in excess nutrition [53]. For insistence, Tagami et al. reported that particulate PTPase (PTP1B is a member of the PTPase family) activities in visceral WAT of insulin-resistant rats were significantly higher than those in non-insulin-resistant control rats. In contrary, a similar phenomenon was not observed in subcutaneous WAT [54]. The above results indicated that the gene expression related to leptin resistance in visceral WAT could differ from those in subcutaneous WAT of HFD-fed obese mice, as demonstrated in our findings.

Our study also had some potential limitations. First, the hypothalamus of HFD-fed mice was not collected in our research. Therefore, it is still unclear whether liraglutide could improve central leptin resistance or not. However, the food intake was not significantly reduced after liraglutide treatment, indicating that the effects of liraglutide in preventing obesity did not possibly rely on the central role of leptin, at least, in the present study. Second, the causal relationship between the liraglutide improving oxidative stress and leptin resistance was unknown. More cellular and knockout animal experiments are needed to clarify this issue.

**Conclusion**

Therefore, liraglutide effectively protected from obesity in HFD-fed mice, which may be related to improved peripheral leptin resistance in adipose tissue and increased antioxidant enzymes levels in adipose and liver tissue.

**Disclosure**

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**Authors’ contributions**

XL wrote the primary manuscript. XL, KY and XG performed the animal experiments. XW and HX performed molecular biology experiments. FG designed the experiment, supervised all of the experiments and revised the primary manuscript. HZ and HP designed...
and supervised the experiments. LW and HY helped to analyzed the data.

Conflict of interest

None of the authors have any potential conflicts of interest associated with this research.

Ethics approval

All the experimental animal procedures were approved by the ethics committee of Peking Union Medical College Hospital.

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