

## Exenatide improves hepatic steatosis by enhancing lipid use in adipose tissue in nondiabetic rats

Kosuke Tanaka, Yuko Masaki, Masatake Tanaka, Masayuki Miyazaki, Munechika Enjoji, Makoto Nakamuta, Masaki Kato, Masatoshi Nomura, Toyoshi Inoguchi, Kazuhiro Kotoh, Ryoichi Takayanagi

Kosuke Tanaka, Yuko Masaki, Masatake Tanaka, Masayuki Miyazaki, Masaki Kato, Masatoshi Nomura, Kazuhiro Kotoh, Ryoichi Takayanagi, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Munechika Enjoji, Department of Clinical Pharmacology, Faculty of Pharmaceutical Science, Fukuoka University, Fukuoka 814-0180, Japan

Makoto Nakamuta, Department of Gastroenterology, Kyushu Medical Center, National Hospital Organization, Fukuoka 810-8563, Japan

Toyoshi Inoguchi, Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka 812-8582, Japan

Author contributions: All the authors contributed equally to this manuscript.

Correspondence to: Masaki Kato, MD, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [mkato11@intmed3.med.kyushu-u.ac.jp](mailto:mkato11@intmed3.med.kyushu-u.ac.jp)

Telephone: +81-92-6425282 Fax: +81-92-6425287

Received: September 28, 2013 Revised: November 12, 2013

Accepted: January 14, 2014

Published online: March 14, 2014

### Abstract

**AIM:** To investigate the metabolic changes in skeletal muscle and/or adipose tissue in glucagon-like peptide-1-induced improvement of nonalcoholic fatty liver disease (NAFLD).

**METHODS:** Male Wistar rats were fed either a control diet (control group) or a high-fat diet (HFD). After 4 wk, the HFD-fed rats were subdivided into two groups; one group was injected with exenatide [HFD-Ex(+) group] and the other with saline [HFD-Ex(-) group] every day for 12 wk. The control group received saline and were fed a control diet. Changes in weight gain, energy intake, and oxygen consumption were analyzed. Glucose tolerance tests were performed after 8

wk of treatment. Histological assessments were performed in liver and adipose tissue. RNA expression levels of lipid metabolism related genes were evaluated in liver, skeletal muscle, and adipose tissue.

**RESULTS:** Exenatide attenuated weight gain [HFD-Ex(-) vs HFD-Ex(+)] and reduced energy intake, which was accompanied by an increase in oxygen consumption and a decrease in the respiratory exchange ratio [HFD-Ex(-) vs HFD-Ex(+)]. However, exenatide did not affect glucose tolerance. Exenatide reduced lipid content in the liver and adipose tissue. Exenatide did not affect the expression of lipid metabolism-related genes in the liver or skeletal muscle. In adipose tissue, exenatide significantly upregulated lipolytic genes, including hormone-sensitive lipase, carnitine palmitoyltransferase-1, long-chain acyl-CoA dehydrogenase, and acyl-CoA oxidase 1 [HFD-Ex(-) vs HFD-Ex(+)]. Exenatide also upregulated catalase and superoxide dismutase 2 [HFD-Ex(-) vs HFD-Ex(+)].

**CONCLUSION:** In addition to reducing appetite, enhanced lipid use by exenatide in adipose tissue may reduce hepatic lipid content in NAFLD, most likely by decreasing lipid influx into the liver.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

**Key words:** Adipose tissue; Energy expenditure; Exenatide; Glucagon-like peptide-1; Hepatic steatosis; Lipolysis; Nonalcoholic fatty liver disease

**Core tip:** Glucagon-like peptide-1 (GLP-1) is reported to improve nonalcoholic fatty liver disease (NAFLD), mainly *via* direct action on the liver. However, organs other than the liver may also be involved in regulation of hepatic lipid contents. In this study, we found significant upregulation of lipolytic genes in adipose tissue in exenatide-treated NAFLD rats. Up-regulation

of catalase, superoxide dismutase and mitochondrial morphological regulators was observed in adipose tissue. These metabolic changes were accompanied by increased oxygen consumption and decreased respiratory exchange ratio. Taken together, enhanced lipid use by GLP-1 in adipose tissue may play an important role in the improvement of NAFLD.

Tanaka K, Masaki Y, Tanaka M, Miyazaki M, Enjoji M, Nakamura M, Kato M, Nomura M, Inoguchi T, Kotoh K, Takayanagi R. Exenatide improves hepatic steatosis by enhancing lipid use in adipose tissue in nondiabetic rats. *World J Gastroenterol* 2014; 20(10): 2653-2663 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i10/2653.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i10.2653>

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is considered a hepatic manifestation of metabolic syndrome. The significant increase in the prevalence of NAFLD in the general population indicates that NAFLD is a burgeoning problem<sup>[1]</sup>. NAFLD is a chronic liver disease that is characterized by steatosis that is histologically similar to that in alcoholic liver injury, without excessive alcoholic intake or hepatitis viral infection<sup>[2,3]</sup>. Nonalcoholic steatohepatitis, a severe stage of NAFLD, frequently progresses into liver cirrhosis and hepatocellular carcinoma<sup>[4-6]</sup>. Body weight reduction and control of complicated diabetes are essential to improve NAFLD<sup>[7,8]</sup>. However, the attempts to restrict food intake and increase physical exercise are often insufficient to treat NAFLD<sup>[9]</sup>.

Glucagon-like peptide-1 (GLP-1), an incretin hormone produced by intestinal L cells, is an effective therapeutic agent for type 2 diabetes mellitus<sup>[10,11]</sup>. GLP-1 regulates plasma glucose levels by promoting insulin secretion and inhibiting glucagon secretion in a glucose-dependent manner<sup>[12,13]</sup>. Exenatide is a GLP-1 receptor agonist, sharing 53% sequence homology with GLP-1<sup>[14,15]</sup>. Exenatide has a longer half-life and enhanced potency compared with GLP-1 because it is less susceptible to degradation by dipeptidyl peptidase-4<sup>[16]</sup>.

GLP-1 may also be able to treat obesity by controlling gastrointestinal motility, which may suppress appetite and promote satiety<sup>[17,18]</sup>. GLP-1 was also reported to reduce hepatic steatosis in animal models of NAFLD<sup>[19-22]</sup>. Although the mechanism underlying this effect of GLP-1 is not completely understood, earlier studies suggested that GLP-1 had direct effects on the liver by improving hepatic insulin sensitivity<sup>[19,20]</sup> and enhancing lipid hydrolysis and oxidation<sup>[21-23]</sup>. Because the GLP-1 receptor is expressed in many organs, including the brain, heart, kidney, stomach, liver, muscle, and adipose tissue<sup>[12,24]</sup>, GLP-1 may reduce hepatic lipid accumulation *via* extrahepatic pathways. In particular, skeletal muscle and adipose tissue are potential targets for GLP-1. Fatty acid influx into the liver is affected by the extent of fatty

acid oxidation in skeletal muscle, as well as triglyceride storage and hydrolysis in adipose tissue. Therefore, changes in lipid metabolic activities in these tissues should reduce hepatic lipid content.

We hypothesized that GLP-1 would affect lipid metabolism in skeletal muscle and/or adipose tissue, leading to the reduction of lipid influx into the liver, resulting in the suppression of hepatic lipid accumulation. In the present study, we show that exenatide enhanced triglyceride hydrolysis and fatty acid oxidation in adipose tissue during the improvement of hepatic steatosis in a high-fat diet (HFD)-induced rat model of NAFLD. Additionally, upregulation of mitochondrial morphologic regulators was observed in adipose tissue. Exenatide increased the systemic energy expenditure and decreased the respiratory exchange ratio (RER). Collectively, the enhancing effects of exenatide (and hence GLP-1) on lipid use in adipose tissue may play a role in the improvement of hepatic steatosis in NAFLD.

## MATERIALS AND METHODS

### Animals

Four-week-old male Wistar rats weighing 80 g were purchased from Japan SLC (Hamamatsu, Japan). Rats were maintained under standard conditions with a 12-h light/dark cycle. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and were approved by the Animal Care Committee of Kyushu University. The rats were divided into two groups and fed a control diet ( $n = 8$ ; control group) or an HFD diet ( $n = 16$ ). The control diet (3.73 kcal/g) comprised 20.8% protein, 4.8% fat, and 58.2% carbohydrate. The HFD (5.06 kcal/g) comprised 18.2% protein, 62.2% fat, and 19.6% carbohydrate. Following 4 wk of feeding, HFD-fed rats were subdivided into two groups ( $n = 8$  per group) and intraperitoneally injected with either 10  $\mu$ g/kg body weight exenatide [Eli Lilly, Indianapolis, IN, United States; HFD-Ex(+) group] or saline [HFD-Ex(-) group] every day for 12 wk. Rats in the control group were injected with saline and fed a control diet. Body weight was measured every 4 wk. Starting from week 6 of feeding (week 2 of exenatide/saline injection), daily food consumption in each cage (2 rats) was measured every 2 wk for 6 wk and averaged levels of energy intakes were calculated. At week 16 of feeding (week 12 of injections), rats were sacrificed after an overnight fast, and the liver, gastrocnemius muscle, and epididymal white adipose tissues were removed.

### Indirect calorimetry

At week 12 of feeding (week 8 of injections), oxygen consumption ( $VO_2$ , mL/kg/h) and RER were determined in the HFD-Ex(-) group and HFD-Ex(+) group ( $n = 4$  per group) using an Oxymax indirect calorimeter (Columbus Instrument, Columbus, OH, United States). After 3 d of acclimation,  $VO_2$  and RER were measured

Table 1 Primer sequences

Gene	Forward	Reverse
<i>SREBP1c</i>	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAGGA
<i>FAS</i>	CTAGGTGGCTTTGGCCTGGA	CGAACGTGCTTGGCTTGGTA
<i>ACC1</i>	GTTCTGTGGACAACGCCCTCA	GTCGAGAAGCAGCCCATTAC
<i>LPL</i>	GTGACCAGGGACATGTGACTTTG	CTGTACTTCGTGTGGTGGGACTA
<i>HSL</i>	TTGCCTACTGCTGGGCTGTC	GACACGGTGATGCAGAGGTTTC
<i>ApoB</i>	TAGCATGCTTGCTGACATAAATGGA	ATGGAGCTGCCGAGGTAATC
<i>CPT-1</i>	CTGCCAGTTCATTAAGCCACA	CAGCTATGCAGCCTTTGACTACCA
<i>LCAD</i>	AAGGCCTGCTTGGCATCAAC	CAGGGCCTGTGCAATTTGAGTA
<i>ACOX1</i>	GGCCGCTATGATGAAATGTG	GGGCTTCAAGTCTTGTGGTAA
<i>Catalase</i>	GAACATTGCCAACACCTGAAAG	GTAGTCAGGGTGGACGTCAGTGAA
<i>SOD2</i>	GACTAGGCCACAGGGCATTCA	ACTCAGAAACCCGTTTGCTCTAC
<i>TNF</i>	TGGCCAGACCCTCACACTC	CTCCTGGTATGAAGTGGCAAATC
<i>MCP1</i>	TCACCAGCAGCAGGTGTCCAAAGA	ACAGAAGTGTGAGGTGGTGTGG
<i>Mfn1</i>	CCTGTACATCGATTCCTGGGTTT	CCTGGGCTGCATTTATCTGGTG
<i>Mfn2</i>	TCAGCCGAGTACACCTACAGAGA	TGAGGGCCAAATGCAAGACA
<i>Opa1</i>	ATGCTCGCTATCACTGCCAAC	CGTTTGCCAGTAAGCAATTTAACC
<i>Dnm1</i>	ATGCCCTGTGGGCTAATGAACAA	GTCTCCGATACAGCGGAAG
<i>GAPDH</i>	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA

SREBP1c: Sterol regulatory element-binding protein-1; FAS: Fatty acid synthase; ACC1: Acetyl-CoA carboxylase 1; LPL: Lipoprotein lipase; HSL: Hormone-sensitive lipase; ApoB: Apolipoprotein B; CPT1: Carnitine palmitoyltransferase-1; LCAD: Long-chain acyl-CoA dehydrogenase; ACOX1: Acyl-CoA oxidase-1; SOD2: Superoxide dismutase 2; TNF: Tumor necrosis factor; MCP1: Monocyte chemoattractant protein-1; Mfn1: Mitofusin 1; Mfn2: Mitofusin 2; Opa1: Optic atrophy-1; Dnm1: Dynamin-1; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

every 4 min for 24 h. The rats were kept in a stable environment with a temperature of 25 °C, 12-h light/dark cycle, and airflow of 2 L/min. RER was calculated as the ratio of the volume of CO<sub>2</sub> produced to O<sub>2</sub> consumed.

### Histological analysis

The liver and epididymal adipose tissue samples were fixed in 10% formalin and embedded in paraffin. Serial sections (5 μm thick) were cut from each block. Histological features were evaluated after staining sections with hematoxylin and eosin. The numbers of lipid droplets in liver tissue sections and the diameters of adipocytes in adipose tissue sections were determined using BIOREVO BZ9000 and BZ II (Keyence, Osaka, Japan). The numbers of hepatic lipid droplets per unit area (/mm<sup>2</sup>) and the diameters in 100 adipocytes were evaluated in five animals from each group.

### Reverse transcription-polymerase chain reaction

Total RNA was prepared from all tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, United States) and cDNA was synthesized from 1.0 μg of RNA by GeneAmp RNA polymerase chain reaction (PCR) (Applied Biosystems, Hammoncton, NJ, United States) with random hexamers. Real-time PCR was performed using LightCycler FastStart DNA Master SYBR Green I (Roche, Basel, Switzerland). The reaction mixture (20 μL) contained Master SYBR Green I, 4 mmol/L MgCl<sub>2</sub>, 0.5 μmol/L of the upstream and downstream PCR primers, and 2 μL of first-strand cDNA as a template. To control for variations in reactions, all PCR data were normalized against glyceraldehyde 3-phosphate dehydrogenase expression. The primer sequences used in this study are listed in Table 1.

### Glucose tolerance test

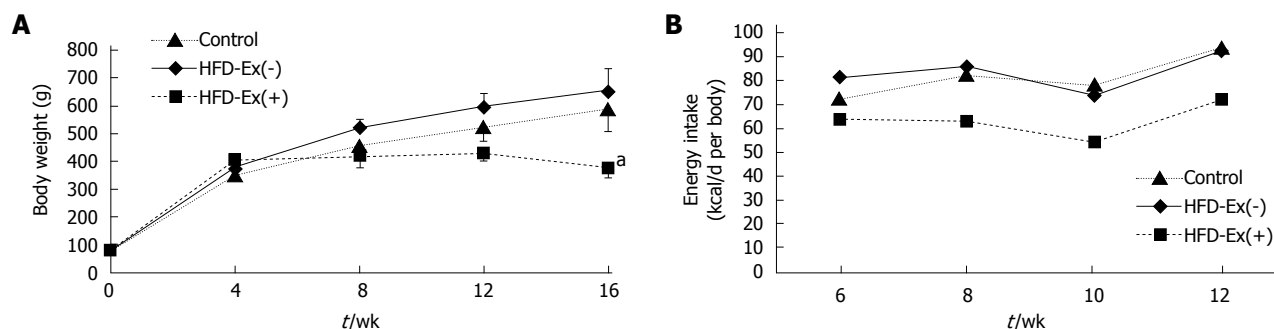
At week 12 of feeding (week 8 of injections), intraperitoneal glucose tolerance tests (IPGTTs) were performed in the HFD-Ex(-) group and HFD-Ex(+) group. After a 14-h fast, the rats were injected with glucose solution (2 g/kg body weight) and serum glucose levels were measured before (0 min) and at 15, 30, 60, 90, and 120 min after glucose injection using a portable glucometer (Lifescan, Bucks, United Kingdom).

### Immunoblotting

Adipose tissue samples (250 mg) were homogenized in 1 mL of lysis buffer (25 mmol/L Tris-HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate). The lysates were centrifuged at 8050 g for 20 min. The upper lipid phase was discarded and the lower aqueous phase was re-centrifuged under the same conditions. The supernatant was collected and loaded onto Mini-Protean TGX gels (Bio-Rad, Hercules, CA, United States) and transferred onto polyvinylidene difluoride membranes. After blocking the membranes with 5% albumin, immunoblotting analyses were performed using antibodies raised against AMP-activated protein kinase (AMPK), phosphorylated AMPK (P-AMPK; Cell Signaling Technology, Beverly, MA, United States), and β-actin (Santa Cruz Biotechnology, Dallas, TX, United States).

### Statistical analysis

All results are expressed as the means ± SD. Statistical analyses were performed using JMP v. 8.01 (SAS Institute, Cary, NC, United States). The differences of means were tested by Tukey-Kramer test (among 3 groups) or Kruskal-Wallis test (between 2 groups) to identify signifi-



**Figure 1** Changes in body weight and energy intake. A: Time-course of body weight. Four-week-old rats were fed a control diet (control group;  $n = 8$ ) or a high-fat diet (HFD) ( $n = 16$ ). After 4 wk of feeding, HFD-fed rats were subdivided into two groups ( $n = 8$  per group) and intraperitoneally injected with either 10  $\mu\text{g}/\text{kg}$  body weight exenatide [HFD-Ex(+) group] or saline [HFD-Ex(-) group] every day for 12 wk. Rats in the control group were injected with saline. At week 16 of feeding, the body weight was significantly lower in the HFD-Ex(+) group than in the control and HFD-Ex(-) groups.  $n = 8$ . <sup>a</sup> $P < 0.05$  vs other groups; B: Daily energy intake during the treatment period. Energy intake was lower in the HFD-Ex(+) group than in the control or HFD-Ex(-) group.

cance. Values of  $P < 0.05$  were considered statistically significant.

## RESULTS

### Exenatide attenuated weight gain and increased oxygen consumption

No apparent differences in weight gain were observed among the three groups after 4 wk of feeding the experimental diets. After this time, body weight gain was generally suppressed in rats treated with exenatide, whereas the body weight of rats in the control and HFD-Ex(-) groups continued to increase. At 16 wk, the body weight of rats in the HFD-Ex(+) group was significantly lower than that of the control and HFD-Ex(-) group ( $376 \pm 36$  g vs  $587 \pm 27$  g and  $376 \pm 36$  g vs  $655 \pm 81$  g, respectively,  $P < 0.05$ ) (Figure 1A). To assess the effects of exenatide on food intake, we measured energy intake (kcal/day per body) between weeks 6 and 12 of the feeding protocol. Energy intake was lower in the HFD-Ex(+) group than in the other groups throughout this time (Figure 1B). Using indirect calorimetry, we determined systemic energy consumption and RER in the HFD-Ex(-) and HFD-Ex(+) groups at week 12 of feeding. Indirect calorimetry revealed that oxygen consumption was significantly greater in the HFD-Ex(+) group than in the HFD-Ex(-) group ( $1269 \pm 67$  mL/kg/h vs  $1114 \pm 97$  mL/kg/h,  $P < 0.05$ ), and this increase was predominant during the dark cycle (Figure 2A). RER was significantly lower in the HFD-Ex(+) group than in the HFD-Ex(-) group ( $0.748 \pm 0.02$  vs  $0.791 \pm 0.01$ ,  $P < 0.05$ ) (Figure 2B). These findings indicate that exenatide enhanced systemic energy consumption by increasing lipid oxidation.

### Exenatide reduced lipid accumulation in the liver and adipose tissue

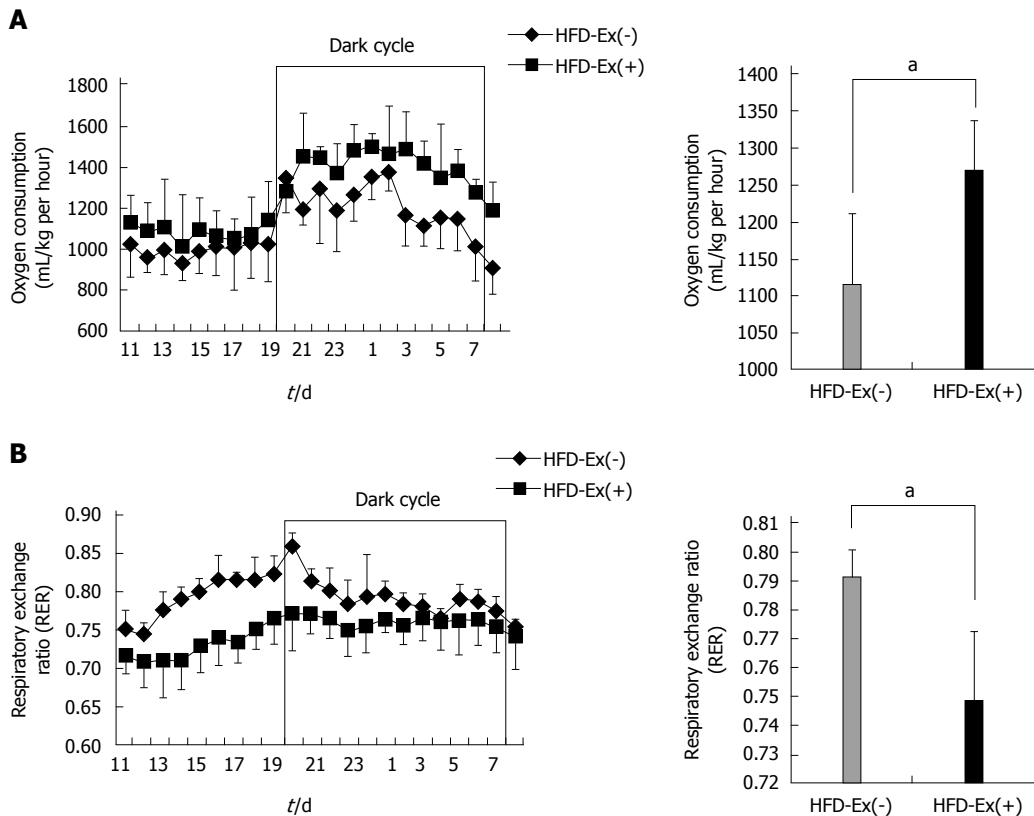
Lipid accumulation in the liver and epididymal white adipose tissue was histologically analyzed at week 16 of feeding. Although marked accumulation of lipid droplets was observed in the livers of the HFD-Ex(-) group, the number of hepatic lipid droplets was significantly

decreased in the HFD-Ex(+) group compared with the HFD-Ex(-) group (Figure 3A, C). In adipose tissue, the adipocytes were frequently enlarged in the HFD-Ex(-) group, reflecting lipid accumulation. However, enlarged adipocytes were not observed in the HFD-Ex(+) or control groups (Figure 3B). Furthermore, the mean diameter of adipocytes in the HFD-Ex(+) group was similar to that in the control group and was significantly smaller in both groups than in the HFD-Ex(-) group (Figure 3D).

### Effects of exenatide on the expressions of genes involved in lipid metabolism in the liver and skeletal muscle

Because exenatide increased oxygen consumption, decreased RER, and decreased lipid accumulation in the liver and adipose tissue, we hypothesized that exenatide altered lipid metabolic activities, including triglyceride hydrolysis and lipid oxidation. To confirm this hypothesis, we determined the expression of lipid metabolism-associated genes in the liver, skeletal muscle, and adipose tissue. In liver, we found that the expression levels of sterol regulatory element-binding protein-1c (SREBP1c), fatty acid synthase (FAS), acetyl-CoA carboxylase-1 (ACC1), hormone-sensitive lipase (HSL), and apolipoprotein B (ApoB) were not significantly different among the three groups [HFD-Ex(-) vs HFD-Ex(+);  $2.20 \pm 1.29$  vs  $0.91 \pm 0.57$ ,  $1.37 \pm 0.51$  vs  $1.15 \pm 0.68$ ,  $1.03 \pm 0.29$  vs  $1.03 \pm 0.29$ ,  $1.61 \pm 0.69$  vs  $1.27 \pm 0.21$ , and  $0.99 \pm 0.29$  vs  $0.89 \pm 0.15$ , respectively,  $P > 0.05$ ] (Figure 4A). Additionally, exenatide did not affect the expression of carnitine palmitoyltransferase-1 (CPT1), long-chain acyl-CoA dehydrogenase (LCAD), or acyl-CoA oxidase 1 (ACOX1) [HFD-Ex(-) vs HFD-Ex(+);  $1.06 \pm 0.50$  vs  $0.95 \pm 0.39$ ,  $1.11 \pm 0.41$  vs  $0.83 \pm 0.21$ , and  $0.85 \pm 0.14$  vs  $0.74 \pm 0.25$ , respectively,  $P > 0.05$ ]. In skeletal muscle, the expression of lipoprotein lipase (LPL) was significantly increased in the HFD-Ex(+) group compared with the control group [control vs HFD-Ex(+);  $1 \pm 0.60$  vs  $5.48 \pm 4.47$ ,  $P < 0.05$ ] but not compared with the HFD-Ex(-) group [HFD-Ex(-) vs HFD-Ex(+);  $3.24 \pm 2.19$  vs  $5.48 \pm 4.47$ ,  $P > 0.05$ ]. As in the liver, exenatide did not affect the





**Figure 2** Oxygen consumption and respiratory exchange ratio evaluated by indirect calorimetry in the high-fat diet-Ex(+) and high-fat diet-Ex(-) groups at week 12 of feeding. A: Oxygen consumption was significantly greater in the high-fat diet (HFD)-Ex(+) group than in the HFD-Ex(-) group, particularly during the dark cycle; B: Respiratory exchange ratio (RER) was significantly lower in the HFD-Ex(+) group than in the HFD-Ex(-) group.  $n = 4$ . <sup>a</sup> $P < 0.05$  between groups.

expression of HSL, CPT1, LCAD, or ACOX1 in skeletal muscle [HFD-Ex(-) *vs* HFD-Ex(+);  $1.44 \pm 0.65$  *vs*  $1.87 \pm 0.88$ ,  $1.26 \pm 0.51$  *vs*  $1.53 \pm 0.63$ ,  $2.15 \pm 0.78$  *vs*  $1.88 \pm 0.77$ , and  $1.40 \pm 0.33$  *vs*  $1.32 \pm 0.61$ , respectively,  $P > 0.05$ ] (Figure 4B). These results imply that exenatide improves hepatic steatosis without affecting lipid metabolism in the liver or skeletal muscle, except for a potential increase in triglyceride hydrolysis in skeletal muscle.

#### Exenatide upregulated genes involved in triglyceride hydrolysis and fatty acid oxidation in adipose tissue

In adipose tissue, exenatide significantly increased the expression of HSL in the HFD-Ex(+) group compared with the control and HFD-Ex(-) groups [HFD-Ex(-) *vs* HFD-Ex(+);  $0.98 \pm 0.37$  *vs*  $1.61 \pm 0.42$ ,  $P < 0.05$ ] (Figure 5A). The expression of LPL was also increased in the HFD-Ex(+) group, albeit not significantly. In terms of genes involved in mitochondrial  $\beta$  oxidation of fatty acids, the expression levels of CPT1, LCAD, and ACOX1 were significantly increased in the HFD-Ex(+) group compared with the control and HFD-Ex(-) groups [HFD-Ex(-) *vs* HFD-Ex(+);  $1.04 \pm 0.27$  *vs*  $1.88 \pm 0.97$ ,  $1.26 \pm 0.23$  *vs*  $2.52 \pm 1.00$ , and  $1.58 \pm 0.45$  *vs*  $2.41 \pm 0.85$ , respectively,  $P < 0.05$ ], suggesting that exenatide enhanced lipid oxidation in adipose tissue.

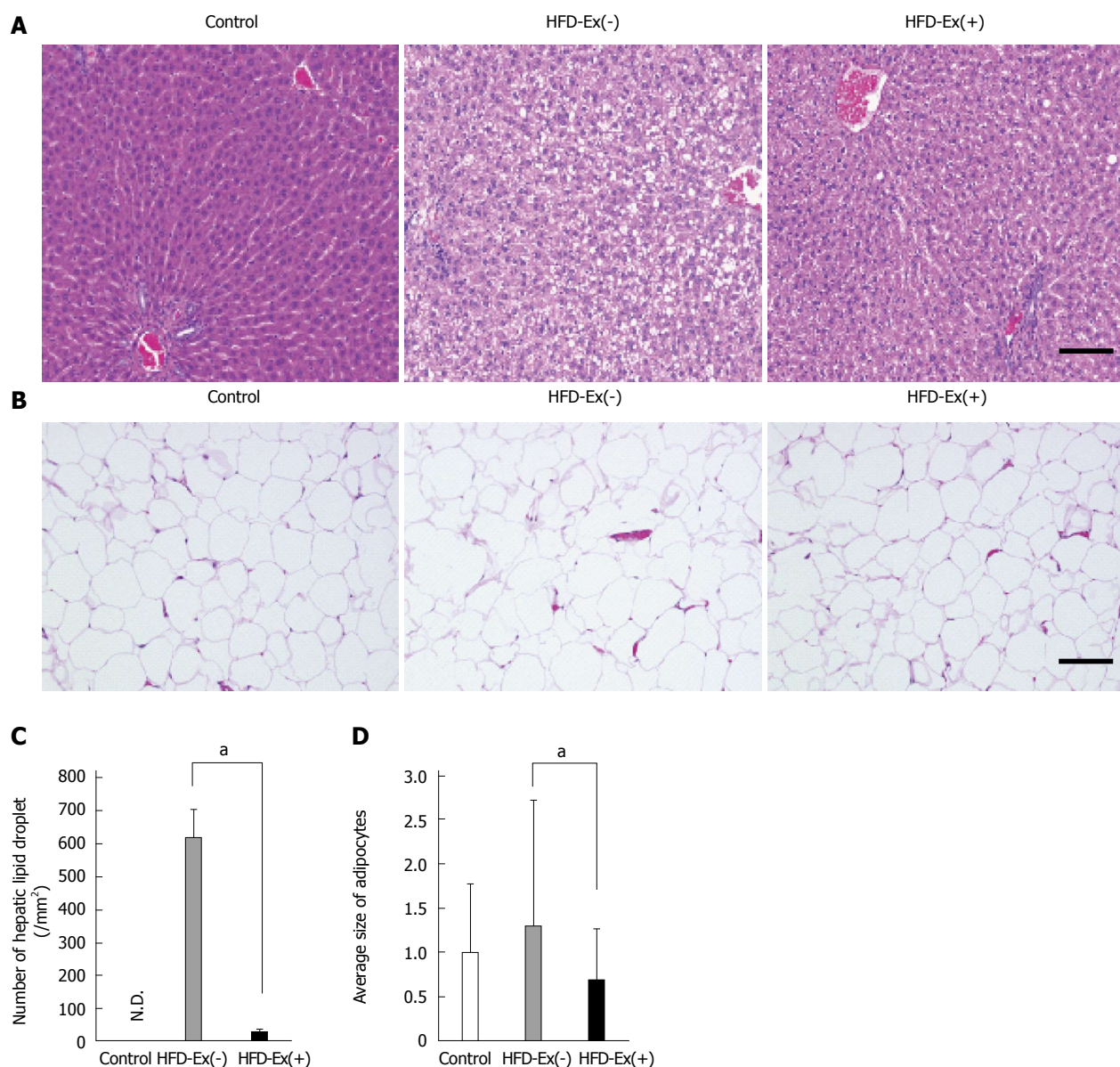
Enhanced lipid oxidation results in the accumulation of intracellular reactive oxygen species (ROS), which induces the cellular response to eliminate this harmful

by-product<sup>[25-27]</sup>. Therefore, we determined the adipose tissue expression levels of catalase and superoxide dismutase (SOD)2 and found that they were significantly greater in the HFD-Ex(+) group than in the control and HFD-Ex(-) groups [HFD-Ex(-) *vs* HFD-Ex(+);  $1.12 \pm 0.29$  *vs*  $2.37 \pm 0.66$  and  $0.99 \pm 0.21$  *vs*  $1.49 \pm 0.23$ , respectively,  $P < 0.05$ ] (Figure 5B).

Because macrophage infiltration into adipose tissue plays an important role in the development of insulin resistance<sup>[28-30]</sup>, we determined the expression levels of tumor necrosis factor and monocyte chemoattractant protein 1. However, the expression levels of these genes were not significantly different among the three groups [HFD-Ex(-) *vs* HFD-Ex(+);  $1.01 \pm 0.40$  *vs*  $1.27 \pm 0.30$  and  $0.90 \pm 0.41$  *vs*  $0.76 \pm 0.49$ , respectively,  $P > 0.05$ ], which suggests that the metabolic changes in adipose tissues induced by exenatide did not involve macrophage activation (Figure 5B).

#### Effects of exenatide on mitochondrial morphologic regulators in adipose tissue

In response to changes in the nutritional environment, mitochondria can change their morphology through two coordinated processes, fusion and fission, which are transcriptionally regulated by a group of genes<sup>[31,32]</sup>. In this group, mitofusin-1 (Mfn1) and mitofusin-2 (Mfn2) regulate mitochondrial fusion of the outer membrane and are believed to play a role in intracellular lipid con-



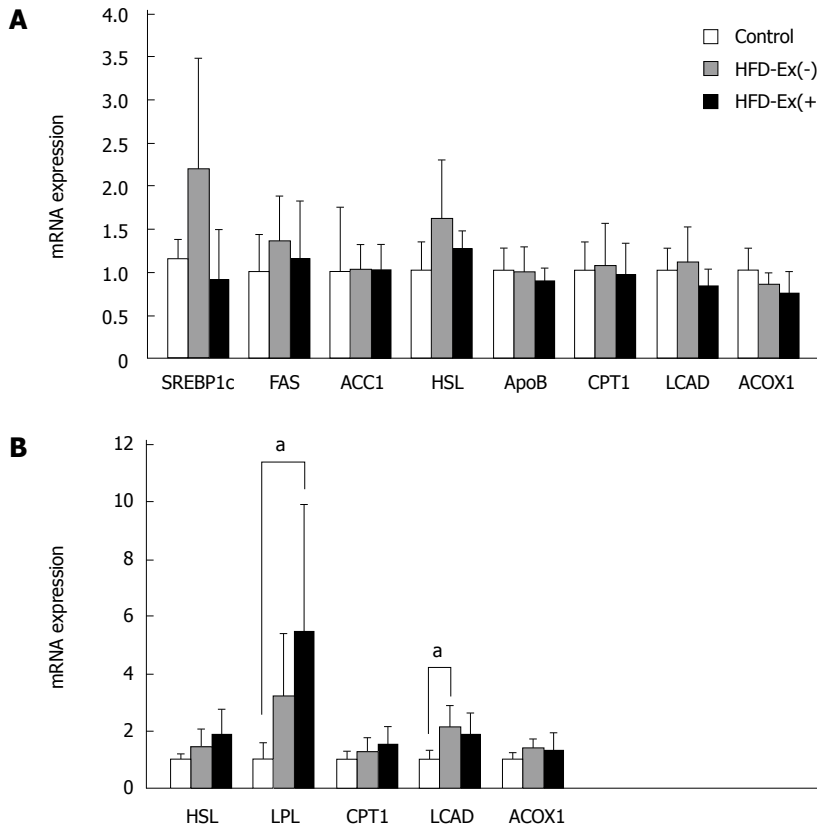
**Figure 3** Histological evaluation of lipid accumulation in the liver and adipose tissue. A: Numerous hepatocytes containing lipid droplets were observed in the high-fat diet (HFD)-Ex(-) group, whereas scant lipid-containing hepatocytes were found in the HFD-Ex(+) group; B: In epididymal white adipose tissue, there were abundant enlarged adipocytes in the HFD-Ex(-) group but not in the HFD-Ex(+) group; C: The number of hepatic lipid droplets was significantly decreased in the HFD-Ex(+) group compared with the HFD-Ex(-) group; D: The mean diameter of adipocytes in the HFD-Ex(+) group was significantly smaller than that in the HFD-Ex(-) group and was similar to that in the control group. The fold changes were calculated as the ratio of the average size of adipocytes in the HFD-Ex(+) or HFD-Ex(-) group to that in the control group.  $n = 5$ , <sup>a</sup> $P < 0.05$  between groups. Scale bar = 100  $\mu\text{m}$ . ND: Not detected.

sumption<sup>[33]</sup>. In addition, optic atrophy-1 (Opa1) regulates the fusion of the inner membrane while dynamin-1 (Dnm1) regulates mitochondrial fission and is involved in intracellular lipid accumulation<sup>[33]</sup>. Therefore, to determine whether the induction of lipid oxidation in adipose tissue is accompanied by changes in mitochondrial morphologic regulation, we determined the expression levels of these regulators. Notably, the expression levels of Mfn1, Mfn2, and Opa1 were significantly greater in the HFD-Ex(+) group than in the control and HFD-Ex(-) group [HFD-Ex(-) *vs* HFD-Ex(+);  $1.13 \pm 0.17$  *vs*  $2.08 \pm 0.40$ ,  $0.99 \pm 0.28$  *vs*  $1.76 \pm 0.50$ , and  $1.08 \pm 0.19$  *vs*  $1.76 \pm 0.30$ , respectively,  $P < 0.05$ ] (Figure 6). Additionally, the expression of Dnm1 was significantly greater in the

HFD-Ex(+) group than in the control group [control *vs* HFD-Ex(+);  $1 \pm 0.50$  *vs*  $1.58 \pm 0.27$ ,  $P < 0.05$ ], but was not significantly greater than that in the HFD-Ex(-) group [HFD-Ex(-) *vs* HFD-Ex(+);  $1.28 \pm 0.23$  *vs*  $1.58 \pm 0.27$ ,  $P > 0.05$ ]. These findings indicate that exenatide not only induces lipid consumption or accumulation, but also that it might regulate mitochondrial reorganization of adipose tissue, most likely reflecting increased use of intracellular lipid.

#### Exenatide had limited effects on glucose tolerance

This NAFLD model was based on nondiabetic, wild-type rats to minimize the effects of exenatide on diabetes control. However, improvement in hepatic lipid accu-



**Figure 4** Effects of glucagon-like peptide-1 on the expression levels of genes associated with lipid metabolism in the liver and skeletal muscle. A: In the liver, there were no significant differences in the expression levels of sterol regulatory element-binding protein-1c (SREBP1c), fatty acid synthase (FAS), acetyl-CoA carboxylase-1 (ACC1), hormone-sensitive lipase (HSL), and apolipoprotein B (ApoB), carnitine palmitoyltransferase-1 (CPT1), long-chain acyl-CoA dehydrogenase (LCAD), or acyl-CoA oxidase 1 (ACOX1) among the three groups; B: In skeletal muscle, the expression of LPL was significantly greater in the high-fat diet (HFD)-Ex(+) group than in the control group. There were no significant differences in the expression levels of HSL, LPL, CPT1, LCAD, or ACOX1 between the HFD-Ex(-) and HFD-Ex(+) group. The fold changes were calculated as the ratio of the expression level in the HFD-Ex(+) or HFD-Ex(-) group to that in the control group.  $n = 8$ ,  $^{\#}P < 0.05$  between groups.

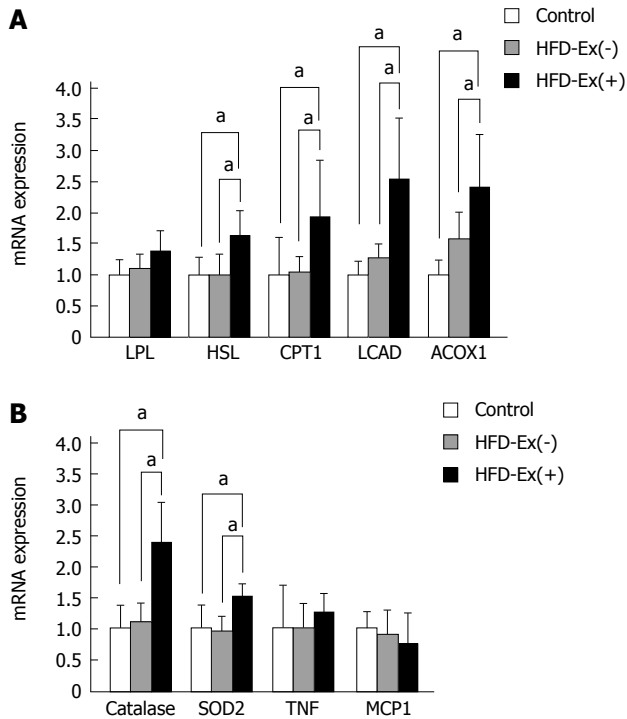
mulation might be due to an improvement in glucose intolerance, which occasionally develops in obese animals. Thus, we performed IPGTTs in rats in the HFD-Ex(-) and HFD-Ex(+) groups. Interestingly, fasting plasma glucose levels were slightly higher in the HFD-Ex(-) group than in the HFD-Ex(+) group, but no significant differences were observed at 15, 30, 60, 90, or 120 min after glucose injection (Figure 7). The nondiabetic profiles and the similar responses in both groups suggest that the effects of exenatide on lipid metabolism are unlikely to be due to improvements in glucose intolerance.

#### Adipose tissue AMPK is not activated by exenatide

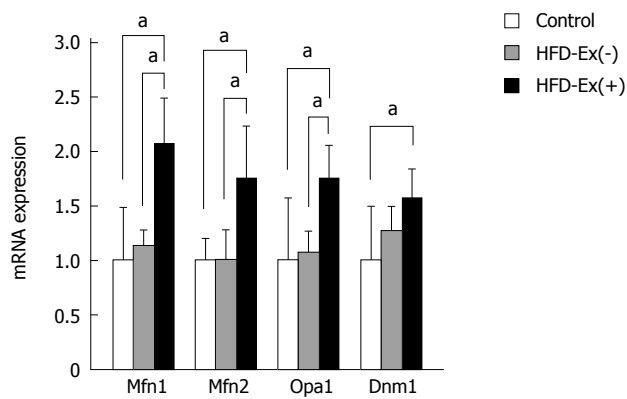
Following an increase in intracellular AMP, AMPK plays an essential role in the consumption of intracellular lipid by suppressing fatty acid synthesis and stimulating fatty acid oxidation<sup>[34]</sup>. To determine whether AMPK activation was involved in the effects of exenatide, we determined the protein expression of total AMPK and P-AMPK in adipose tissue. As shown in Figure 8, there were no obvious differences in AMPK or P-AMPK levels among the three groups.

## DISCUSSION

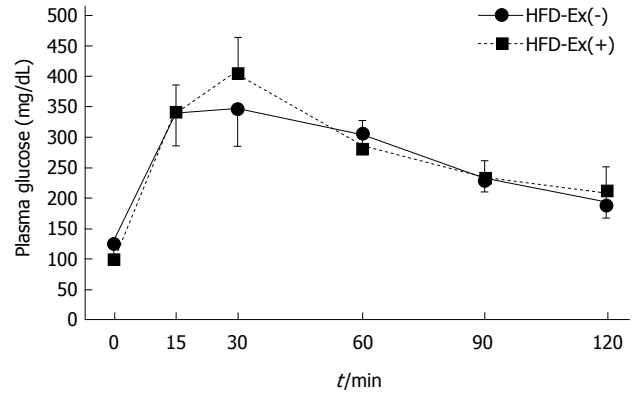
The mechanism by which GLP-1 and its analogs improve hepatic steatosis is still not fully understood, although changes in hepatic lipid metabolism are thought to be involved in these effects<sup>[20-23]</sup>. Because hepatic lipid content is determined by intrahepatic lipogenesis and lipolysis, as well as the extent of fatty acid influx into the liver, changes in lipid use in skeletal muscle and adipose tissue may contribute to hepatic lipid metabolism. Thus, we investigated the effects of exenatide, a GLP-1 receptor agonist, on lipid metabolism in the liver, skeletal muscle, and adipose tissue. To minimize the effects of exenatide on glycemic control in the diabetic state, we used a nondiabetic, HFD-induced rat NAFLD model. Exenatide reduced lipid accumulation in the liver and in adipose tissue and decreased the size of adipocytes. The reduction of body weight gain by exenatide was accompanied by a significant reduction in food intake. Using indirect calorimetry, we showed that exenatide increased oxygen consumption and reduced the RER. These findings suggest that reduced food intake and increased



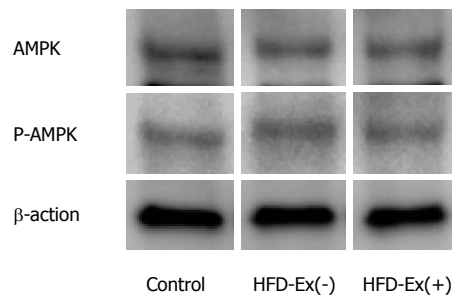
**Figure 5** Effects of exenatide on the expression levels of genes associated with lipid metabolism, reactive oxygen species elimination, and macrophage activation in adipose tissue. A: The expression levels of hormone-sensitive lipase (HSL), carnitine palmitoyltransferase-1 (CPT1), long-chain acyl-CoA dehydrogenase (LCAD), and acyl-CoA oxidase 1 (ACOX1) were significantly greater in the high-fat diet (HFD)-Ex(+) group than in the control and HFD-Ex(-) groups; B: The expression levels of catalase and superoxide dismutase (SOD2) were significantly greater in the HFD-Ex(+) group than in the control and HFD-Ex(-) groups. There were no significant differences in tumor necrosis factor (TNF) or monocyte chemoattractant protein 1 (MCP1) expression levels among the three groups. The fold changes were calculated as the ratio of the expression level in the HFD-Ex(+) or HFD-Ex(-) group to that in the control group.  $n = 8$ ,  $^aP < 0.05$  between groups.



**Figure 6** Effects of exenatide on the expression levels of mitochondrial morphologic regulators in adipose tissue. Genes involved in mitochondrial fusion [mitofusin-1 (Mfn1) and mitofusin-2 (Mfn2) and optic atrophy-1 (Opa1)] were significantly greater in the high-fat diet (HFD)-Ex(+) group than in the control and HFD-Ex(-) groups. The expression of dynamin-1 (Dnm1), which is involved in mitochondrial fission, was not significantly different between the HFD-Ex(+) and HFD-Ex(-) groups. The fold changes were calculated as the ratio of the expression level in the HFD-Ex(+) or HFD-Ex(-) group to that in the control group.  $n = 8$ ,  $^aP < 0.05$  between groups.



**Figure 7** Effect of exenatide on glucose tolerance. Intraperitoneal glucose tolerance tests (IPGTTs) were performed in the high-fat diet (HFD)-Ex(+) and HFD-Ex(-) groups at week 12 of feeding. Fasting plasma glucose levels were slightly lower in the HFD-Ex(+) group than in the HFD-Ex(-) group, but no significant differences were observed at the other times during the IPGTTs.



**Figure 8** Effects of exenatide on AMP-activated protein kinase activation in adipose tissue. Immunoblotting for total AMP-activated protein kinase (AMPK), phosphorylated AMPK (P-AMPK), and  $\beta$ -actin were performed. The protein levels of total AMPK and P-AMPK were not significantly different among the three groups. HFD: High-fat diet.

energy consumption, most likely through increased lipid use, contribute to the exenatide-induced reduction in systemic lipid accumulation. We then determined the expression levels of lipid metabolism-related genes in the liver, skeletal muscle, and adipose tissue. Surprisingly, the hepatic expression levels of lipogenic genes (*SREBP1c*, *FAS*, and *ACC1*) and lipolytic genes (*HSL*, *CPT1*, *LCAD*, and *ACOX1*) were unaffected by exenatide. The expression of ApoB was also unaffected. These results are inconsistent with those of previous reports, which revealed that GLP-1 and its analogs directly modulate hepatic lipid metabolism<sup>[20,23,35,36]</sup>. Because these findings were mainly observed in diabetic animals or in cultured hepatocytes, we speculated that organs other than the liver might be more sensitive to GLP-1 or its analogs in the nondiabetic state, resulting in the absence of a hepatic response. In skeletal muscle, exenatide did not affect the expression levels of HSL, CPT1, LCAD, or ACOX1, but LPL expression was significantly higher in the HFD-Ex(+) group compared with the control, but not compared with the HFD-Ex(-) group, which suggests that lipid consumption is not increased in skeletal



muscle. However, lipolytic genes were upregulated by exenatide in adipose tissue. Notably, the expression levels of HSL, CPT1, LCAD, and ACOX1 were significantly greater in the HFD-Ex(+) group than in the control and HFD-Ex(-) groups. To evaluate whether these changes were associated with changes in nutrient oxidation, we analyzed the expression levels of catalase and SOD2, which are responsible for eliminating ROS produced during oxidative phosphorylation<sup>[37,38]</sup>. We showed that exenatide significantly upregulated the expression of both enzymes. Therefore, exenatide seemed to promote nutrient oxidation, especially of lipid, in adipose tissue. To analyze the effects of exenatide on lipid use, we determined the expression levels of genes regulating mitochondrial morphology, which responds to changes in the nutritional environment<sup>[33,39,40]</sup>. In particular, intracellular lipid content is greatly affected by the correlation between mitochondrial fusion and fission<sup>[31,32]</sup>. In this study, we showed that exenatide significantly upregulated the expression levels of Mfn1, Mfn2, and Opa1, which regulate mitochondrial fusion and promote the consumption of intracellular lipid<sup>[31,32]</sup>. Taken together, our observations suggest that exenatide enhanced lipid use in adipose tissue, which contributed to the improvement in hepatic steatosis, most likely by reducing lipid influx into the liver.

The mechanisms by which GLP-1 modulates lipid use in adipose tissue are largely unknown. The GLP-1 receptor has been detected in 3T3-L1 adipocytes and in human adipose tissue<sup>[24,41,42]</sup>, and GLP-1 was reported to stimulate lipolysis in a receptor-dependent manner<sup>[42]</sup>. These findings suggest that exenatide enhances lipid use by signaling *via* the GLP-1 receptor in adipocytes. Another mechanism may involve activation of the sympathetic nervous system. It was reported that treatment with a dipeptidyl peptidase 4 inhibitor increased lipolysis in adipose tissues, and this was associated with elevated plasma norepinephrine levels<sup>[43]</sup>. Furthermore, intracerebroventricular infusion of GLP-1 decreased lipid storage in white adipose tissue in a manner that was partially mediated *via* sympathetic nerve activation<sup>[44]</sup>. These findings are consistent with our observation that exenatide enhanced adipose tissue expression of HSL, which is activated by the sympathetic nervous system *via* the cAMP-dependent pathway<sup>[45,46]</sup>. Not only white adipose tissue, but also brown adipose tissue might be involved in the actions of GLP-1 observed in this study. Recently, Lockie *et al.*<sup>[47]</sup> reported that intracerebroventricular injection of GLP-1 induced thermogenesis in brown adipose tissue, accompanied with increased activity of innervated sympathetic fibers. Taken together, we hypothesize that GLP-1 enhances lipid utility in both the adipose tissues, lipolysis in white adipose tissue and thermogenesis in brown adipose tissue, leading to increased energy consumption, resulting in the improvement of hepatic steatosis. However, the precise roles of these adipose tissues need further investigation.

In conclusion, this study showed that reduced food

intake and enhanced lipid use by exenatide in adipose tissue contributed to an improvement in hepatic steatosis in a rat model of HFD-induced NAFLD. The mechanism by which exenatide (and therefore GLP-1) modulates lipid metabolism in adipose tissue should be investigated further.

## COMMENTS

### Background

Nonalcoholic fatty liver disease (NAFLD) is considered a hepatic manifestation of metabolic syndrome. Recently, with the prevalence of metabolic syndrome, NAFLD-patients are increasing. If untreated, NAFLD or nonalcoholic steatohepatitis (NASH), a severe stage of NAFLD, frequently progresses into liver cirrhosis and hepatocellular carcinoma. Body weight reduction and control of complicated diabetes are essential to improve NAFLD. However, attempts to restrict food intake and increase physical exercise are often insufficient to treat NAFLD.

### Research frontiers

Glucagon-like peptide-1 (GLP-1) was reported to reduce hepatic steatosis in animal models of NAFLD. In addition to suppressing appetite, direct action of GLP-1 on the liver is reported to enhance hepatic lipolysis to prevent lipid accumulation. Because GLP-1 receptor distributes widely in various tissues, authors supposed that organs other than the liver might also be involved in the regulation of hepatic lipid contents. In this study using a rat model of NAFLD, authors evaluated the changes in lipid metabolism induced by GLP-1 treatment in liver, skeletal muscle, and adipose tissue.

### Innovations and breakthrough

In the high-fat diet (HFD)-induced NAFLD model, GLP-1 treatment reduced lipid accumulations in liver and adipose tissues. Authors found that increased expressions of genes were involved in lipolysis and lipid oxidation in adipose tissue, but not in the liver or skeletal muscle. In adipose tissue, GLP-1 significantly upregulated catalase, superoxide dismutase 2, and mitochondrial morphological regulators. Because the improvement of hepatic steatosis by GLP-1 was accompanied with increased energy expenditure and decreased respiratory exchange ratio, enhanced lipid utility by GLP-1 in adipose tissue may reduce lipid influx into the liver, resulting in the reduction of hepatic lipid accumulation.

### Application

In this study, authors show that GLP-1 improves hepatic steatosis in the HFD-induced nondiabetic NAFLD model, which seems to be mediated by enhanced lipolysis with increased systemic energy expenditure. These actions of GLP-1 would be ideal for the treatment of human disease of NAFLD, with or without diabetes.

### Terminology

NAFLD is a chronic liver disease that is characterized by steatosis that is histologically similar to that in alcoholic liver injury, without excessive alcoholic intake or hepatitis viral infection. NASH, a severe stage of NAFLD, frequently progresses into liver cirrhosis and hepatocellular carcinoma. GLP-1, an incretin hormone produced by intestinal L cells, is an effective therapeutic agent for type 2 diabetes mellitus but is immediately degraded by dipeptidyl peptidase-4 (DPP4). Exenatide, a GLP-1 receptor agonist, has a longer half-life and enhanced potency compared with GLP-1 because it is less susceptible to degradation by DPP4.

### Peer review

The authors focused on a different point, *i.e.*, adipose tissue to elucidate the mechanism of improving NAFLD by GLP-1. This unique study related the enhanced lipid metabolism of adipose tissue by GLP-1 with improvement of NAFLD.

## REFERENCES

- 1 Page J. Nonalcoholic fatty liver disease: the hepatic metabolic syndrome. *J Am Acad Nurse Pract* 2012; **24**: 345-351 [PMID: 22672485 DOI: 10.1111/j.1745-7599.2012.00716.x]
- 2 Paredes AH, Torres DM, Harrison SA. Nonalcoholic fatty liver disease. *Clin Liver Dis* 2012; **16**: 397-419 [PMID: 22541706]

- DOI: 10.1016/j.cld.2012.03.005]
- 3 **Kleiner DE**, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. *Semin Liver Dis* 2012; **32**: 3-13 [PMID: 22418883 DOI: 10.1055/s-0032-1306421]
  - 4 **Brunt EM**. Pathology of nonalcoholic steatohepatitis. *Hepatology* 2005; **33**: 68-71 [PMID: 16214395]
  - 5 **Hashimoto E**, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. *J Gastroenterol* 2011; **46** Suppl 1: 63-69 [PMID: 20844903 DOI: 10.1007/s00535-010-0311-8]
  - 6 **White DL**, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatology* 2012; **10**: 1342-1359.e2 [PMID: 23041539 DOI: 10.1016/j.cgh.2012.10.001]
  - 7 **Yki-Järvinen H**. Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 709-714 [PMID: 20842026 DOI: 10.1097/MCO.0b013e32833f4b34]
  - 8 **Khan R**, Foster GR, Chowdhury TA. Managing diabetes in patients with chronic liver disease. *Postgrad Med* 2012; **124**: 130-137 [PMID: 22913901 DOI: 10.3810/pgm.2012.07.2574]
  - 9 **Adams LA**, Angulo P. Treatment of non-alcoholic fatty liver disease. *Postgrad Med J* 2006; **82**: 315-322 [PMID: 16679470]
  - 10 **Russell S**. Incretin-based therapies for type 2 diabetes mellitus: a review of direct comparisons of efficacy, safety and patient satisfaction. *Int J Clin Pharm* 2013; **35**: 159-172 [PMID: 23263796 DOI: 10.1007/s11096-012-9729-9]
  - 11 **Nielsen LL**, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycemic control of type 2 diabetes. *Regul Pept* 2004; **117**: 77-88 [PMID: 14700743]
  - 12 **Baggio LL**, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131-2157 [PMID: 17498508]
  - 13 **Mojsos S**, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest* 1987; **79**: 616-619 [PMID: 3543057]
  - 14 **Eng J**, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem* 1992; **267**: 7402-7405 [PMID: 1313797]
  - 15 **Göke R**, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, Göke B. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem* 1993; **268**: 19650-19655 [PMID: 8396143]
  - 16 **Gallwitz B**. Exenatide in type 2 diabetes: treatment effects in clinical studies and animal study data. *Int J Clin Pract* 2006; **60**: 1654-1661 [PMID: 17109672]
  - 17 **Holst JJ**. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007; **87**: 1409-1439 [PMID: 17928588]
  - 18 **Dailey MJ**, Moran TH. Glucagon-like peptide 1 and appetite. *Trends Endocrinol Metab* 2013; **24**: 85-91 [PMID: 23332584 DOI: 10.1016/j.tem.2012.11.008]
  - 19 **Gedulin BR**, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes DG, Young AA. Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. *Endocrinology* 2005; **146**: 2069-2076 [PMID: 15618356]
  - 20 **Svegliati-Baroni G**, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* 2011; **31**: 1285-1297 [PMID: 21745271 DOI: 10.1111/j.1478-3231.2011.02462.x]
  - 21 **Mells JE**, Fu PP, Sharma S, Olson D, Cheng L, Handy JA, Saxena NK, Sorescu D, Anania FA. Glp-1 analog, liraglutide, ameliorates hepatic steatosis and cardiac hypertrophy in C57BL/6J mice fed a Western diet. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G225-G235 [PMID: 22038829 DOI: 10.1152/ajpgi.00274.2011]
  - 22 **Lee J**, Hong SW, Chae SW, Kim DH, Choi JH, Bae JC, Park SE, Rhee EJ, Park CY, Oh KW, Park SW, Kim SW, Lee WY. Exendin-4 improves steatohepatitis by increasing Sirt1 expression in high-fat diet-induced obese C57BL/6J mice. *PLoS One* 2012; **7**: e31394 [PMID: 22363635 DOI: 10.1371/journal.pone.0031394]
  - 23 **Ding X**, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 2006; **43**: 173-181 [PMID: 16374859]
  - 24 **Mérida E**, Delgado E, Molina LM, Villanueva-Peñacarrillo ML, Valverde I. Presence of glucagon and glucagon-like peptide-1-(7-36)amide receptors in solubilized membranes of human adipose tissue. *J Clin Endocrinol Metab* 1993; **77**: 1654-1657 [PMID: 8263154]
  - 25 **Jolly SR**, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* 1984; **54**: 277-285 [PMID: 6697450]
  - 26 **Matés JM**, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. *Clin Biochem* 1999; **32**: 595-603 [PMID: 10638941]
  - 27 **Orr WC**, Sohal RS. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 1994; **263**: 1128-1130 [PMID: 8108730]
  - 28 **Bastard JP**, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006; **17**: 4-12 [PMID: 16613757]
  - 29 **Aldhahi W**, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. *Curr Diab Rep* 2003; **3**: 293-298 [PMID: 12866991]
  - 30 **Lee YS**, Park MS, Choung JS, Kim SS, Oh HH, Choi CS, Ha SY, Kang Y, Kim Y, Jun HS. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia* 2012; **55**: 2456-2468 [PMID: 22722451 DOI: 10.1007/s00125-012-2592-3]
  - 31 **Bereiter-Hahn J**, Vöth M. Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech* 1994; **27**: 198-219 [PMID: 8204911]
  - 32 **Chan DC**. Mitochondrial fusion and fission in mammals. *Annu Rev Cell Dev Biol* 2006; **22**: 79-99 [PMID: 16704336]
  - 33 **Kita T**, Nishida H, Shibata H, Niimi S, Higuti T, Arakaki N. Possible role of mitochondrial remodelling on cellular triacylglycerol accumulation. *J Biochem* 2009; **146**: 787-796 [PMID: 19671539 DOI: 10.1093/jb/mvp124]
  - 34 **Bijland S**, Mancini SJ, Salt IP. Role of AMP-activated protein kinase in adipose tissue metabolism and inflammation. *Clin Sci (Lond)* 2013; **124**: 491-507 [PMID: 23298225 DOI: 10.1042/CS20120536]
  - 35 **Gupta NA**, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, Anania FA. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. *Hepatology* 2010; **51**: 1584-1592 [PMID: 20225248 DOI: 10.1002/hep.23569]
  - 36 **Ben-Shlomo S**, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *J Hepatol* 2011; **54**: 1214-1223 [PMID: 21145820 DOI: 10.1016/j.jhep.2010.09.032]
  - 37 **Gaetani GF**, Ferraris AM, Rolfo M, Mangerini R, Arena S, Kirkman HN. Predominant role of catalase in the disposal of

- hydrogen peroxide within human erythrocytes. *Blood* 1996; **87**: 1595-1599 [PMID: 8608252]
- 38 **Miriyala S**, Holley AK, St Clair DK. Mitochondrial superoxide dismutase--signals of distinction. *Anticancer Agents Med Chem* 2011; **11**: 181-190 [PMID: 21355846]
- 39 **Zorzano A**, Liesa M, Palacín M. Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. *Int J Biochem Cell Biol* 2009; **41**: 1846-1854 [PMID: 19703653 DOI: 10.1016/j.biocel.2009.02.004]
- 40 **Civitarese AE**, Ravussin E. Mitochondrial energetics and insulin resistance. *Endocrinology* 2008; **149**: 950-954 [PMID: 18202132]
- 41 **Montrose-Rafizadeh C**, Yang H, Wang Y, Roth J, Montrose MH, Adams LG. Novel signal transduction and peptide specificity of glucagon-like peptide receptor in 3T3-L1 adipocytes. *J Cell Physiol* 1997; **172**: 275-283 [PMID: 9284947]
- 42 **Vendrell J**, El Bekay R, Peral B, García-Fuentes E, Megia A, Macías-González M, Fernández Real J, Jiménez-Gómez Y, Escoté X, Pachón G, Simó R, Selva DM, Malagón MM, Tinahones FJ. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. *Endocrinology* 2011; **152**: 4072-4079 [PMID: 21862620 DOI: 10.1210/en.2011-1070]
- 43 **Boschmann M**, Engeli S, Dobberstein K, Budziarek P, Strauss A, Boehnke J, Sweep FC, Luft FC, He Y, Foley JE, Jordan J. Dipeptidyl-peptidase-IV inhibition augments postprandial lipid mobilization and oxidation in type 2 diabetic patients. *J Clin Endocrinol Metab* 2009; **94**: 846-852 [PMID: 19088168 DOI: 10.1210/jc.2008-1400]
- 44 **Nogueiras R**, Pérez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, Patterson JT, Disse E, Pfluger PT, López M, Woods SC, DiMarchi R, Diéguez C, Rahmouni K, Rohner-Jeanrenaud F, Tschöp MH. Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. *J Neurosci* 2009; **29**: 5916-5925 [PMID: 19420258 DOI: 10.1523/JNEUROSCI.5977-08.2009]
- 45 **Carmen GY**, Victor SM. Signalling mechanisms regulating lipolysis. *Cell Signal* 2006; **18**: 401-408 [PMID: 16182514]
- 46 **Bartness TJ**, Song CK. Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. *J Lipid Res* 2007; **48**: 1655-1672 [PMID: 17460327]
- 47 **Lockie SH**, Heppner KM, Chaudhary N, Chabenne JR, Morgan DA, Veyrat-Durebex C, Ananthakrishnan G, Rohner-Jeanrenaud F, Drucker DJ, DiMarchi R, Rahmouni K, Oldfield BJ, Tschöp MH, Perez-Tilve D. Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes* 2012; **61**: 2753-2762 [PMID: 22933116]

**P- Reviewers:** Buchler C, Ganesh Pai C, Rocha R, Santoro N

**S- Editor:** Gou SX **L- Editor:** A **E- Editor:** Liu XM





百世登

**Baishideng**®

Published by **Baishideng Publishing Group Co., Limited**

Flat C, 23/F., Lucky Plaza,

315-321 Lockhart Road, Wan Chai, Hong Kong, China

Fax: +852-65557188

Telephone: +852-31779906

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045