World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i43.16227

World J Gastroenterol 2014 November 21; 20(43): 16227-16235
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

MK-0626, a selective DPP-4 inhibitor, attenuates hepatic steatosis in *ob/ob* mice

Tatsuya Ohyama, Ken Sato, Yuichi Yamazaki, Hiroaki Hashizume, Norio Horiguchi, Satoru Kakizaki, Masatomo Mori, Motoyasu Kusano, Masanobu Yamada

Tatsuya Ohyama, Ken Sato, Yuichi Yamazaki, Hiroaki Hashizume, Norio Horiguchi, Satoru Kakizaki, Masatomo Mori, Masanobu Yamada, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Gunma 371-8511, Japan
Motoyasu Kusano, Department of Endoscopy and Endoscopic Surgery, Gunma University Hospital, Gunma 371-8511, Japan
Author contributions: Sato K designed the research, wrote the paper, and analyzed the data; Ohyama T and Yamazaki Y performed the experiment; Hashizume H, Horiguchi N and Kakizaki S analyzed the data; Mori M and Kusano M revised the draft critically for important intellectual content; Yamada M gave final approval of the version to be published.
Supported by Merck Sharp and Dohme
Correspondence to: Ken Sato, MD, PhD, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. satoken@gunma-u.ac.jp
Telephone: +81-27-2208127 Fax: +81-27-2208136
Received: November 13, 2013 Revised: January 6, 2014
Accepted: June 14, 2014
Published online: November 21, 2014

Abstract

AIM: To investigate the mechanism and *in vivo* effects of MK-0626, a dipeptidyl peptidase-4 inhibitor, on hepatic steatosis using *ob/ob* mice.

METHODS: We analyzed obese (*ob/ob*) 8-wk-old male mice that had been randomly divided into two groups of *ob/ob* mice (n = 16 each) and were treated with 1.5 or 3 mg/kg MK-0626 and two control groups of untreated *ob/ob* mice and lean littermates (n = 16 each). All mice were fed a normal chow diet with or without MK-0626 for either four or eight weeks. Blood samples were collected, and total hepatectomy was performed.

RESULTS: The administration of dietary MK-0626 ameliorated the hepatic lipid accumulation in *ob/ob* mice treated with 3 mg/kg MK-0626 (3 MK), $P < 0.05$, *vs* untreated *ob/ob mice* (*ob/ob*). The MK-0626 treatment reduced the serum alanine aminotransferase levels (both treatment groups, $P < 0.05$ *vs ob/ob*) and glucoses/insulin levels/calculated HOMA scores (1.5 MK, $P < 0.05$ *vs ob/ob*; 3 MK, $P < 0.01$ *vs ob/ob*) and increased the serum adiponectin levels (3 MK, $P < 0.05$ *vs ob/ob*) in a dose-dependent manner. The MK-0626 treatment increased the mRNA expression of peroxisome proliferator-activated receptor α/microsomal triglyceride transfer protein (1.5 MK, $P < 0.05$ *vs ob/ob*; 3 MK, $P < 0.01$ *vs ob/ob*) but reduced the sterol regulatory element binding transcription factor-1c/fatty acid synthase/stearoyl-CoA desaturase-1 (both treatment groups, $P < 0.01$ *vs ob/ob*). The MK-0626 treatment increased the activity of AMP-activated protein kinase (AMPK) (both treatment groups, $P < 0.01$ *vs ob/ob*).

CONCLUSION: MK-0626 could attenuate hepatic steatosis through enhancing AMPK activity, inhibiting hepatic lipogenic gene expression, enhancing triglyceride secretion from liver and increasing serum adiponectin levels.

Key words: Dipeptidyl peptidase-4 inhibitor; Hepatic steatosis; *ob/ob* mice; AMP-activated protein kinase; Microsomal triglyceride transfer protein; Adiponectin

Core tip: Administration of MK-0626, a dipeptidyl peptidase-4 inhibitor, ameliorated hepatic steatosis; reduced serum alanine aminotransferase, glucose, and insulin levels; reduced HOMA scores; and increased serum adiponectin levels in *ob/ob* mice. MK-0626 treatment significantly increased the mRNA expression of peroxisome proliferator-activated receptor α and microsomal triglyceride transfer protein but significantly reduced sterol regulatory element binding transcription factor-1c, fatty acid synthase and stearoyl-CoA desaturase-1.

AMP-activated protein kinase (AMPK) activity was significantly increased. These results suggest that MK-0626 could attenuate hepatic steatosis by enhancing AMPK activity, inhibiting hepatic lipogenic gene expression, enhancing triglyceride secretion from the liver and increasing serum adiponectin levels.

Ohyama T, Sato K, Yamazaki Y, Hashizume H, Horiguchi N, Kakizaki S, Mori M, Kusano M, Yamada M. MK-0626, a selective DPP-4 inhibitor, attenuates hepatic steatosis in *ob/ob* mice. *World J Gastroenterol* 2014; 20(43): 16227-16235 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i43/16227.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i43.16227

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome. Other comorbidities of metabolic syndrome include obesity, type 2 diabetes mellitus, hypertension and dyslipidemia coupled with insulin resistance, which is a central feature of metabolic syndrome. Thus, improving insulin sensitivity would decrease hepatic fat deposition and accordingly inhibit hepatocyte vulnerability to oxidative stress[1].

The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) play a pivotal role in controlling blood glucose levels[2,3]. In response to meal ingestion, both hormones facilitate insulin synthesis and release[4-6] and, as a result, an increase in insulin sensitivity is observed. GLP-1 inhibits glucagon release, delays gastric emptying and increases satiety[4-6]. These incretin hormones are degraded and inactivated by dipeptidyl peptidase-4 (DPP-4)[7]. DPP-4 inhibitors prevent the degradation/inactivation of the biologically active form of GLP-1 and GIP, thereby augmenting the biological activity of GLP-1 and GIP[8], and have been approved for the treatment of type 2 diabetes.

Previous studies showed that inhibition of DPP-4 prevents hepatic steatosis in animal models[9-13], and a clinical pilot study with 30 NAFLD patients with type 2 diabetes mellitus showed that the DPP-4 inhibitor sitagliptin improved elevated liver enzymes[14]. However, the mechanisms by which the DPP-4 inhibitor prevents hepatic steatosis remain to be elucidated.

ob/ob mice have a naturally occurring spontaneous point mutation in the leptin gene that prevents the peptide from being produced[15] and are well-recognized as a naturally occurring model of hepatic steatosis and type 2 diabetes. The characteristics of the *ob/ob* mouse include several metabolic and neuroendocrine abnormalities such as obesity, hyperphagia, hyperinsulinemia, hyperlipidemia, hyperglycemia and insulin resistance. In addition, *ob/ob* mice have a decreased metabolic rate and body temperature. Because *ob/ob* mice have several characteristics that mimic metabolic syndrome in humans, these mice form one of the most widely studied mouse models of obesity and metabolic syndrome[16-18].

MK-0626 is a potent, orally active DPP-4 inhibitor (IC_{50} = 6.3 nmol/L) with excellent selectivity and oral bioavailability in preclinical species and *in vivo* efficacy in animal models[19]. The objectives of our study were to characterize the *in vivo* effects and mechanism of action of the α-amino amide DPP-4 inhibitor, MK-0626, on hepatic steatosis using *ob/ob* mice.

MATERIALS AND METHODS

Animals, treatment and specimen collection

Obese (*ob/ob*) 6-wk-old male mice and their lean littermates were purchased from Charles Liver Co. Ltd. (Tokyo, Japan). All mice were housed in cages and maintained on a 12-h light/dark cycle with free access to food and water. The mice were acclimatized for 2 wk, during which time they were fed a normal chow diet (CLEA Rodent Diet CE-2) from CLEA Japan, Inc. (Tokyo, Japan). At 8 wk of age, they were placed on a normal chow diet (D12450B) from Research Diets (Tokyo, Japan) as a transition to MK-0626 supplemented D12450B chow. Mice were randomly divided into two groups of *ob/ob* mice (n = 16 each) and were fed either a normal chow diet or a normal chow diet supplemented with MK-0626 (1.5 mg/kg) or MK-0626 (3 mg/kg). In addition, two control groups (n = 16 each) of untreated *ob/ob* mice and lean littermates were fed a normal chow diet. After the mice were switched to D12450B, body weight and food intake were monitored weekly. All mice were fed an experimental diet for either four or eight weeks. At the completion of the study, fasting blood samples were drawn to analyze glucose and insulin levels and the homeostatic model assessment (HOMA). Further sera were drawn to measure serum active GLP-1 concentrations and biochemical parameters such as alanine aminotransferase (ALT). Total hepatectomy was performed at the time of euthanasia, and liver samples were divided for histopathology and other analyses. For protein or RNA analysis, tissues were frozen in liquid nitrogen and stored at -80 ℃ until needed. To achieve statistical power for the study, 64 mice were used for the experiment, and 16 mice were included in each treatment arm. All mouse procedures were performed in accordance with the guidelines for animal care and use established by the Gunma University School of Medicine.

Hepatic lipid profiles

Total liver lipids were extracted from liver homogenate using methanol and chloroform, and they were then reacted with a vanillin-phosphoric acid reagent[20]. The hepatic lipid content was measured enzymatically with triglyceride GPO-Trinder and Infinity cholesterol reagents (Sigma-Aldrich, St. Louis, MO).

Biochemical and histological analysis

Serum glucose and serum ALT were measured with an auto-analyzer. Adiponectin concentration was measured

Table 1 Primary pairs for real-time polymerase chain reaction of genes related to hepatic fatty acid metabolism

Gene name	Accession	Forward	Reverse
PPARα	Mm00440939		
SREBP-1c	Mm00550338		
SCD-1		GTTGGCTCCATCCATTGC	AACCATGGGAAGCCAAGTTT
FAS		AACCTCAGCAACACATCT	CGTTTACAAAGGGCATGCA
MTP	Mm00435015		
CPT1		CCCTGGGCATGATTGCAA	AAGAGGACGCCACTCACGAT

PPARα: Peroxisome proliferator-activated receptor α; SREBP-1c: Sterol regulatory element binding transcription factor-1c; SCD-1: Stearoyl-CoA desaturase-1; FAS: Fatty acid synthase; MTP: Microsomal triglyceride transfer protein activity; CPT-1: Carnitine palmitoyltransferase-1.

using a Quantikine ELISA Kit (RD Systems, Minneapolis, MN, United States). Insulin concentration was measured using a Mouse Ultrasensitive Insulin ELISA (ALPCO Diagnosis, Salem, NH). HOMA was calculated using serum glucose and insulin concentrations. Serum active GLP-1 was measured using a GLP-1 (Active) ELISA KIT (Shibayagi, Gunma, Japan). For histological examination, paraffin-embedded liver tissue specimens were stained with Hematoxylin-Eosin and Oil Red O. Quantitative analysis of Oil Red O-stained areas was performed using the NIH image software program in 10 microscopic fields at a 400-fold magnification.

RNA expression analysis of the liver specimen

Total RNA was isolated with the ISOGEN RNA extraction reagent (Nippon Gene, Toyama, Japan), quantified by spectrophotometry and reverse-transcribed to cDNA. Then, the cDNA was used for polymerase chain reaction (PCR). It was amplified using iQ-SYBR Green Supermix with specific oligonucleotide primers for target sequences or glyceraldehyde-3-phosphate dehydrogenase (for normalization). Taqman Universal PCR Master Mix and the Taqman Gene Expression Assay (Applied Biosystems Japan Ltd., Tokyo, Japan) were used to analyze the genes of interest, which included peroxisome proliferator-activated receptor (PPAR)α, sterol regulatory element binding transcription factor (SREBP)-1c, stearoyl-CoA desaturase (SCD)-1, fatty acid synthase (FAS), microsomal triglyceride transfer protein (MTP) activity and carnitine palmitoyltransferase (CPT)-1. The specific oligonucleotide primers are shown in Table 1.

Western blotting analysis

The protein extracted from the liver tissue was run on a sodium dodecyl sulfate-polyacrylamide electrophoresis gel and then transferred to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare United Kingdom Ltd., Buckinghamshire, England). After the membranes were blocked in PVDF Blocking Reagent from Can Get Signal (TOYOBO, Osaka, Japan) for one hour at room temperature, they were probed with primary antibodies to phospho-AMP-activated protein kinase (AMPK) (1:1000) and AMPK (1:5000).

Statistical analysis

Statistical analysis was performed using SPSS v.16.0 (IBM Corp., Armonk, NY, United States). Data were analyzed by one-way analysis of variance. Values are expressed as the mean ± SD. A minimum of three independent experiments were performed, unless indicated otherwise. *P* values of less than 0.05 were considered statistically significant.

RESULTS

MK-0626 treatment increased active GLP-1 concentrations in the serum of ob/ob mice compared to MK-0626-untreated ob/ob mice

Serum active GLP-1 concentrations in *ob/ob* mice were significantly lower than those in lean mice (Figure 1A). MK-0626 treatment of *ob/ob* mice increased serum active GLP-1 concentrations in a dose-dependent manner (Figure 1A).

MK-0626 treatment reduced serum ALT, glucose and, insulin levels and HOMA scores and increased serum adiponectin levels in ob/ob mice compared to MK-0626-untreated ob/ob mice

Treatment with MK-0626 for 4 wk in *ob/ob* mice reduced serum ALT, glucose, and insulin levels and the calculated HOMA scores and increased serum adiponectin levels compared to MK-0626-untreated *ob/ob* mice in a dose-dependent manner. However, the effect of 1.5 mg/kg MK-0626 on serum adiponectin levels did not reach statistical significance (Table 2).

MK-0626 treatment did not significantly change body weight, food intake or the ratio of liver weight to body weight in ob/ob mice

There was no significant change in body weight in either the low or high dose MK-0626 treatment groups compared with MK-0626-untreated *ob/ob* mice, a trend that was sustained throughout the treatment period (Figure 1B). In addition, the food intake of *ob/ob* mice was not significantly altered by MK-0626 treatment, and this trend was sustained over the entire treatment period (Figure 1C). Body weight and food intake of the mice at the end of treatment are shown in Figure 1D and E, respectively. Body weight and food intake of the *ob/ob* mice significantly increased throughout the experimental period compared to their lean littermates regardless of

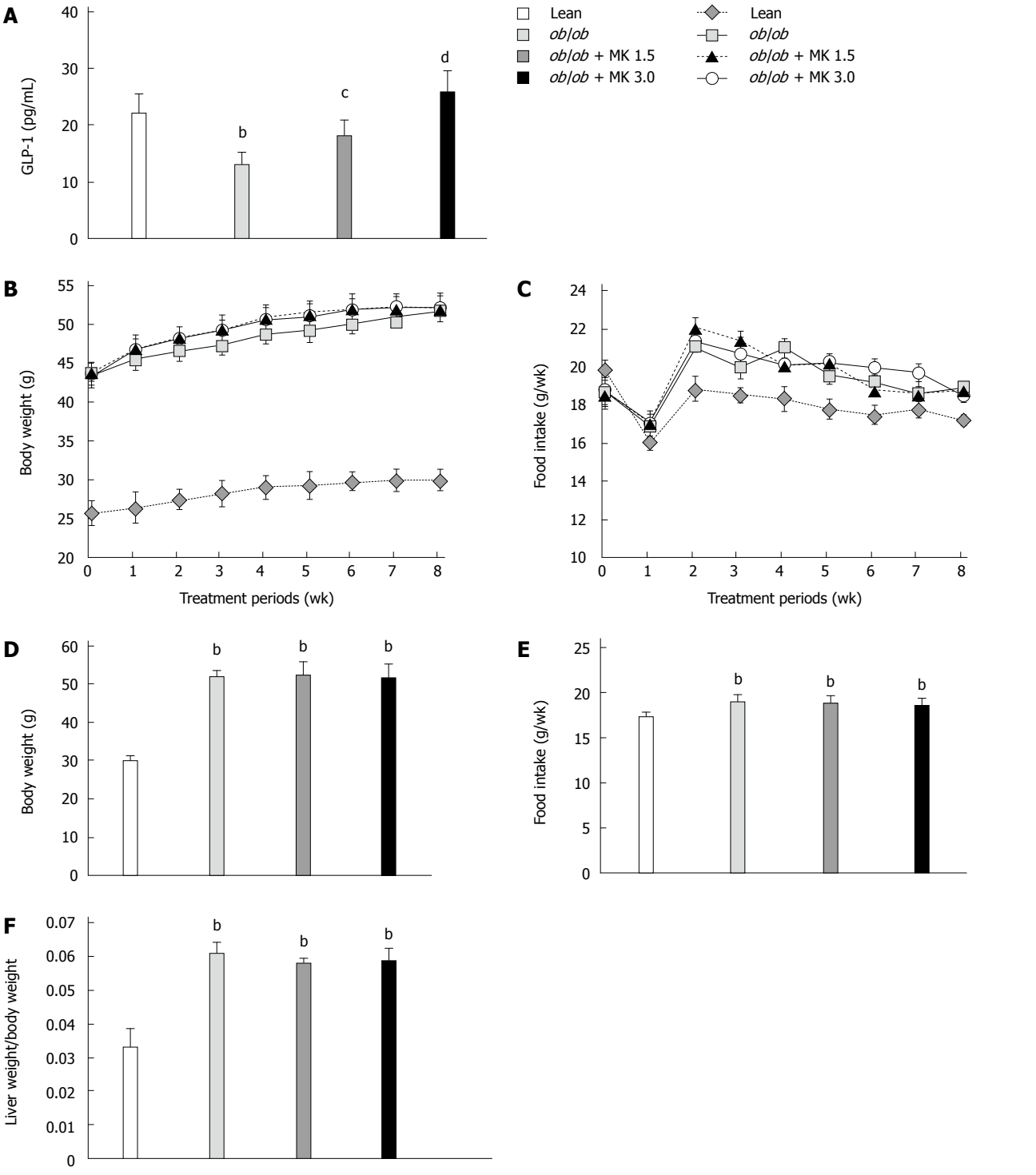

Figure 1 Serum active glucagon-like peptide-1 concentrations, body weight, food intake or ratio of liver weight to body weight in *ob/ob* mice and their lean littermates. A: Serum active glucagon-like peptide-1 (GLP-1) concentrations (pg/mL) in *ob/ob* mice that were fed either a normal chow diet (*ob/ob*) or a normal chow diet supplemented with MK-0626 (1.5 mg/kg) (*ob/ob* + MK 1.5) or MK-0626 (3 mg/kg) (*ob/ob* + MK 3.0) and their lean littermates fed a normal chow diet (lean). Serum samples were collected at the end of the 8-wk treatment period; B: Changes in body weight in lean, *ob/ob, ob/ob* + MK 1.5 and *ob/ob* + MK 3.0 groups during the treatment; C: Changes in food intake in lean, *ob/ob, ob/ob* + MK 1.5 and *ob/ob* + MK 3.0 groups during the treatment; D: A comparison of body weight at the end of the 8-wk treatment period; E: A comparison of food intake at the end of the 8-wk treatment period; F: A comparison of the ratio of liver weight to body weight at the end of the 8-wk treatment period. $^{b}P < 0.01$ *vs* the lean group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the *ob/ob* group ($n = 16$ mice per group).

MK-0626 treatment, except for food intake at week 1 and 7 of therapy (Figure 1B and C). MK-0626 treatment did not significantly change the ratio of liver weight to body weight at the end of treatment (Figure 1F).

MK-0626 treatment reduced hepatic fat content in ob/ob mice

MK-0626-treated *ob/ob* mice had a significant reduction in Oil Red O-stained areas in the liver, and the reduction

Table 2 Metabolic laboratory findings from each experimental mice group

Laboratory findings	Lean	*ob/ob*	*ob/ob* + MK-0626 (1.5 mg/kg)	*ob/ob* + MK-0626 (3 mg/kg)
Glucose (mg/dL)	141 ± 43.8	349 ± 15.7	288 ± 17.6[a]	247 ± 42.5[b]
Insulin (μU/mL)	1.83 ± 0.61	11.54 ± 0.59	8.42 ± 1.03[a]	7.83 ± 0.44[b]
HOMA score	0.63 ± 0.06	9.96 ± 2.32	5.98 ± 0.52[a]	4.78 ± 0.166[b]
ALT (U/L)	43.3 ± 10.3	590 ± 109.5	278 ± 70.5[a]	272.5 ± 131.5[a]
Adiponectin (μg/mL)	31.15 ± 2.07	34.57 ± 1.25	37.86 ± 3.40	39.90 ± 1.28[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs ob/ob* mice group. HOMA: Homeostatic model assessment; ALT: Alanine aminotransferase.

occurred in a dose-dependent manner (Figure 2A, B). Correspondingly, the hepatic lipid content was significantly reduced in MK-0626-treated *ob/ob* mice in a dose-dependent manner (Figure 2C).

MK-0626 treatment increased PPARα and MTP mRNA expression and decreased the mRNA expression of SREBP-1c, SCD-1 and FAS

MK-0626 treatment significantly increased hepatic PPARα mRNA, a key element involved in β-oxidation of free fatty acids, compared with the MK-0626-untreated *ob/ob* control mice (Figure 3). MK-0626 treatment significantly reduced hepatic mRNA expression levels of SREBP-1c, FAS and SCD-1, key regulators of *de novo* hepatic lipogenesis, compared with MK-0626-untreated *ob/ob* control mice (Figure 3). In addition, MK-0626 treatment significantly increased MTP mRNA, a key factor responsible for intracellular lipid transport in the intestine and liver, compared with MK-0626-untreated *ob/ob* mice (Figure 3). However, MK-0626 treatment did not significantly change hepatic mRNA levels of CPT1, a key factor involved in lipolysis, compared with MK-0626-untreated *ob/ob* control mice (Figure 3).

MK-0626 treatment increased AMPK activation in whole liver in a dose-dependent manner

In a dose-dependent manner, MK-0626 treatment increased activation of AMPK, which is a sensor of cellular energy status and acts as a regulator of hepatic lipogenesis, in whole liver obtained from *ob/ob* mice compared with MK-0626-untreated *ob/ob* mice (Figure 4).

DISCUSSION

The major finding of our study was that MK-0626, a selective α-amino amide DPP-4 inhibitor, attenuated liver steatosis in experimental obese and diabetic mice. This conclusion is based on the result that MK-0626 decreased the degree of Oil red O-staining and hepatic lipid content in whole livers of *ob/ob* mice. However, the mechanism of this hypolipidemic effect was not due to a decrease in food intake and/or body weight because MK-0626 treatment did not significantly alter food intake and body weight in *ob/ob* mice. As for weight effects, a GLP-1 receptor agonist generally causes a weight loss of 1-4 kg in human studies[21-23] and a measurable weight loss in animal studies[10,24]. Conversely, DPP-4 inhibitors are weight neutral in patients with type 2 diabetes[25-27] and in animal studies[10,11,28], although a contradictory human study[29] exists. Our study showed that treatment with MK-0626 did not change body weight, which is consistent with the majority of prior reports. As for food intake, a DPP-4 inhibitor does not cause a decrease in food intake in animal studies[10,28], which is consistent with our study. Thus, the effect of MK-0626 on fatty liver was not due to a change in weight loss or food intake.

A possible mechanism of MK-0626 action on fatty livers is through enhancement of AMPK activity and the resultant inhibition of hepatic lipogenic gene expression. This hypothetical mechanism is supported by prior studies that demonstrated that high fat diet fed DPP4-deficient rats[9] reduce hepatic fat content and that wild-type and β-cell-specific glucokinase haploinsufficient (Gck+/-) diabetic mice treated with des-fluoro-sitagliptin (DFS), a DDP-4 inhibitor[11] also reduce grade of hepatic steatosis. A DPP4-deficient rat model is very useful for evaluating the action of DPP-4 inhibition. Although we also used *ob/ob* mice, one of the genetically modified animals, our model of oral administration of DPP4 inhibitor was also important in view of reflecting a more physiological state. Thus, we were able to achieve a dose-dependent effect of the DPP4 inhibitor on hepatic steatosis, which was supported by a dose-dependent increase in serum active GLP-1 concentrations in *ob/ob* mice. In diet-induced fatty livers from wild-type and $Gck^{+/-}$ diabetic mice, DFS treatment increased the mRNA expression of PPARα, which is one possible mechanism of ameliorating fatty liver[11] and is consistent with our results. In our study, we elucidated the increase in mRNA expression of microsomal triglyceride transfer protein as a new possible mechanism of ameliorating fatty liver. Another possible mechanism of ameliorating fatty liver is an increase in serum adiponectin, which was supported by Souza-Mello *et al*[13]. Adiponectin reportedly enhances AMPK and the PPARα pathway in the liver and skeletal muscle[30], which correlates well with our results. Interestingly, our results were also supported by data that demonstrate that plasma DPP-4 activity negatively correlates with plasma adiponectin in healthy young people[31].

In our model, MK-0626 improved the HOMA score, which is a surrogate measure of insulin resistance in a fasting state and tends to represent hepatic insulin resistance[32], which is consistent with the therapeutic effect of a DPP-4 inhibitor (P32/98) in the Vancouver diabetic fatty

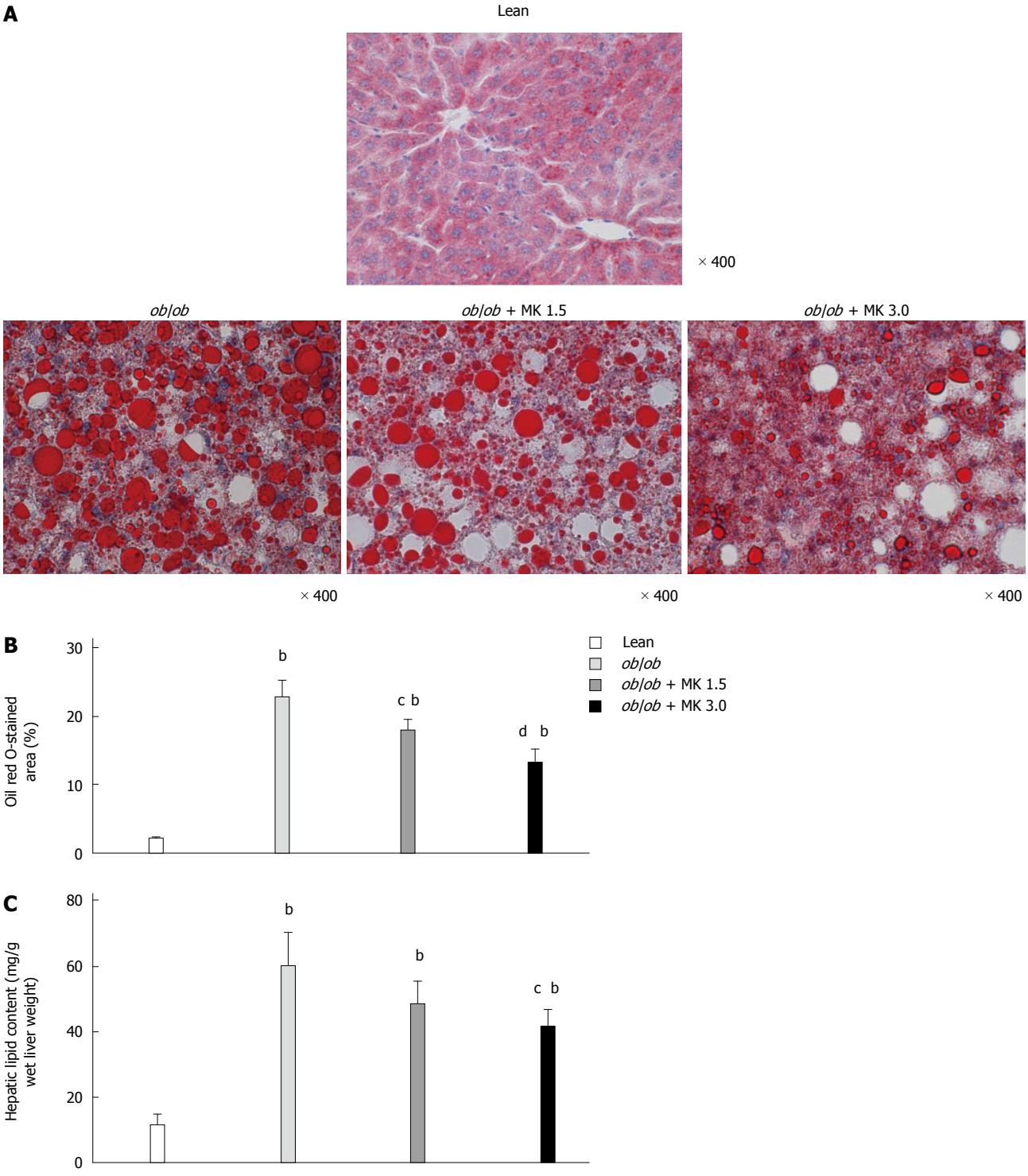

Figure 2 Assessment of hepatic histology and lipid content in the livers of *ob/ob* mice and their lean littermates at the end of an 8-wk treatment period. The groups are the same as those shown in Figure 1; A: Representative hepatic histology of lean, *ob/ob*, *ob/ob* + MK 1.5 and *ob/ob* + MK 3.0 groups. Liver sections were stained with Oil red O; B: Quantitative histomorphometric analysis for total lipid content of all hepatic histology for each experimental group. Oil red O-stained areas were quantified in 10 microscopic fields at a 400-fold magnification; C: Quantification of the lipid content of the liver from each experimental group. Results are expressed as the mean ± SD; n =16 for each group. $^{b}P < 0.01$ *vs* the lean group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the *ob/ob* group.

Zucker Rat[33] and a fructose-rich diet in normal rats[10] or sitagliptin in high-fat diet-induced obese rats[12]. However, normoglycemic DPP4-deficient rats did not show improved hepatic insulin sensitivity *in vivo* and maintained constant high levels of active GLP-1, implying that active GLP-1 has a direct effect on fat metabolism in a DPP4-deficient rat model[9]. Thus, dietary MK-0626 may improve insulin sensitivity when coupled with the direct effect of serum active GLP-1. A DPP-4 inhibitor may also be effective for nonalcoholic steatohepatitis in which insulin resistance plays a central role. However, there is a contradictory report that the HOMA-IR was not signifi-

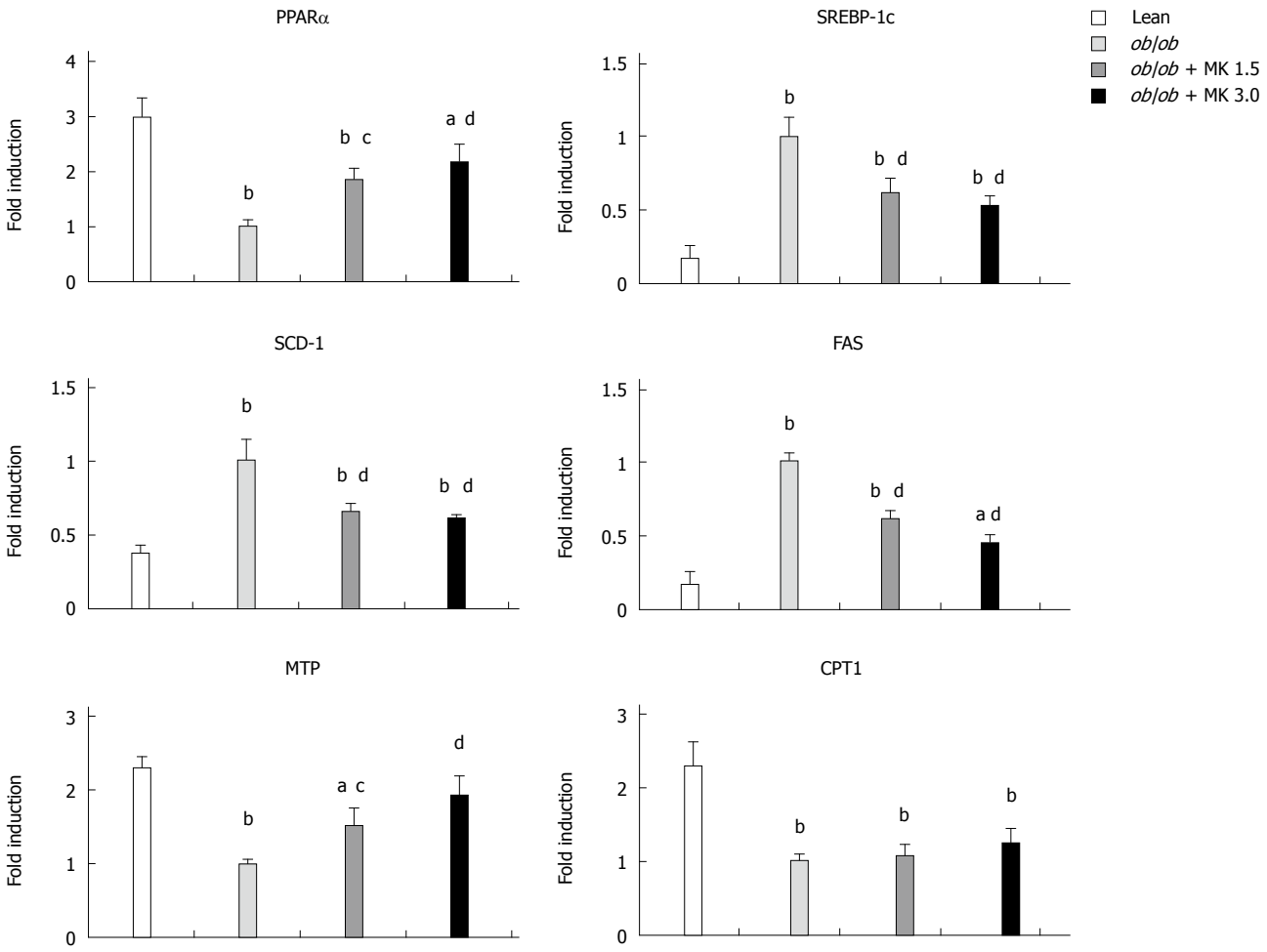

Figure 3 Effect of MK-0626 on the mRNA expression of genes related to hepatic fatty acid metabolism from whole livers at the end of the 8-wk treatment period in each experimental group. The groups are the same as those shown in Figure 1. Total RNA extracted from liver tissues was used for mRNA expression analysis of peroxisome proliferator-activated receptor (PPAR)α, sterol regulatory element binding transcription factor (SREBP)-1c, stearoyl-CoA desaturase (SCD)-1, fatty acid synthase (FAS), microsomal triglyceride transfer protein (MTP) activity and carnitine palmitoyltransferase (CPT)-1. Results are expressed as the mean ± SD; n = 16 for each group. [a]$P < 0.05$, [b]$P < 0.01$ *vs* the lean group; [c]$P < 0.05$, [d]$P < 0.01$ *vs* the *ob/ob* group.

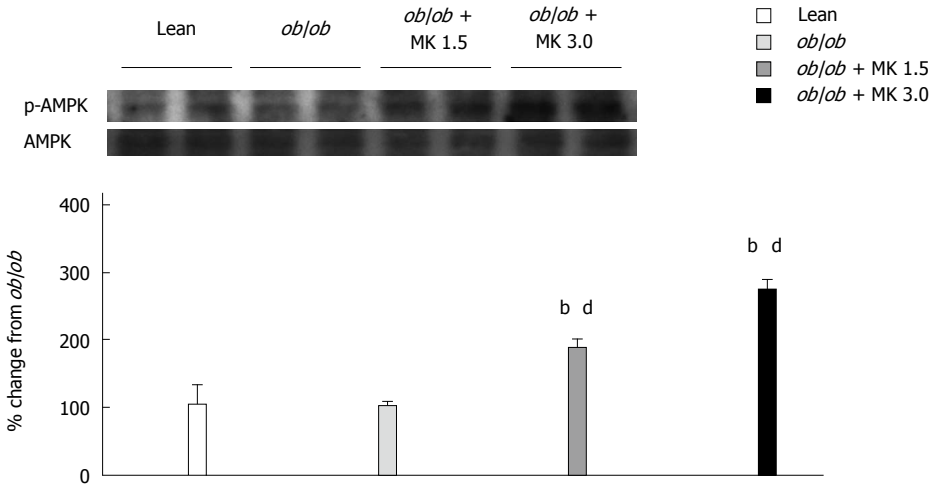

Figure 4 Phosphorylation of AMP-activated protein kinase following MK-0626 treatment in whole livers from each experimental group. The groups are the same as those shown in Figure 1. A representative western blot shows phosphorylation of AMP-activated protein kinase (AMPK) at the end of the 8-wk treatment period. The histogram below shows the expression of p-AMPK normalized to AMPK. Results are expressed as the mean ± SD; n = 16 for each group. [b]$P < 0.01$ *vs* the lean group; [d]$P < 0.01$ *vs* the *ob/ob* group.

cantly changed with sitagliptin treatment in patients with type 2 diabetes[27].

In summary, we demonstrated that dietary MK-0626, a DPP-4 inhibitor, could attenuate hepatic steatosis by enhancing AMPK activity, inducing inhibition of hepatic lipogenic gene expression, enhancing triglyceride secretion from the liver and increasing serum adiponectin levels. Thus, some novel mechanisms of a DPP-4 inhibitor on hepatic steatosis were discovered in our study. Moreover, our study design was original and persuasive because of the following reasons: (1) we evaluated the dose response effect of a DPP-4 inhibitor on hepatic steatosis; (2) we used a naturally occurring model of hepatic steatosis and type 2 diabetes; (3) we used two distinct control groups; and (4) we used a sufficient number of mice in each group to achieve statistical power of the data outcomes. Because DPP-4 inhibitor medications are widely used in clinical practice and are characterized by good safety and tolerability profiles, these inhibitors may provide an effective treatment strategy for patients with hepatic steatosis induced by type Ⅱ diabetes. Thus, the effects of a DPP-4 inhibitor on hepatic steatosis should be further evaluated in a large, prospective human study in the future.

ACKNOWLEDGMENTS

The Nature Publishing Group Language Editing service provided writing assistance.

COMMENTS

Background

Nonalcoholic fatty liver disease is the hepatic manifestation of metabolic syndrome. Other comorbidities of metabolic syndrome include obesity, type 2 diabetes mellitus, hypertension and dyslipidemia coupled with insulin resistance, which is a central feature of metabolic syndrome. Thus, improving insulin sensitivity would decrease hepatic fat deposition and accordingly inhibit hepatocyte vulnerability to oxidative stress.

Research frontiers

Administration of MK-0626, a dipeptidyl peptidase-4 inhibitor, ameliorated hepatic steatosis; reduced serum alanine aminotransferase, glucose, and insulin levels; reduced homeostatic model assessment scores; and increased serum adiponectin levels in *ob/ob* mice.

Innovations and breakthroughs

MK-0626 could attenuate hepatic steatosis through enhancing AMP-activated protein kinase activity, inhibiting hepatic lipogenic gene expression, enhancing triglyceride secretion from liver and increasing serum adiponectin levels.

Peer review

The major finding of this experimental study was that MK-0626 attenuated liver steatosis. Interestingly authors demonstrated that this effect is not due to a decrease in food intake and/or body weight. They suggested that possible mechanisms are the inhibition of hepatic lipogenic gene expression, the enhancement of triglyceride secretion from the liver and the increase of serum adiponectin levels.

REFERENCES

1 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]

2 **Kieffer TJ**, Habener JF. The glucagon-like peptides. *Endocr Rev* 1999; **20**: 876-913 [PMID: 10605628]

3 **Meier JJ**, Nauck MA. Clinical endocrinology and metabolism. Glucose-dependent insulinotropic polypeptide/gastric inhibitory polypeptide. *Best Pract Res Clin Endocrinol Metab* 2004; **18**: 587-606 [PMID: 15533777]

4 **Drucker DJ**, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696-1705 [PMID: 17098089]

5 **Drucker DJ**. Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* 2002; **122**: 531-544 [PMID: 11832466]

6 **Drucker DJ**. The biology of incretin hormones. *Cell Metab* 2006; **3**: 153-165 [PMID: 16517403]

7 **Mentlein R**, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993; **214**: 829-835 [PMID: 8100523]

8 **Weber AE**. Dipeptidyl peptidase IV inhibitors for the treatment of diabetes. *J Med Chem* 2004; **47**: 4135-4141 [PMID: 15293982]

9 **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]

10 **Maiztegui B**, Borelli MI, Madrid VG, Del Zotto H, Raschia MA, Francini F, Massa ML, Flores LE, Rebolledo OR, Gagliardino JJ. Sitagliptin prevents the development of metabolic and hormonal disturbances, increased β-cell apoptosis and liver steatosis induced by a fructose-rich diet in normal rats. *Clin Sci* (Lond) 2011; **120**: 73-80 [PMID: 20795946 DOI: 10.1042/cs20100372]

11 **Shirakawa J**, Fujii H, Ohnuma K, Sato K, Ito Y, Kaji M, Sakamoto E, Koganei M, Sasaki H, Nagashima Y, Amo K, Aoki K, Morimoto C, Takeda E, Terauchi Y. Diet-induced adipose tissue inflammation and liver steatosis are prevented by DPP-4 inhibition in diabetic mice. *Diabetes* 2011; **60**: 1246-1257 [PMID: 21330637 DOI: 10.2337/db10-1338]

12 **Akaslan SB**, Degertekin CK, Yilmaz G, Cakir N, Arslan M, Toruner FB. Effects of sitagliptin on nonalcoholic fatty liver disease in diet-induced obese rats. *Metab Syndr Relat Disord* 2013; **11**: 243-250 [PMID: 23544853 DOI: 10.1089/met.2012.0128]

13 **Souza-Mello V**, Gregório BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. *Clin Sci* (Lond) 2010; **119**: 239-250 [PMID: 20415664 DOI: 10.1042/CS20100061]

14 **Iwasaki T**, Yoneda M, Inamori M, Shirakawa J, Higurashi T, Maeda S, Terauchi Y, Nakajima A. Sitagliptin as a novel treatment agent for non-alcoholic Fatty liver disease patients with type 2 diabetes mellitus. *Hepatogastroenterology* 2011; **58**: 2103-2105 [PMID: 22024083 DOI: 10.5754/hge11263]

15 **Maffei M**, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995; **1**: 1155-1161 [PMID: 7584987]

16 **Campfield LA**, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; **269**: 546-549 [PMID: 7624778]

17 **Halaas JL**, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543-546 [PMID: 7624777]

18 **Pelleymounter MA**, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on

body weight regulation in ob/ob mice. *Science* 1995; **269**: 540-543 [PMID: 7624776]

19 **Edmondson SD**, Mastracchio A, Mathvink RJ, He J, Harper B, Park YJ, Beconi M, Di Salvo J, Eiermann GJ, He H, Leiting B, Leone JF, Levorse DA, Lyons K, Patel RA, Patel SB, Petrov A, Scapin G, Shang J, Roy RS, Smith A, Wu JK, Xu S, Zhu B, Thornberry NA, Weber AE. (2S,3S)-3-Amino-4-(3,3-difluoropyrrolidin-1-yl)-N,N-dimethyl-4-oxo-2-(4-[1-2,4]triazolo[1,5-a]-pyridin-6-ylphenyl)butanamide: a selective alpha-amino amide dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* 2006; **49**: 3614-3627 [PMID: 16759103]

20 **Folch J**, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; **226**: 497-509 [PMID: 13428781]

21 **Garber A**, Henry R, Ratner R, Garcia-Hernandez PA, Rodriguez-Pattzi H, Olvera-Alvarez I, Hale PM, Zdravkovic M, Bode B. Liraglutide versus glimepiride monotherapy for type 2 diabetes (LEAD-3 Mono): a randomised, 52-week, phase III, double-blind, parallel-treatment trial. *Lancet* 2009; **373**: 473-481 [PMID: 18819705 DOI: 10.1016/s0140-6736(08)61246-5.Epub-2008Sep24]

22 **Nelson P**, Poon T, Guan X, Schnabel C, Wintle M, Fineman M. The incretin mimetic exenatide as a monotherapy in patients with type 2 diabetes. *Diabetes Technol Ther* 2007; **9**: 317-326 [PMID: 17705687]

23 **Blonde L**, Klein EJ, Han J, Zhang B, Mac SM, Poon TH, Taylor KL, Trautmann ME, Kim DD, Kendall DM. Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes. *Diabetes Obes Metab* 2006; **8**: 436-447 [PMID: 16776751]

24 **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]

25 **Scott R**, Wu M, Sanchez M, Stein P. Efficacy and tolerability of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy over 12 weeks in patients with type 2 diabetes. *Int J Clin Pract* 2007; **61**: 171-180 [PMID: 17156104]

26 **Hanefeld M**, Herman GA, Wu M, Mickel C, Sanchez M, Stein PP. Once-daily sitagliptin, a dipeptidyl peptidase-4 inhibitor, for the treatment of patients with type 2 diabetes. *Curr Med Res Opin* 2007; **23**: 1329-1339 [PMID: 17559733]

27 **Rosenstock J**, Sankoh S, List JF. Glucose-lowering activity of the dipeptidyl peptidase-4 inhibitor saxagliptin in drug-naive patients with type 2 diabetes. *Diabetes Obes Metab* 2008; **10**: 376-386 [PMID: 18355324 DOI: 10.1111/j.1463-1326.2008.00876.x.Epub2008Mar18]

28 **Furuta Y**, Horiguchi M, Sugaru E, Ono-Kishino M, Otani M, Sakai M, Masui Y, Tsuchida A, Sato Y, Takubo K, Hochigai H, Kimura H, Nakahira H, Nakagawa T, Taiji M. Chronic administration of DSP-7238, a novel, potent, specific and substrate-selective DPP IV inhibitor, improves glycaemic control and beta-cell damage in diabetic mice. *Diabetes Obes Metab* 2010; **12**: 421-430 [PMID: 20415690]

29 **Yanai H**, Adachi H, Hamasaki H, Masui Y, Yoshikawa R, Moriyama S, Mishima S, Sako A. Effects of 6-month sitagliptin treatment on glucose and lipid metabolism, blood pressure, body weight and renal function in type 2 diabetic patients: a chart-based analysis. *J Clin Med Res* 2012; **4**: 251-258 [PMID: 22870172 DOI: 10.4021/jocmr975w.Epub2012Jul20]

30 **Shehzad A**, Iqbal W, Shehzad O, Lee YS. Adiponectin: regulation of its production and its role in human diseases. *Hormones* (Athens) 2012; **11**: 8-20 [PMID: 22450341]

31 **Kirino Y**, Sei M, Kawazoe K, Minakuchi K, Sato Y. Plasma dipeptidyl peptidase 4 activity correlates with body mass index and the plasma adiponectin concentration in healthy young people. *Endocr J* 2012; **59**: 949-953 [PMID: 22785237]

32 **Rutter MK**, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes* 2005; **54**: 3252-3257 [PMID: 16249452]

33 **Pospisilik JA**, Stafford SG, Demuth HU, McIntosh CH, Pederson RA. Long-term treatment with dipeptidyl peptidase IV inhibitor improves hepatic and peripheral insulin sensitivity in the VDF Zucker rat: a euglycemic-hyperinsulinemic clamp study. *Diabetes* 2002; **51**: 2677-2683 [PMID: 12196458]

P- Reviewer: Di Minno MND, Navarro-Jarabo JM
S- Editor: Gou SX **L- Editor**: A **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327