GLP-1 analog liraglutide protects against cardiac steatosis, oxidative stress and apoptosis in streptozotocin-induced diabetic rats

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Objective: Accumulating evidence has implicated that GLP-1 may have a beneficial effect on cardiovascular but the mechanism is not fully understood. Here we show that GLP-1 analog, liraglutide, inhibits cardiac steatosis, oxidative stress and apoptosis in streptozotocin (STZ)-induced type 1 diabetic rats, via activation of AMPK-Sirt1 pathway.

Methods: Diabetic rats were treated with subcutaneous injections of liraglutide (0.3 mg/kg/12 h) for 4 weeks. Myocardial steatosis (detected by oil red O staining and myocardial triglyceride and diacylglycerol (DAG) contents assay), expression of protein kinase C (PKC), heart NADPH oxidase activity, oxidative stress markers (8-hydroxy-2’-deoxyguanosine staining), apoptosis (TUNEL analysis) and genes that affect apoptosis and lipid metabolism were evaluated.

Results: Administration of liraglutide did not affect plasma glucose and insulin levels, body weights in STZ-induced diabetic rats, but normalized myocardial steatosis, expression of PKC, NADPH oxidase activity, oxidative stress markers and apoptosis, all of which were significantly increased in diabetic hearts. Additionally, expression of genes mediating lipid uptake, synthesis and oxidation were increased in the diabetic hearts, and these increases were all reduced by liraglutide. In addition, liraglutide increased expression of Sirt1 and phosphorylated AMPK in the diabetic hearts.

Conclusions: Liraglutide may have a beneficial effect on cardiac steatosis, DAG-PKC-NADPH pathway, oxidative stress and apoptosis via activation of AMPK-Sirt1 pathway, independently of a glucose-lowering effect.

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1. Introduction

Diabetes mellitus is a well-recognized risk factor for developing heart failure. Diabetic cardiomyopathy is defined as left ventricular (LV) dysfunction that occurs independent of coronary artery disease and hypertension [1], and it is well established as a cause of heart failure in diabetic patients. Myocardial triglyceride (TG) content is significantly higher in patients with prediabetes or diabetes than in healthy individuals [2,3] and is associated with impaired left ventricular diastolic function [2]. Lipid overload results in the accumulation of lipid intermediates, such as diacylglycerol (DAG), which activate protein kinase C (PKC) [4] and the production of reactive oxygen species (ROS) [5,6], which can promote apoptosis [7]. Activation of PKC, oxidative stress and myocardial apoptosis are implicated in diabetes-induced cardiovascular complications [5,9].

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid gut hormone secreted in a nutrient-dependent manner that stimulates insulin secretion and inhibits glucagon secretion and gastric emptying, resulting in reduced postprandial glycemia [10,11]. The GLP-1 receptor was originally identified in ileal beta cells but is widely expressed in extrapancreatic tissues, including the lung, kidney, central nervous system (CNS), enteric and peripheral

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nervous system lymphocytes, blood vessels, and heart [12–14]. GLP-1 agonists are a class of drugs approved for the treatment of diabetes that have significant beneficial cardiovascular effects [14]. In fact, GLP-1 receptor agonists have been reported to have direct beneficial effects, such as improving left ventricular performance after myocardial infarction [15] and protecting against cardiac ischemia [16] and the progression of atherosclerosis [17]. Additionally, it has been suggested that incretin-based therapy may represent a novel therapeutic strategy for the treatment of heart failure patients with diabetes, in particular for their cardioprotective effects independent of those attributable to tight glycemic control [18]. However, the mechanisms underlying the effect of GLP-1 receptor agonists on the diabetic heart have not been elucidated. In this study, we show that GLP-1 analog liraglutide protects against myocardial steatosis, oxidative stress and apoptosis via activation of the S' AMP-activated protein kinase (AMPK)-Sirt1 pathway in the streptozotocin (STZ)-induced type 1 diabetes mellitus rat model.

2. Methods

2.1. Animals

Male Wistar rats were purchased from Clea Japan Inc. (Tokyo, Japan) and given standard rat chow and water ad libitum. Diabetes was induced in 7-week-old rats by injecting STZ (Sigma, St. Louis, MO, USA) in 0.1 mol/L citrate buffer pH 4.5 at a dose of 80 mg/kg body weight. Rats with plasma blood glucose levels >16.7 mmol/L were considered diabetic. Rats given injection of citrate buffer alone served as non-diabetic controls. One week after the induction of diabetes, half of the non-diabetic rats (n = 8) and half of the diabetic rats (n = 8) were randomly selected and treated with subcutaneous injections of the GLP-1 analog liraglutide for 4 weeks at a dose of 0.3 mg/kg/12 h as previously reported [19,20]. Twice daily dosing was used because the pharmacokinetic half-life of liraglutide is only approximately 4 h in rats [20]. The remaining non-diabetic rats (n = 8) and diabetic rats (n = 8) were treated with injections of phosphate buffer and served as controls. After 4 weeks, blood samples were obtained from the dorsal tail vein. Plasma glucose and insulin concentrations were determined using the glucose oxidase method and enzyme-linked immunosorbent assays (ELISA) (Morinaga Institute of Biological Science, Yokohama, Japan), respectively. All rats were anesthetized by pentobarbital (0.1 mg/kg intraperitoneal injection) and sacrificed. The heart was rapidly dissected, frozen in liquid nitrogen, and stored at -80 °C until use. All protocols were reviewed and approved by the Committee on the Ethics of Animal Experiments, Graduate School of Medical Sciences, Kyushu University.

2.2. Tissue preparation and histological analysis

Serial 10-μm-thick sections of each heart were prepared using a sliding Coldtrime (Sakura Fine Technical Co. Ltd., Tokyo, Japan). For histological analysis, the sections were collected on glass slides, stained with oil red O and counterstained with hematoxylin to identify intramyocardial lipid deposits. The stained sections were observed under a bright-field illumination microscope (BZ-9000, Keyence, Osaka, Japan).

2.3. Measurement of cardiac TG and DAG contents

Heart TG content was assayed using a Triglyceride Quantification Kit (BioVision, Mountain View, CA, USA), in accordance with the manufacturer’s instructions. Briefly, the heart tissue was perfused with phosphate-buffered saline (PBS) and homogenized in 5% Triton-X100 in water. Samples were slowly heated to 80 °C for 5 min. Insoluble materials were removed by centrifugation. The TG concentration in the supernatant was determined using the enzyme-based colorimetric assay.

For biochemical analysis of DAG, lipids were extracted from heart tissue using the Folch partition method [21]. Briefly, the heart tissue was homogenized in 2 ml of 100% methanol for 30 s. After adding 2 ml of chloroform and 1 ml of H2O to the homogenates, the samples were allowed to stand for 30 min at room temperature. After centrifugation at 500 ×g for 10 min, the lower phase was collected. The upper phase was mixed with 4 ml chloroform and DAGs were re-extracted as outlined above. The lower phases (containing lipid) from both centrifugation steps were combined and dried under nitrogen gas. The total DAG contents were measured by high-performance liquid chromatography–tandem mass spectrometry as previously described [6,22].

2.4. Immunohistochemistry

Heart tissues were fixed in 10% formaldehyde and embedded in paraffin. Sections (5-μm-thick) were deparaffinized and dehydrated with xylene and ethanol. Antigen retrieval was performed in 10 mmol/L citrate buffer with 0.1% Nonidet P-40 (Sigma) in a microwave oven. After inactivation of endogenous peroxidase with 3% H2O2 in methanol for 15 min at room temperature, the sections were preincubated for 30 min with 1% bovine serum albumin in PBS. The samples were subsequently incubated with anti-pan PKC mouse polyclonal antibody (1:50 dilution) overnight at 4 °C, washed in PBS, and probed with anti-rabbit IgG antibody labeled with peroxidase (Histofine Simple Stain MAX PO(R), Nichirei Corp., Tokyo, Japan) for 30 min at room temperature. As a negative control, the primary antibody was replaced with sera obtained from a rabbit before immunization. The sites of peroxidase were visualized with diaminobenzidine (Nichirei Corp.). The sections were counterstained with hematoxylin before being examined under a bright-field illumination microscope (BZ-9000, Keyence). For 8-hydroxy-2'-deoxyguanosine (8-OHdG) staining, the samples were incubated with anti-8-OHdG mouse monoclonal antibody (4 μg/ml) (Japan Institute for the Control of Aging, Fukuroi, Japan) overnight at 4 °C. The sections were then incubated with biotinylated anti-mouse immunoglobulin serum for 30 min, followed by incubation with peroxidase-labeled streptavidin using a Histofine SAB-PO kit (Nichirei Corp.) for 15 min at room temperature. The peroxidase was then visualized with diaminobenzidine. The density of 8-OHdG staining was analyzed using Adobe Photoshop (version 8.0; Adobe Systems, Mountain View, CA, USA), as previously reported [23].

2.5. Western blotting analysis

For total protein extracts and western blotting analysis of PKC, phospho-PKC, CD36, PPARγ, acetyl-CoA carboxylase (ACC), Sirt1, AMPK and phospho-AMPK, heart tissues were homogenized in lysis buffer (0.25 mol/L sucrose, 1 mmol/L EDTA) supplemented with protease inhibitor cocktail (Sigma) and phosphatase inhibitors (Sigma) and centrifuged for 10 min at 16,000 ×g. Protein concentrations were determined using a BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). Subsequently, 30 μg protein/lane was separated discontinuously on 4–15% sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA, USA). After blocking the nonspecific binding, the membranes were incubated overnight at 4 °C with anti-pan PKC (α, β, β', γ, δ, ε, η, θ, and z) (1:1000; Novus Biologicals, Littleton, CO, USA), phospho-specific anti-pan PKC (1:1000; Cell Signaling Technology, Danvers, MA, USA), anti-CD36 (1:1000; Abcam, Cambridge, UK), anti-PPARγ (1:3000; Abcam),
anti-ACC (1:1000; Cell Signaling Technology), anti-Sirt1 (1:1000; Santa Cruz, Santa Cruz, CA, USA), anti-AMPK α (1:1000; Cell Signaling Technology), phospho-specific anti-AMPK α (1:1000; Cell Signaling Technology) or anti-β-tubulin mouse polyclonal (1:10,000; Santa Cruz) antibodies, followed by horseradish peroxidase-conjugated sheep anti-mouse IgG antibody (1:10,000; Amersham Pharmacia Biosciences, Buckinghamshire, UK) or donkey anti-rabbit IgG antibody (1:10,000; Amersham) as a secondary antibody. We used the ECL Plus system (Amersham) to detect the protein bands.

2.6. NAD(P)H oxidase activity

NAD(P)H oxidase activity was assayed in heart homogenates as NAD(P)H-dependent superoxide generation measured using lucigenin-enhanced chemiluminescence as previously described [24]. After heart homogenates were preincubated with dark-adapted lucigenin (10 μM/L) for 10 min at 37 °C, NAD(P)H (100 μM/L) was added immediately before recording. Light emission was recorded every 15 s for 60 min and was expressed as relative light units (RLU). An NAD(P)H oxidase inhibitor, diphenyleneiodonium chloride (DPI) (10 μM), was preincubated with heart homogenates for 10 min to demonstrate the experimental specificity for NAD(P)H oxidase activity. Experiments were performed in triplicate; all results are from at least three independent experiments. Superoxide production was calculated as the sum of the RLU per micromass of protein.

2.7. RNA extraction and quantitative RT-PCR

Total RNA was extracted from frozen heart samples using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer’s instructions. Extracted RNA (1 μg) was converted to single-stranded cDNA using a QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA). The mRNA levels were quantified by quantitative RT-PCR using an iTag SYBR Green mix (Bio-Rad) with the Bio-Rad Chromo 4/Option system. PCR reactions for each target cDNA were done at the conditions shown in Supplemental Tables 1, 2, and 3.

The linearity of the amplifications as a function of cycle number was tested in preliminary experiments. The mRNA expression levels of each gene were normalized to the expression levels of the housekeeping gene β-actin.

2.8. TUNEL analysis

Heart tissues were fixed in 10% formaldehyde and embedded in paraffin, and 5-μm sections were prepared. The DeadEnd Fluorometric TUNEL System kit (Promega, Madison, WI, USA) was used to detect apoptotic nuclei according to the manufacturer’s instructions. The Vectashield with DAPI mounting solution (Vector, Burlingame, CA, USA) was used for visualization of nuclei. Photographs were taken with a Nikon A1Si confocal microscope (Nikon Instech Co. Ltd., Tokyo, Japan) and the results are expressed as the percentage of TUNEL-positive nuclei.

2.9. Statistical analysis

All data are expressed as the means ± SEM. Statistical analysis was performed with Student’s t-test or one-way analysis of variance (ANOVA) with Fisher’s protected least significant difference (PLSD) test. A value of P < 0.05 was considered statistically significant.

3. Results

3.1. Myocardial lipid contents

Administration of liraglutide (0.3 mg/kg/12 h) was started 1 week after the onset of diabetes. Characteristics of the experimental rats are shown in Supplementary Table 4 and Supplementary Fig. 1. Liraglutide significantly decreased body weight by day 28 in control rats but not in diabetic rats. It did not affect the plasma glucose and insulin levels in either group. Liraglutide also decreased food intake significantly in control rats but not in diabetic rats.

After administration of liraglutide for 4 weeks, we first evaluated myocardial steatosis and found that it was more pronounced in the hearts of diabetic rats and was reduced by liraglutide (Fig. 1A–D). Next, we evaluated intramyocardial lipid deposition using oil red O staining. Lipid depositions in the hearts of diabetic rats were significantly pronounced compared with the hearts of control rats and were significantly reduced by liraglutide (Fig. 1E–I). Consistent with the histological findings, the heart TG and DAG contents were higher in the diabetic rats than in the control rats and were significantly reduced by liraglutide (Fig. 1M,N). The heart DAG contents were lower in the liraglutide-treated nondiabetic rats than in the control rats, and TG contents in the hearts of liraglutide-treated nondiabetic rats were presented a decreased trend compared with the control rats, but had no statistical significance (P = 0.07).

3.2. PKC-NAD(P)H activity, oxidative stress and apoptosis in the heart

Lipid overload results in an increased accumulation of lipid intermediates such as DAG, which activate PKC in the diabetic heart [4]. Therefore, we examined the effect of liraglutide on PKC expression. Immunostaining analysis for PKC using anti-pan PKC antibody showed that the expression of PKC was remarkably increased in the cardiomyocytes of STZ-induced diabetic rats (Fig. 2CG) compared with that of the control rats (Fig. 2AE). These increases in PKC expression were completely suppressed by liraglutide (Fig. 2D,H). We did not observe differences in PKC expression between control rats and liraglutide-treated nondiabetic rats (Fig. 2BF). Western blotting analyses of the LV tissue homogenates using anti-pan PKC antibody showed that the protein expression of PKC was 2.5-fold higher in STZ-induced diabetic rats compared with the control rats (P < 0.0001), and this increase was significantly reduced by liraglutide (Fig. 2I). Phosphorylated forms of PKC, which are activated PKC, were also significantly higher in the STZ-induced diabetic rats compared with the control rats (P < 0.0005), and this increase was reduced by liraglutide to the control levels (Fig. 2J). PKC activates NAD(P)H oxidase, which produces ROS. Therefore, we evaluated the NAD(P)H oxidase activities using the lucigenin-enhanced chemiluminescence method. Liraglutide administration significantly suppressed diabetes-induced activation of NAD(P)H oxidase in heart homogenates to control levels (Fig. 3A). Next, we performed immunostaining for 8-OHdG in the LV sections of rats to evaluate oxidative stress status in the heart. Diabetic rats showed high 8-OHdG staining compared with the control rats (Fig. 3D,H), and it was remarkably ameliorated by liraglutide (Fig. 3EJ). Additionally, we examined the effect of liraglutide on apoptosis in the hearts of diabetic rats. The mRNA levels of Bax and Caspase-3 were markedly increased in diabetic hearts, and this increase was reduced by liraglutide (Fig. 4A). In addition, cardiomyocyte apoptosis was evaluated by TUNEL assay. TUNEL-positive cells were markedly
increased in diabetic hearts, and this increase was reduced by liraglutide (Fig. 4B–F).

3.3. Effect of liraglutide treatment on the heart expressions of genes that affect lipid metabolism

Next, we investigated whether the reduction, observed in liraglutide-treated diabetic rats in the myocardial lipid content, is the result of liraglutide modulation of heart lipid metabolism. We evaluated the expression of genes mediating lipid uptake, synthesis and oxidation, such as fatty acid translocase (CD36), fatty acid synthase (ACC1), fatty acid oxidation (ACC2), DAG synthase (glycerol-3-phosphate acyltransferase (GPAT) and monoaclglycerol acyltransferase (MGAT)) and TG synthesis (diacylglycerol acyltransferase (DGAT)). CD36 is a major transporter for long chain fatty acids in the heart. Peroxisome proliferator-activated receptor gamma (PPARγ) is essential for the basal regulation of CD36, and many factors regulate CD36 expression through a PPARγ-dependent mechanism [25]. The mRNA and protein levels of CD36 were markedly increased in diabetic hearts, and this increase was reduced by liraglutide (Fig. 5A,B). Likewise, we found that PPARγ mRNA and protein levels were increased in
diabetic hearts, and this increase was reduced by liraglutide (Fig. 5A,C). We then evaluated the effect of liraglutide treatment on GPAT, MGATs and DGATs, which catalyze two consecutive steps in the synthesis of TG. The mRNA levels of GPAT, MGAT1, MGAT2 and DGAT2 were increased in the hearts of STZ-induced diabetic rats, and these increases were all reduced by liraglutide (Fig. 5A). Furthermore, we examined the effect of liraglutide treatment on the expression of ACC, which regulates fatty acid synthesis and oxidation [26]. The mRNA levels of ACC were markedly increased in diabetic hearts, and this increase was reduced by liraglutide (Fig. 5A). Western blot analysis confirmed that the protein levels of ACC in the heart homogenates of diabetic rats were significantly decreased by liraglutide (Fig. 5D).

3.4. Effects of liraglutide treatment on regulation of Sirt1 and AMPK

Sirt1 and AMPK are signaling molecules that control lipid metabolism by deacetylation of acetylated lysine residues on intracellular NAD+/NADH ratios and phosphorylation via an increase in the AMP/ATP ratio, respectively [27,28]. The effect of liraglutide treatment on mRNA expression of heart Sirt1 was measured by RT-PCR. As shown in Fig. 5A, mRNA expression of Sirt1 was significantly increased in the hearts of liraglutide-treated diabetic rats. Western blot analysis confirmed that the protein levels of Sirt1 in the heart homogenates of diabetic rats were significantly increased by liraglutide (Fig. 5E).

Next, we evaluated the effect of liraglutide on phosphorylated
Fig. 3. Effect of liraglutide on oxidative stress. (A) NAD(P)H-dependent ROS generation in heart homogenates were assessed by lucigenin-enhanced chemiluminescence. Bars represent the mean ± SEM (n = 6); #P < 0.0001 vs. control, *P < 0.0001 vs. DPI and "P < 0.0001 (ANOVA). Open bars, control rats; closed bars, diabetic rats. (B–I) The sections were cut through whole hearts and then immunostained with anti-8-OHdG antibody. Brown, diamino-benzidine stain. (B, F) control rats, (C, G) liraglutide-treated control rats, (D, H) diabetic rats or (E, I) liraglutide-treated diabetic rats (B–E, at ×20-magnification, F–I, at ×200-magnification). (J) Relative intensities of 8-OHdG staining levels were compared to controls. Bars represent the means ± SEM: *P < 0.0001 (ANOVA). Open bars, control rats; closed bars, diabetic rats.
AMPK and found that these were decreased in the hearts of diabetic rats, and this decrease was reversed by liraglutide (Fig. 5F).

**4. Discussion**

Cardiac steatosis recently has been described as an important feature of disturbed myocardial substrate metabolism [29,31]. This most likely contributes to the development of heart failure in patients with diabetes, even in the absence of coronary artery disease and arterial hypertension (diabetic cardiomyopathy) [1]. In the present study, we initially found that liraglutide prevents cardiac steatosis in the heart of diabetic rats (Fig. 1) independent of a glucose-lowering effect. These data suggest that liraglutide may have a direct beneficial effect on diabetic cardiomyopathy. The lipid overload results in activation of the DAG-PKC-NADPH pathway, which produces ROS [5]. In recent years, oxidative stress has been considered an important pathogenic factor in the development of diabetic vascular complications [28–31]. Accumulating evidence shows that many protein, lipid and DNA markers of oxidative stress are increased in the kidney and vascular tissues from animals and patients with diabetes [31–33]. In the present study, we showed that liraglutide prevents increased PKC-NADPH activity and oxidative stress markers in the diabetic heart. These data suggest that liraglutide may have a cardioprotective effect by inhibiting oxidative stress. PKC activation and oxidative stress stimulate cellular apoptosis [34], and myocyte apoptosis is suggested to be involved in the development of diabetic cardiomyopathy [9]. Diabetes-induced cardiomyocyte apoptosis has been shown in human patients [35] and various animal models [36]. The rates of apoptotic cells in cardiomyocytes of diabetic rats that we showed here was as the same as those in previous report [36]. The present study showed that liraglutide prevents cardiomyocyte apoptosis in
Fig. 5. Effect of liraglutide on expressions of genes and proteins that affect lipid metabolism in the hearts of diabetic rats. (A) The mRNA expressions of CD36, PPARγ, GPAT, MGAT1, MGAT2, DGAT1, ACC1, ACC2, SREBP1c and Sirt1 were detected by real-time RT-PCR. Bars represent the means ± SEM (n = 8); *P < 0.05, **P < 0.005 and ***P < 0.0005 (ANOVA). (B–F) Western blotting was performed using anti-CD36, anti-PPARγ, anti-ACC, anti-Sirt1, anti-AMPK, anti-phospho-specific (p)-AMPK or anti-β-tubulin antibodies. Bars represent the means ± SEM (n = 6); *P < 0.05, **P < 0.005 and ***P < 0.0005 (ANOVA). Open bars, control rats; closed bars, diabetic rats.
diabetic rats in parallel with the prevention of cardiac steatosis and oxidative stress.

Cardiac steatosis could be due to an increased myocardial fatty acid uptake. Fatty acids can be taken up by passive diffusion (20%) although most of the transport is protein-mediated (80%) [37]. This protein-mediated transport of fatty acid depends largely on the plasma membrane transporter CD36 [38]. In this present study, we found that iraglutide regulates the expression of CD36 in the diabetic heart. PPARα is essential for the basal regulation of CD36, and many factors regulate CD36 expression through a PPAR-dependent mechanism [25]. We showed that iraglutide down-regulates PPARα expression in the hearts of diabetic rats in parallel with downregulation of CD36. It has been reported that Sirt1 represses PPARα by docking with its cofactors NCoR (nuclear receptor co-repressor) and SMRT (silencing mediator of retinoid and thyroid hormone receptors) [39] and that AMPK activates Sirt1 by increasing the NAD+/NADH ratio [40]. The present study showed that iraglutide increased expression of Sirt1 and phosphorylated AMPK in the diabetic heart. Additionally, we showed that iraglutide increases phosphorylation of AMPK and inhibits DAG–PKC pathway in vitro study [41]. In consisting with our findings, Baiteru et al. [42] recently reported that GLP-1 induces AMPK activation and inhibits PKC phosphorylation in cardiomyocytes, and the effect of GLP-1 is lost in AMPK knockout cells. Therefore, it was suggested that iraglutide may inhibit cardiac steatosis and DAG–PKC pathway in the diabetic heart by downregulation of PPARα and CD36 via activation of the AMPK-Sirt1 pathway.

TG and DAG are synthesized through two major pathways, the glycerol phosphate pathway and the monoacylglycerol (MAG) pathway. MAG catalysis is the first step in the glycerol phosphate pathway by the acylation of glycerol 3-phosphate, and MCAT catalysis is the first step in the MAG pathway by acylating MAG. The final step in the synthesis of TG involves the esterification of DAG by DGATs [43]. In the present study, we showed that iraglutide attenuated the increased expression of DGAT, MCAT and DGAT in the diabetic heart. These data suggest that iraglutide has a preventive effect of increased lipid synthesis in the diabetic heart. Sterol regulatory element-binding protein 1c (SREBP1c) is a transcription factor controlling the expression of key genes of lipidogenesis and thus may represent a suitable candidate for the regulation of triglyceride stores in various tissues [44]. In the present study, we did not observe differences in cardiac SREBP-1c mRNA levels between diabetic rats and iraglutide-treated diabetic rats. However, it has been reported that Sirt1 deacetylates and inhibits SREBP1c activity [45]. Therefore, iraglutide may downregulate lipogenesis via inhibition of SREBP1c activity by the AMPK-Sirt1 pathway.

ACC plays a critical role in the regulation of fatty acid metabolism and its two isoforms, ACC1 and ACC2, appear to have distinct functions in the control of fatty acid synthesis and fatty acid oxidation, respectively [26]. It has been reported that AMPK inhibits the enzymatic activity of ACC [46]. In the present study, we found that iraglutide downregulates the expression of ACC1 and ACC2 in the diabetic heart. These data suggest that iraglutide may prevent cardiac steatosis in the diabetic heart, at least in part, by decreasing fatty acid synthesis and increasing oxidation of fatty acid via activation of AMPK. Although the mRNA expression levels of both ACC1 and ACC2 were significantly increased in diabetic hearts, the ACC protein level was not significantly higher in diabetic hearts compared to control hearts. Therefore, the ACC protein degradation may be increased in the diabetic heart. The detailed molecular mechanisms should be evaluated in further studies.

In the present study, the mechanisms underlying the protective effect of iraglutide on diabetic heart appeared to be mediated by activation of AMPK via the GLP-1 receptor. However, a recent study has shown that cardiac GLP-1 receptor expression is localized to the cardiac atria [47], although a previous study showed direct cardiovascular benefit of iraglutide [48]. It has been reported that GLP-1 receptor is expressed in areas of the CNS involved in the control of energy balance and that CNS GLP-1 receptor signaling controls peripheral lipid deposition mediated by the sympathetic nervous system [49]. In addition, α-adrenergic receptors activate AMPK in rat hearts [50]. Therefore, it is also possible that iraglutide might activate AMPK through CNS GLP-1 receptor signaling in the diabetic heart. It should be evaluated in further studies whether the effect of iraglutide on heart is directly mediated by GLP-1 receptor or mediated by its stimulation of sympathetic nerve system.

In conclusion, we showed for the first time that iraglutide administration protected against myocardial steatosis and oxidative stress at least in part, by activation of the AMPK-Sirt1 pathway, independently of a glucose-lowering effect in an animal model of diabetes. Iraglutide ameliorate high glucose-induced oxidative. Iraglutide may have a direct beneficial effect on diabetes-related heart diseases via these mechanisms.

Conflict of interest

The authors have no conflict of interest to report in relation to this manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.03.026.

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