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RESEARCH PAPER

Effects of vildagliptin versus sitagliptin, on cardiac function, heart rate variability and mitochondrial function in obese insulin-resistant rats

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BACKGROUND AND PURPOSE

Long-term high-fat diet (HFD) consumption has been shown to cause insulin resistance, which is characterized by hyperinsulinaemia with metabolic inflexibility. Insulin resistance is associated with cardiac sympathovagal imbalance, cardiac dysfunction and cardiac mitochondrial dysfunction. Dipeptidyl peptidase-4 (DPP-4) inhibitors, vildagliptin and sitagliptin, are oral anti-diabetic drugs often prescribed in patients with cardiovascular disease. Therefore, in this study, we sought to determine the effects of vildagliptin and sitagliptin in a murine model of insulin resistance.

EXPERIMENTAL APPROACH

Male Wistar rats weighing 180–200 g, were fed either a normal diet (20% energy from fat) or a HFD (59% energy from fat) for 12 weeks. These rats were then divided into three subgroups to receive vildagliptin (3 $mg \cdot kg^{-1} \cdot day^{-1}$), sitagliptin (30 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle for another 21 days. Metabolic parameters, oxidative stress, heart rate variability (HRV), cardiac function and cardiac mitochondrial function were determined.

KEY RESULTS

Rats that received HFD developed insulin resistance characterized by increased body weight, plasma insulin, total cholesterol and oxidative stress levels along with a decreased high-density lipoprotein (HDL) level. Moreover, cardiac dysfunction, depressed HRV, cardiac mitochondrial dysfunction and cardiac mitochondrial morphology changes were observed in HFD rats. Both vildagliptin and sitagliptin decreased plasma insulin, total cholesterol and oxidative stress as well as increased HDL level. Furthermore, vildagliptin and sitagliptin attenuated cardiac dysfunction, prevented cardiac mitochondrial dysfunction and completely restored HRV.

CONCLUSIONS AND IMPLICATIONS

Both vildagliptin and sitagliptin share similar efficacy in cardioprotection in obese insulin-resistant rats.

Abbreviations

DBP, diastolic BP; DPP-4, dipeptidyl peptidase-4; EDP, end-diastolic pressure; ESP, end-systolic pressure; FFT, fast Fourier transform; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HF, high-frequency; HFD, high-fat diet; HFDSi, high-fat diet group treated with sitagliptin; HFDV, high-fat diet group treated with vehicle; HFDVil, high-fat diet group treated with vildagliptin; HOMA, homeostasis model assessment; HR, heart rate; HRV, heart rate variability;

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JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra ethylbenzimidazolcarbocyanine iodide; LDL, low-density lipoprotein; LF, low-frequency; MDA, malondialdehyde; ND, normal diet; NDSi, normal-diet group treated with sitagliptin; NDV, normal-diet group treated with vehicle; NDVil, normal-diet group treated with vildagliptin; ROS, reactive oxygen species; SBP, systolic BP; SV, stroke volume; SW, stroke work; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; VLDL, very low-density lipoprotein; VLF, very low-frequency

Introduction

Long-term high-fat diet (HFD) consumption is known to cause insulin resistance (Pratchayasakul *et al.*, 2011; Pipatpiboon *et al.*, 2012). Insulin resistance is a risk factor for developing cardiac sympathovagal dysregulation (Pongchaidecha *et al.*, 2009), cardiac dysfunction (Ouwens *et al.*, 2005) and cardiac mitochondrial dysfunction (Dong *et al.*, 2007). Dipeptidyl peptidase-4 (DPP-4) inhibitors, including vildagliptin and sitagliptin, are oral anti-diabetic drugs that inhibit the DPP-4 enzyme, resulting in a prolonged action of the glucagon-like peptide-1 (GLP-1) hormone. GLP-1 is an incretin hormone secreted from intestinal L-cells. Several studies have reported that GLP-1 possesses beneficial effects resulting in decreased plasma glucose levels and improved cardiac function in clinical studies and animal models (Buse *et al.*, 2004; Bose *et al.*, 2005; Poornima *et al.*, 2008). Moreover, DPP-4 inhibitors show a beneficial effect on metabolic parameters and the heart (Ahren *et al.*, 2004; Lenski *et al.*, 2011; Apaijai *et al.*, 2012). Clinical and animal studies have reported that vildagliptin and sitagliptin can increase plasma insulin and decrease glucose levels in type 2 diabetes models (Mari *et al.*, 2005; Tremblay *et al.*, 2011). Moreover, vildagliptin shows a cardioprotective effect in the hearts of swine (Chinda *et al.*, 2012) and insulin-resistant rats (Apaijai *et al.*, 2012) and sitagliptin prevents cardiac fibrosis in diabetic mice (Lenski *et al.*, 2011). Although the effects of the two DPP-4 inhibitors, vildagliptin and sitagliptin, have been studied, their comparative effects on metabolic parameters as well as cardiac function and mitochondrial function of HFD-induced insulin-resistant rats are still unclear.

This study aimed to determine the effects of vildagliptin and sitagliptin on metabolic parameters, oxidative stress levels, heart rate (HR) variability (HRV), cardiac function, cardiac mitochondrial function and cardiac mitochondrial morphology in long-term HFD consumption induced insulin-resistant rats. We hypothesized that vildagliptin and sitagliptin would improve metabolic parameters and prevent an increase in oxidative stress levels, preserve HRV and cardiac function, and cardiac mitochondrial function in HFD-induced insulin-resistant rats.

Methods

Animals and diet

All experiment protocols in this study were approved by the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines, and in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010). Thirty-six male Wistar rats, weighing 180–200 g, were obtained from the National Animal Center, Salaya Campus, Mahidol University, Thailand. Rats were housed in a 12 h light/dark cycle with controlled temperature (25°C). All rats were allowed to acclimate for 7 days and then divided into either the normal-diet (ND) group, fed the standard laboratory pelleted diet containing 20% energy from fat or HFD group fed a diet containing 59% energy from fat. The rats were fed with their respective diets for 12 weeks (Pratchayasakul *et al.*, 2011; Apaijai *et al.*, 2012; Pipatpiboon *et al.*, 2012). Each diet group was further divided into three treatment groups (n = 6/group) consisting of vildagliptin 3 mg kg^{-1}·day^{-1} (Gulvus, Novartis, Bangkok, Thailand; Burkey *et al.*, 2005; Apaijai *et al.*, 2012), sitagliptin 30 mg kg^{-1}·day^{-1} (Januvia, MSD, Bangkok, Thailand; Chen *et al.*, 2011) and vehicle (0.9% normal saline solution in an equal volume). These concentrations were chosen as they were shown previously to be effective in improving insulin sensitivity (Burkey *et al.*, 2005; Chen *et al.*, 2011). Rats were fed *via* gavage feeding for 21 days. Body weight and food intake were recorded weekly. Blood samples were drawn from the tail vein at week 0, week 12 and upon completion of treatment. The plasma was separated and kept frozen at –85°C until use. HRV was determined at the baseline (week 0), week 4, week 8, week 12 and post-treatment. Cardiac function parameters were determined using the pressure-volume catheter (Scisence Inc., ON, Canada; Apaijai *et al.*, 2012). After the cardiac function study was finished, the heart was rapidly removed and the left ventricular tissue was used to determine cardiac mitochondrial function and cardiac malondialdehyde (MDA) levels (Apaijai *et al.*, 2012).

Determination of metabolic parameters

Plasma glucose and total cholesterol levels were determined using a commercial colorimetric assay kit (Biotech, Bangkok, Thailand) (Pipatpiboon *et al.*, 2012). Plasma high-density lipoprotein (HDL) and low-density lipoprotein (LDL)/very low-density lipoprotein (VLDL) levels were determined using a commercial colorimetric assay kit (Biovision, Milpitas, CA, USA; Singh *et al.*, 2008). Plasma insulin levels were determined using the sandwich ELISA kit (LINCO Research, St. Charles, MO, USA; Pratchayasakul *et al.*, 2011; Pipatpiboon *et al.*, 2012). The homeostasis model assessment (HOMA) index, a mathematical model, was used to assess insulin resistance. The HOMA index is calculated from fasting plasma glucose levels and fasting plasma insulin concentration. A higher HOMA index indicates a higher degree of insulin resistance (Pratchayasakul *et al.*, 2011; Pipatpiboon *et al.*, 2012).

Determination of plasma and cardiac MDA levels

Plasma and cardiac MDA levels were determined using a HPLC-based assay (Thermo Scientific, Bangkok, Thailand;

Apaijai *et al.*, 2012). Plasma and cardiac MDA were mixed with H_3PO_4 and thiobarbituric acid (TBA) to produce TBA reactive substances (TBARS). The plasma and cardiac TBARS concentration were determined directly from a standard curve and reported as equivalent to the MDA concentration (Apaijai *et al.*, 2012).

Determination of HRV

Lead II ECG was recorded in all conscious rats using PowerLab (ADInstruments, Colorado Springs, CO, USA) equipped with the Chart 5.0 program. ECG was recorded for 20 min in each rat. The stable ECG trace and the relationship between the RR interval and the beat numbers (Tachogram) are shown in Figure 1A and 1B, respectively. Power spectra of RR interval variability (Figure 1C) were obtained using fast Fourier transform (FFT) algorithm (Chattipakorn *et al.*, 2007; Pongchaidecha *et al.*, 2009; Apaijai *et al.*, 2012; Kumfu *et al.*, 2012). Three major oscillatory components were detected as high-frequency (HF; 0.6–3 Hz) band, low-frequency (LF; 0.2–0.6 Hz) band and very low-frequency (VLF; below 0.2 Hz) band. Each spectral component was calculated as integrals under the respective part of the power spectral density function and was presented in the absolute unit (ms^2). To minimize the effect of changes in total power on the LF and HF bands, LF and HF were expressed as normalized units by dividing it by the total power minus VLF (Chattipakorn *et al.*, 2007; Incharoen *et al.*, 2007). LF (0.2–0.6 Hz) and HF (0.6–3 Hz) ratios were analysed using MATLAB program (Pongchaidecha *et al.*, 2009; Apaijai *et al.*, 2012). LF/HF ratio is considered as an index of sympathovagal balance (Chattipakorn *et al.*, 2007; Kumfu *et al.*, 2012). Increased LF/HF ratio indicated depressed HRV (Apaijai *et al.*, 2012).

Cardiac function

Rats were anaesthetized using Zoletil (50 $mg \cdot kg^{-1}$; Virbac Laboratories, Carros, France) and Xylazine (0.15 $mg \cdot kg^{-1}$,

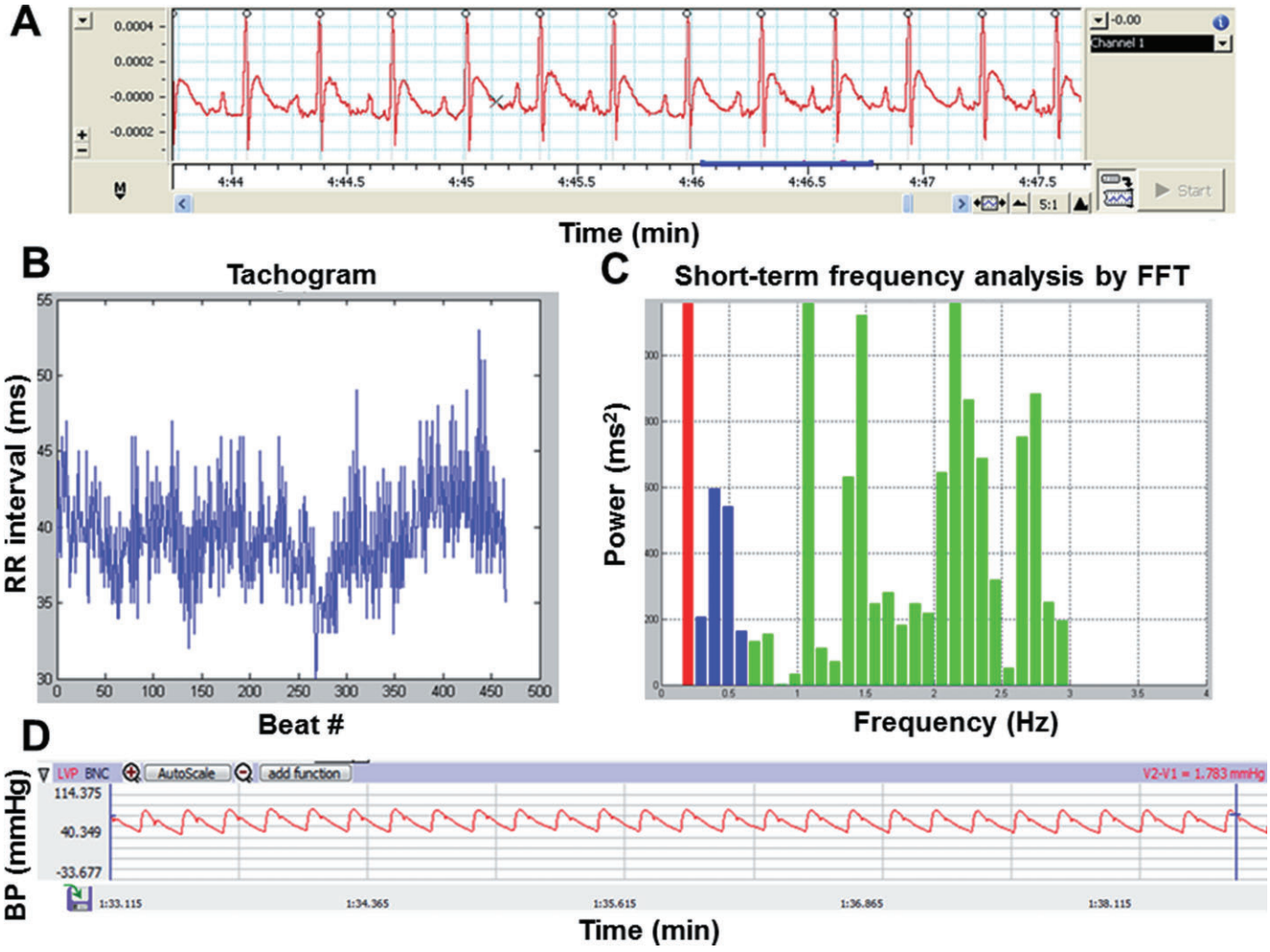

Figure 1

Representative figure of stable ECG trace (A), The RR interval and the beat numbers (Tachogram) (B), Power spectra of RR interval variability (C), and stable BP trace (D). The different colours in panel C represent the different frequency intervals for VLF, LF and HF for HRV analysis.

Laboratorios Calier, Barcelona, Spain) injected intramuscularly. Tracheostomy was performed and rats were ventilated with room air. The right carotid artery was identified and a pressure–volume (P–V) catheter was inserted. Systolic BP (SBP) and diastolic blood BP (DBP) were determined from the carotid artery during the P–V loop measurement (Figure 1D). The catheter was then advanced into the left ventricle. Rats were allowed to stabilize for 5 min and then data were recorded for 20 min. The cardiac function parameters, including heart rate (HR), end-systolic and diastolic pressure (ESP and EDP), maximum and minimum dP/dt (±dP/dt), stroke work (SW) and stroke volume (SV), were determined using an analytical program (Labscribe, Dover, NH, USA) (Apaijai *et al.*, 2012; Kumfu *et al.*, 2012).

Cardiac mitochondrial isolation and determination of mitochondrial function

Cardiac mitochondrial isolation was performed as previously described (Thummasorn *et al.*, 2011; Chinda *et al.*, 2012). The heart of each rat was perfused with normal saline solution and rapidly removed. The left ventricular tissue was minced and homogenized in ice-cold buffer containing 300 mM sucrose, 5 mM N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic sodium salt, and 0.2 mM EGTA. The homogenate was centrifuged at 800 g for 5 min. The supernatant was then collected and centrifuged at 8800 g for 5 min. The pellet was resuspended in respiration buffer containing 50 mM sucrose, 100 mM KCl, 10 mM HEPES and 5 mM KH_2PO_4. In the present study, cardiac mitochondrial function, detected as cardiac mitochondrial reactive oxygen species (ROS) production, cardiac mitochondrial membrane potential change and cardiac mitochondrial swelling was determined.

Cardiac mitochondrial ROS production was determined by incubating cardiac mitochondria with 2-μM 2′,7′-dichlorfluorescein-diacetate dye at 25°C for 20 min. ROS production was detected using a fluorescent microplate reader (BioTek Instruments, Winooski, VT, USA). The dye was excited at λ_{ex} 485 nm and detected at λ_{em} 530 nm (Thummasorn *et al.*, 2011; Apaijai *et al.*, 2012; Chinda *et al.*, 2012).

Cardiac mitochondrial membrane potential change was determined by incubating cardiac mitochondria with 5-μM 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra ethylbenzimidazolcarbocyanine iodide (JC-1) dye at 37°C for 30 min. Cardiac mitochondrial membrane potential changes were detected using a fluorescent microplate reader. JC-1 monomer form (green) fluorescence was excited at λ_{ex} 485 nm and detected at λ_{em} 590 nm. JC-1 aggregate form (red) fluorescence was excited at λ_{ex} 485 nm and detected at λ_{em} 530 nm. A decrease in the red/green fluorescence intensity ratio was considered an indicator of cardiac mitochondrial membrane depolarization (Thummasorn *et al.*, 2011; Apaijai *et al.*, 2012; Chinda *et al.*, 2012).

Cardiac mitochondrial swelling was determined after incubation in respiration buffer. The absorbance was measured using a spectrophotometer. A decrease in absorbance was considered an indicator of cardiac mitochondrial swelling (Thummasorn *et al.*, 2011; Apaijai *et al.*, 2012; Chinda *et al.*, 2012).

Cardiac mitochondrial morphology determination

Cardiac mitochondria were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, post fixed in 1% cacodylate-buffer osmium tetroxide for 2 h at room temperature, and dehydrated in a graded series of ethanol. Then, cardiac mitochondria were embedded in Epon-Aradite. Ultrathin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Cardiac mitochondrial morphology was observed with a transmission electron microscope (Thummasorn *et al.*, 2011).

Statistical analysis

Data were presented as mean ± SE. One way ANOVA followed by Fisher's least significant difference *post hoc* was used to test the difference among the groups. $P < 0.05$ was considered statistically significant.

Results

Metabolic parameters

We found that the body weight, food intake, plasma insulin, glucose, total cholesterol and MDA levels, did not vary between ND and HFD rats at the baseline (Table 1). After 12 weeks of HFD consumption, body weight, plasma insulin, HOMA index, total cholesterol and plasma MDA levels were significantly increased compared with ND rats (Table 1). We found that plasma insulin, total cholesterol, HDL levels and HOMA index were significantly improved in HFD rats treated with vildagliptin and sitagliptin. Plasma and cardiac MDA levels were also improved in HFD rats treated with vildagliptin and sitagliptin. However, body weight, visceral fat weight and plasma glucose level were unaffected by both drugs (Table 2). In ND rats, no difference was found among the vildagliptin-, sitagliptin- and vehicle-treated groups.

HRV

At baseline, the LF/HF ratio did not differ between the ND and HFD groups (Figure 2A). We found that the LF/HF ratio was increased in week 8 of HFD consumption and markedly increased in week 12 (0.19 ± 0.02 at baseline, 0.26 ± 0.03 at week 8 and 0.33 ± 0.01 at week 12) (Figure 2A). After 21 days of treatment, LF/HF ratio was increased in HFD group treated with vehicle (HFDV) rats [HFDV 0.37 ± 0.02, $P < 0.05$ versus ND group treated with vehicle (NDV)]. Both vildagliptin and sitagliptin returned the LF/HF ratio to the normal level [HFD group treated with vildagliptin (HFDVil) 0.19 ± 0.01, HFD group treated with sitagliptin (HFDSi) 0.22 ± 0.03, $P < 0.05$ versus HFDV] (Figure 2B).

Cardiac function parameters

In the ND groups, cardiac function parameters did not differ among the three treatment groups (Table 3). In the HFD groups treated with vehicle, cardiac dysfunction was observed as shown by an increase in HR, EDP and –dP/dt, and a decrease in ESP, +dP/dt and SV. We found that ESP, EDP, +dP/dt, –dP/dt and SV were significantly improved in HFD rats treated with vildagliptin and sitagliptin. However,

Table 1

Effects of vildagliptin and sitagliptin on metabolic parameters and oxidative stress levels in ND and HFD rats

	Baseline		Week 12	
Metabolic parameters	**ND**	**HFD**	**ND**	**HFD**
Body weight (g)	209 ± 9	208 ± 9	465 ± 6*	565 ± 13*,†
Food intake (g·day^{-1})	20 ± 1	19 ± 5	23 ± 1	28 ± 3
Plasma insulin (ng·mL^{-1})	2.0 ± 0.4	2 ± 0.8	2 ± 0.3	4 ± 0.4*,†
Plasma glucose (mg·dL^{-1})	136 ± 8	138 ± 8	141 ± 8	144 ± 3
HOMA index	17 ± 1	17 ± 2	18 ± 3	27 ± 4*,†
Plasma total cholesterol (mg·dL^{-1})	83 ± 5	83 ± 8	83 ± 9	152 ± 7*,†
Plasma MDA (μmol·mL^{-1})	2 ± 0.2	2 ± 0.1	3 ± 0.4	6 ± 0.1*,†

*P < 0.05 versus Baseline, †P < 0.05 versus ND week 12.

Table 2

Effects of vildagliptin and sitagliptin on metabolic parameters and oxidative stress levels in ND and HFD rats treated with vehicle, vildagliptin and sitagliptin

Metabolic parameters	**NDV**	**NDVil**	**NDSi**	**HFDV**	**HFDVil**	**HFDSi**
Body weight (g)	434 ± 5	442 ± 12	450 ± 9	588 ± 12*	563 ± 2*	577 ± 19*
Food intake (g)	21 ± 1	22 ± 2	21 ± 2	24 ± 2	23 ± 2	23 ± 2
Visceral fat (g)	23 ± 2	24 ± 2	23 ± 3	56 ± 3*	50 ± 5*	50 ± 4*
Plasma insulin (ng·mL^{-1})	2.5 ± 0.4	2.3 ± 0.4	2.7 ± 0.4	3.7 ± 0.4*	2.4 ± 0.5†	2.8 ± 0.6†
Plasma glucose (mg·dL^{-1})	144 ± 5	146 ± 8	146 ± 7	149 ± 10	146 ± 9	144 ± 8
HOMA index	18.2 ± 3.7	19.7 ± 4.1	19.2 ± 5.9	27.8 ± 5.5*	17.4 ± 5.2†	18.4 ± 6.1†
Plasma total cholesterol (mg·dL^{-1})	86 ± 7	84 ± 5	87 ± 6	167 ± 7*	104 ± 7†	104 ± 4†
HDL cholesterol (mg·dL^{-1})	0.9 ± 0.1	1.1 ± 0.0	1.4 ± 0.2	0.6 ± 0.1*	1.3 ± 0.4†	1.2 ± 0.0†
LDL/VLDL cholesterol (mg·dL^{-1})	0.9 ± 0.3	0.8 ± 0.0	0.8 ± 0.1	1.0 ± 0.3	0.5 ± 0.1	0.6 ± 0.2
Plasma MDA (μmol·mL^{-1})	2.4 ± 0.1	2.7 ± 0.2	2.4 ± 0.1	7.07 ± 0.1*	6.4 ± 0.2*,†	6.2 ± 0.3*,†
Cardiac MDA (μmol·mg^{-1} protein)	5.4 ± 1.8	5.5 ± 2.4	5.9 ± 1.3	10.5 ± 2.6*	7.9 ± 1.6*,†	7.9 ± 1.2*,†

*P < 0.05 versus NDV, †P < 0.05 versus HFDV.

Table 3

Effects of vildagliptin and sitagliptin on cardiac function in ND and HFD rats treated with vehicle, vildagliptin and sitagliptin

Cardiac function	**NDV**	**NDVil**	**NDSi**	**HFDV**	**HFDVil**	**HFDSi**
HR (bpm)	313 ± 26	322 ± 32	344 ± 0.3	412 ± 14*	334 ± 26†	378 ± 27*,†
SBP (mmHg)	134 ± 1	132 ± 1	132 ± 3	130 ± 2	136 ± 3	134 ± 3
DBP (mmHg)	107 ± 1	107 ± 1	108 ± 2	111 ± 1	109 ± 2	110 ± 3
ESP (mmHg)	134 ± 6	137 ± 5	137 ± 3	116 ± 14*	136 ± 13†	143 ± 21†
EDP (mmHg)	18 ± 2	17 ± 1	17 ± 1	38 ± 2*	23 ± 4†	25 ± 2†
+dP/dt (mmHg·s^{-1})	8 804 ± 385	8 763 ± 230	8 532 ± 360	6 787 ± 284*	8 072 ± 234*,†	7 595 ± 382*,†
–dP/dt (mmHg·s^{-1})	–5 309 ± 279	–5 753 ± 100	–5 011 ± 185	–3 923 ± 394*	–5 566 ± 858†	–4 701 ± 687†
SW (mmHg·mL^{-1})	10 466 ± 95	10 288 ± 75	10 456 ± 73	10 406 ± 80	10 330 ± 62	10 263 ± 66
SV (μL·g^{-1})	1.02 ± 0.01	1.03 ± 0.06	0.99 ± 0.03	0.77 ± 0.04*	0.93 ± 0.01†	0.96 ± 0.03†

*P < 0.05 versus NDV, †P < 0.05 versus HFDV.

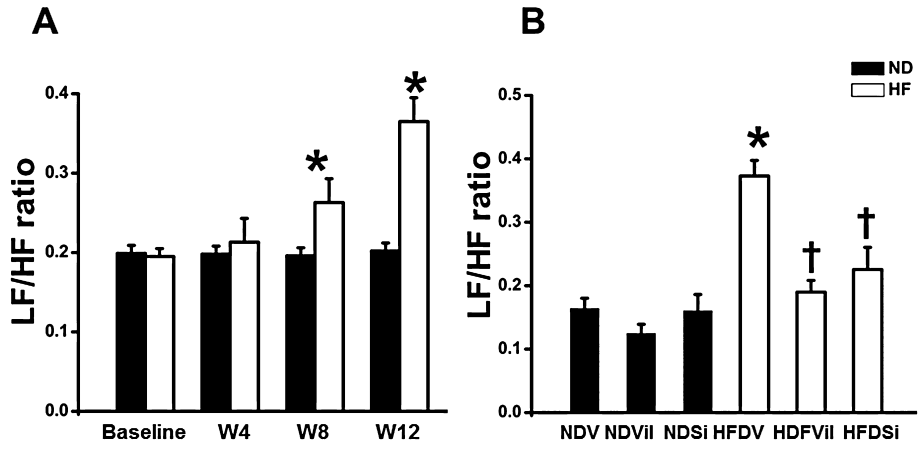

Figure 2

LF/HF ratio in ND and HFD rats (A). The LF/HF ratio significantly increased in weeks 8 and 12 of HFD consumption, in comparison with the baseline. *$P < 0.05$ versus baseline. LF/HF ratio in ND and HFD rats treated with vehicle, vildagliptin, and sitagliptin (B). In HFD rats, vildagliptin and sitagliptin restored the LF/HF ratio, in comparison with the vehicle. *$P < 0.05$ versus NDV, †$P < 0.05$ versus HFDV.

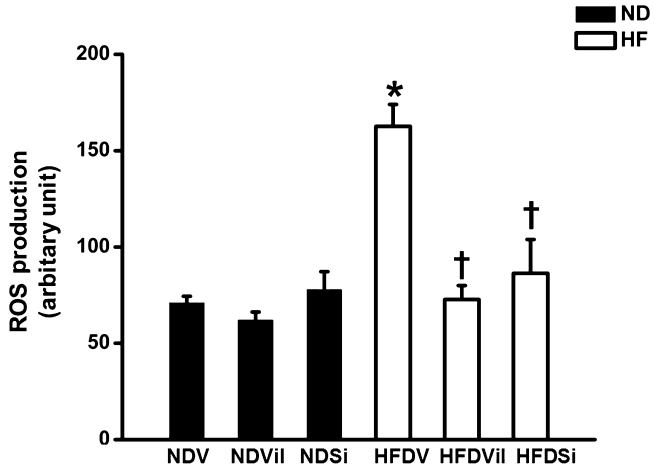

Figure 3

Cardiac mitochondrial ROS production in ND and HFD rats treated with vehicle, vildagliptin, and sitagliptin. In HFD rats, vildagliptin and sitagliptin reduced cardiac mitochondrial ROS production, in comparison with the vehicle. *$P < 0.05$ versus NDV, †$P < 0.05$ versus HFDV.

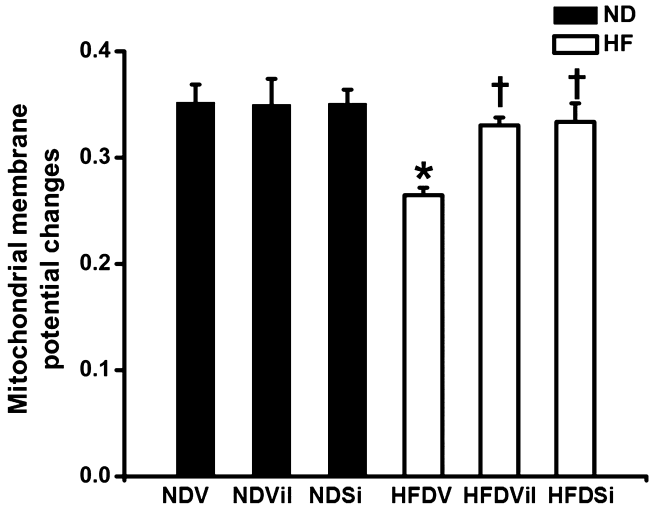

Figure 4

Cardiac mitochondrial membrane potential changes in ND and HFD rats treated with vehicle, vildagliptin and sitagliptin. In HFD rats, vildagliptin and sitagliptin prevented cardiac mitochondrial membrane depolarization, in comparison with the vehicle. *$P < 0.05$ versus NDV, †$P < 0.05$ versus HFDV.

treatment with vildagliptin, but not sitagliptin, eliminated pathophysiologic elevation of HR compared with the mean HR of the group treated with vehicle only (Table 3). Moreover, we found that SBP, DBP and SW did not differ among treatment groups.

Cardiac mitochondrial function and morphology

In the ND group, cardiac mitochondrial ROS production [NDV 71 ± 4 au, ND group treated with vildagliptin (NDVil) 62 ± 4 au, ND group treated with sitagliptin (NDSi) 78 ± 10 au, Figure 3], the red/green fluorescent intensity ratio, which indicated cardiac mitochondrial membrane potential change (NDV 0.35 ± 0.01, NDVil 0.35 ± 0.02, NDSi 0.35 ± 0.01, Figure 4), and the absorbance, which indicated mitochondrial swelling (NDV 0.95 ± 0.03 au, NDVil 0.94 ± 0.02 au, NDSi 0.95 ± 0.01 au, Figure 5), were not different among the three treatment groups of the ND rats. In HFD rats, an increase in cardiac mitochondrial ROS production (HFDV 164 ± 11 au, $P < 0.05$ versus ND group, Figure 3), cardiac mitochondrial depolarization (HFDV 0.27 ± 0.01, $P < 0.05$ versus ND group, Figure 4), and cardiac mitochondrial swelling (HFDV 0.83 ± 0.02 au, $P < 0.05$ versus ND group, Figure 5) were observed. Both vildagliptin (HFDVil 73 ± 7 au) and sitagliptin (HFDSi 86 ± 18 au) returned cardiac mitochon-

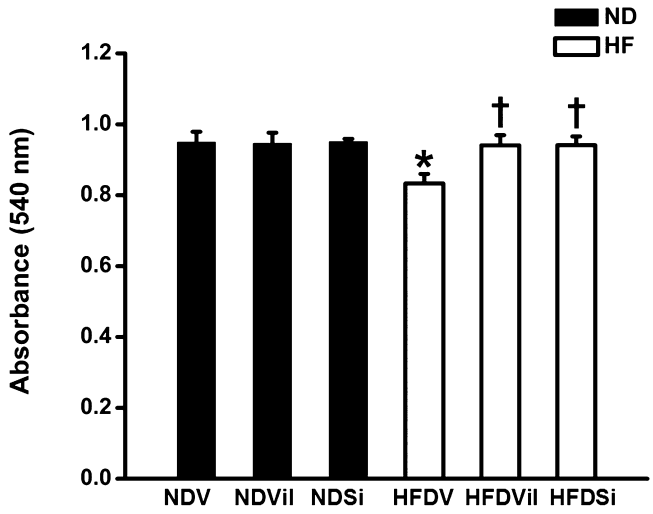

Figure 5

Cardiac mitochondrial swelling in ND and HFD rats treated with vehicle, vildagliptin and sitagliptin. In HFD rats, vildagliptin and sitagliptin reduced cardiac mitochondrial swelling, in comparison with the vehicle. *$P < 0.05$ versus NDV, †$P < 0.05$ versus HFDV.

drial ROS production to the normal level (Figure 3). Moreover, vildagliptin and sitagliptin prevented cardiac mitochondrial depolarization (Figure 4) and cardiac mitochondrial swelling (Figure 5). In HFD rats, representative electron microscope picture illustrated unfolded cristae in cardiac mitochondrion, comparison with that in ND rats (Figure 6), indicating cardiac mitochondrial swelling. Both vildagliptin and sitagliptin prevented cardiac mitochondrial swelling in HFD rats (Figure 6).

Discussion

Findings from this study show that vildagliptin and sitagliptin improve metabolic parameters and decrease oxidative stress in HFD-induced insulin-resistant rats. Vildagliptin and sitagliptin completely preserved HRV, and attenuated cardiac dysfunction in HFD-induced insulin-resistant rats. However, only vildagliptin returned HR to the normal level. Moreover, vildagliptin and sitagliptin prevented cardiac mitochondrial dysfunction and preserved cardiac mitochondrial morphology in rats with insulin resistance induced by HFD consumption.

In the present study, our model is a high-fat diet-induced insulin resistance, which is characterized by hyperinsulinaemia with euglycaemia. Our result showed that plasma glucose level was not different between ND and high-fat diet group at baseline and at week 12, whereas plasma insulin level was significantly increased in rats at week 12 of high-fat diet consumption. This result confirmed that these rats developed an insulin resistance at week 12 after high-fat diet consumption. These findings were also consistent with previous reports using high-fat diet-induced insulin-resistant rats (Pratchayasakul *et al.*, 2011; Apaijai *et al.*, 2012; Pipatpiboon *et al.*, 2012).

Long-term HFD consumption is known to induce insulin resistance (Pongchaidecha *et al.*, 2009; Pratchayasakul *et al.*, 2011; Pipatpiboon *et al.*, 2012). Previous studies have shown that DPP-4 inhibitors, vildagliptin and sitagliptin specifically, improved metabolic parameters, and reduced plasma insulin levels in animal and clinical studies (Ahren *et al.*, 2004; Mari *et al.*, 2005; Dobrian *et al.*, 2011; Briand *et al.*, 2012). In the current study, plasma insulin levels were decreased in HFD rats treated with both vildagliptin and sitagliptin. Moreover, we found that both vildagliptin and sitagliptin reduced total plasma cholesterol levels in HFD rats. This finding is consistent with previous studies reporting that vildagliptin and sitagliptin decreased plasma cholesterol levels in normal rats (Yin *et al.*, 2011), HFD mice (Flock *et al.*, 2007) and type 2 diabetes patients (Kleppinger and Helms, 2007; Tremblay *et al.*, 2011). Furthermore, our study is the first showing that vildagliptin and sitagliptin could increase plasma HDL levels in long-term HFD-induced insulin-resistant rats, whereas both drugs did not alter plasma glucose and LDL/VLDL levels. Previous clinical studies as well as in normal and type 2 diabetes rats also showed that vildagliptin and sitagliptin reduced levels of oxidative stress (Read *et al.*, 2010; Matsui *et al.*, 2011; Chinda *et al.*, 2012; Goncalves *et al.*, 2012). In this study, we found that plasma and cardiac MDA levels were decreased in HFD rats treated with vildagliptin and sitagliptin. These findings indicate that both vildagliptin and sitagliptin could attenuate insulin-resistant condition and reduce oxidative stress in obese insulin-resistant rats induced by long-term HFD consumption.

HRV is an indicator used to determine cardiac sympathovagal balance, a commonly used metric associated with autonomic regulatory function (Chattipakorn *et al.*, 2007; Incharoen *et al.*, 2007; Kumfu *et al.*, 2012). Our study demonstrated that the LF/HF ratio was increased in HFD rats during week 8 and markedly increased during week 12 of HFD consumption, indicating cardiac sympathovagal imbalance. This study showed that both vildagliptin and sitagliptin restore the HRV by returning the LF/HF ratio to normal levels. Because increased sympathetic activity such as stress and insulin resistance could cause depressed HRV (i.e. increased LF/HF ratio; McCann *et al.*, 1995; Sun *et al.*, 2011; Apaijai *et al.*, 2012), HRV improvement by vildagliptin and sitagliptin could be due to the modulation of autonomic regulatory function derived from improved insulin sensitivity and reduced oxidative stress levels. The improved HRV found in this study could also be due to the anti-inflammatory effect. Future studies are needed to investigate the effects of the DPP-4 inhibitors on the association between anti-inflammatory role and HRV.

Previous studies and ours have shown that cardiac dysfunction can be induced by various diets in obese insulin-resistant rats (McCann *et al.*, 1995; Sun *et al.*, 2011; Apaijai *et al.*, 2012). Consistent with previous reports, this study demonstrated that long-term HFD consumption leads to cardiac dysfunction. Treatment with vildagliptin and sitagliptin significantly improved cardiac function in HFD-induced insulin-resistant rats. This cardioprotective effects could be due to their protection of cardiac mitochondrial dysfunction.

Cardiac mitochondria are responsible for supplying appropriate energy for maintaining normal cardiac function. Previous studies have reported that insulin resistance is

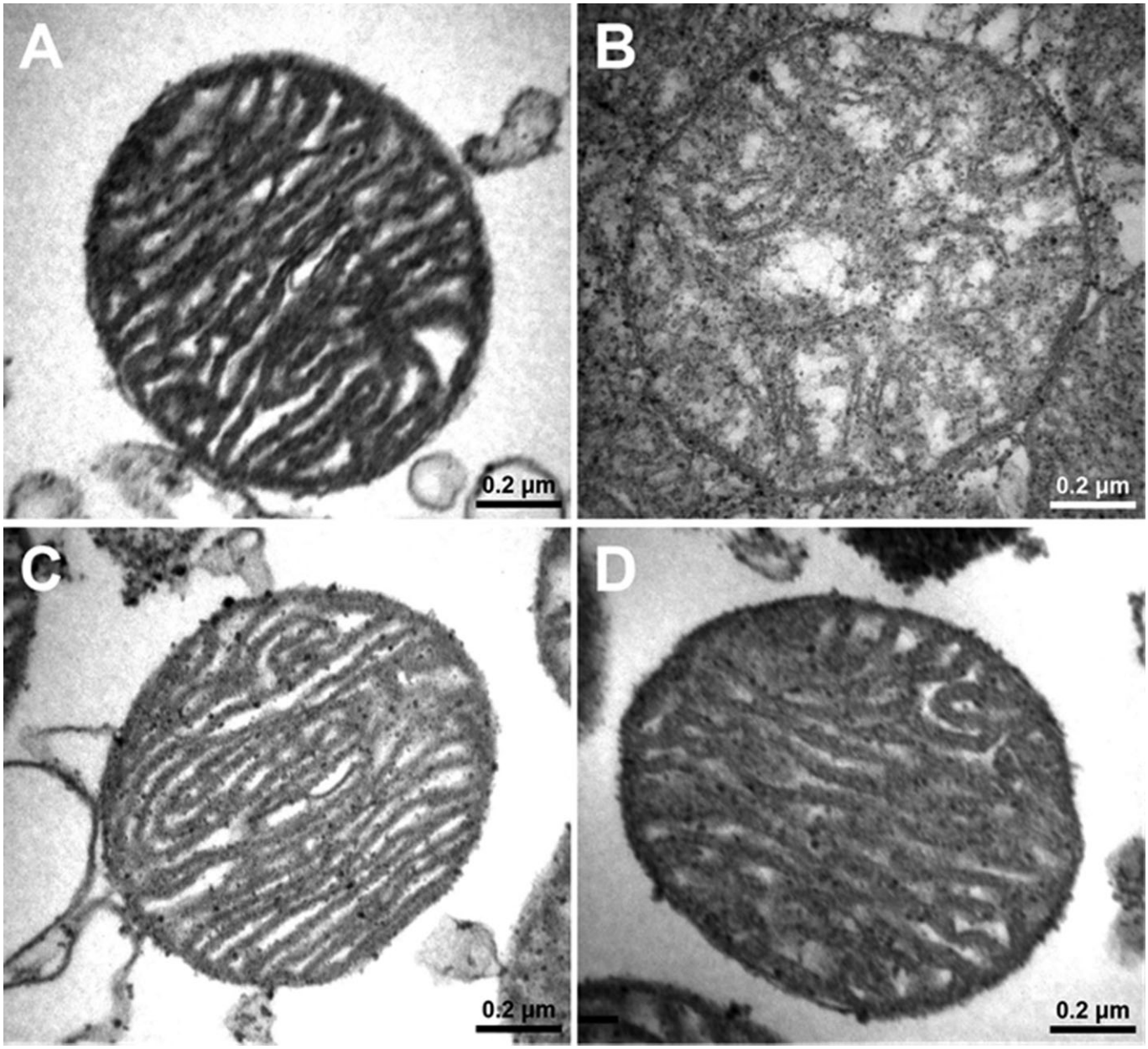

Figure 6

Electron microscope pictures of cardiac mitochondria in NDV (A) and HFD rats treated with vehicle (B), vildagliptin (C) and sitagliptin (D). In HFD rats, vildagliptin and sitagliptin prevented cardiac mitochondrial morphology changes, in comparison with the vehicle.

related to mitochondrial dysfunction in various insulin target tissues including myocardium (Kraegen *et al.*, 2008; Coletta and Mandarino, 2011). We found that cardiac mitochondrial dysfunction occurred in HFD rats, as characterized by increased cardiac mitochondrial ROS production, mitochondrial membrane depolarization, and cardiac mitochondrial swelling (Thummasorn *et al.*, 2011; Chinda *et al.*, 2012). Chinda *et al.* reported that vildagliptin could attenuate cardiac mitochondrial ROS production and mitochondrial membrane depolarization in isolated cardiac mitochondria experiencing oxidative stress (Chinda *et al.*, 2012). Moreover, Thummasorn *et al.* reported that severe oxidative stress could damage cardiac mitochondrial ultrastructure (Thummasorn *et al.*, 2011). In the present study, we found that both vildagliptin and sitagliptin prevented cardiac mitochondrial dysfunction in obese insulin-resistant rats caused by long-term HFD consumption.

In this study, we demonstrated that our obese insulin-resistant rats demonstrated cardiac autonomic dysfunction as shown by depressed HRV, cardiac mechanical dysfunction as shown by abnormal cardiac pressure–volume data, and cardiac mitochondrial dysfunction as shown by increased mitochondrial ROS production, mitochondrial membrane depolarization and mitochondrial swelling. In the present

study, we found that both vildagliptin and sitagliptin provided cardioprotection *via* their benefits on prevention of cardiac mitochondrial dysfunction including decreased ROS production, restored mitochondrial membrane potential and prevented mitochondrial swelling. Because it is known that increased ROS production is mainly contributed to mitochondrial depolarization and mitochondrial swelling, the major effect that both drugs attenuated mitochondrial membrane depolarization could be due to their ability to decrease the mitochondrial ROS production in these obese insulin-resistant rats. The anti-oxidative effect of both drugs could also be seen in their ability to decrease plasma MDA as well. These findings suggested that the anti-oxidative effects of these DPP-4 inhibitors could play a major role in their cardioprotective effects in these obese insulin-resistant rats.

In conclusion, insulin resistance, increased oxidative stress, cardiac sympathovagal imbalance, cardiac dysfunction and cardiac mitochondrial dysfunction were observed in long-term HFD-fed rats. Both vildagliptin and sitagliptin ameliorated these undesirable effects by decreasing oxidative stress and cardiac mitochondrial dysfunction, and improving insulin-resistant condition, HRV and cardiac function in long-term HFD consumption induced insulin-resistant rats.

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Conflict of interest

None.

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