CHAPTER 3

Results

3.1 Effects of probiotics improved inflammation and lipid profile in HFD-induced obese-insulin resistant rats.

At the baseline, all among groups were not different in metabolic parameters including body weight, food intake, plasma glucose, insulin, HOMA index, TC, TG, HDL and LDL levels. After 12 weeks of HFD consumption, HFD-fed rats had a higher body weight, food intake, plasma insulin, HOMA index, TC and LDL levels than ND-fed rats. However, plasma glucose, HDL and TG levels were not different between ND and HFD-fed rats (Table 3.1). Probiotics reduced plasma insulin, TC, and HOMA index in HFD-fed rats while food intake, body weight, visceral fat, plasma glucose, HDL and TG levels were unchanged compared to HFV (Table 3.2). We also performed an OGTT to determine insulin sensitivity in HFD-fed rats after they received probiotics, and we found that AUCg was increased in HFD-fed rats, and this treatment decreased AUCg in HFD-fed rats compared to HFV (Table 3.2). Moreover, probiotics did not alter AUCg in ND-fed rats, compared with NDV (Table 3.2).

Serum LPS was used as an indicator for systemic inflammation after HFD-consumption. Our results showed that serum LPS levels were increased after 12 weeks of HFD consumption (Figure 3.1). In ND-fed rats, probiotics treatment did not alter serum LPS levels compared to NDV (Figure 3.1). Probiotics reduced serum LPS levels in HFD-fed rats, compared to HFV (Figure 3.1).

Table 3.1 The metabolic parameters at baseline and after 12-weeks of either ND or HFD consumption. ND; normal diet, HFD; high-fat diet.

Metabolic parameters	Baseline		12-weeks of HFD consumption	
	ND	**HF**	**ND**	**HF**
Body weight (g)	226± 2	225± 2	486 ± 8	581 ± 24*
Food intake (g/day)	23.1 ± 0.4	23.5 ± 0.9	20.7 ± 0.1	22.9 ± 0.6*
Plasma glucose (mg/dl)	132.6 ± 5.9	140.7 ± 17.6	130.3 ± 6.9	136.9 ± 6.9
Plasma insulin (ng/ml)	2.05 ± 0.4	1.99 ± 0.2	3.9 ± 0.4	6.4 ± 0.4*
HOMA index	22.4 ± 4.9	21.7 ± 7.8	43.4 ± 7.4	61.1 ± 7.0*
Plasma total cholesterol (mg/dl)	74.5 ± 5.3	75.9 ± 5.9	74.7 ± 2.6	89.4 ± 5.0*
Plasma total triglyceride (mg/dl)	89.3 ± 6.6	92.1 ± 12.8	116.1 ± 5.9	113.6 ± 6.4
Plasma HDL (mg/dl)	24.6 ± 1.3	25.2 ± 1.6	25.7 ± 0.6	26.5 ± 0.7
LDL cholesterol (mg/dl)	22.5 ± 3.6	20.9 ± 4.1	22.5 ± 1.7	39.1 ± 4.0*

*$p<0.05$ vs. ND group.

Table 3.2 The metabolic parameters after 12-weeks of vehicle and probiotics administration in ND-fed rats and HFD-fed rats. ND; normal diet, HF; high-fat diet, NDV; normal diet plus vehicles. ND; normal diet, HF; high-fat diet, NDV; normal diet with vehicles, NDPO; normal diet with prebiotics, HFV; high-fat with vehicles, HFPO; high-fat diet with probiotics.

Metabolic parameters	ND		HF	
	NDV	NDPO	HFV	HFPO
Body weight (g)	507 ± 10	513 ± 15	698 ± 24*	648 ± 29*
Food intake (g/day)	20.3 ± 1.0	20.1 ± 0.9	23.6 ± 0.4*	24.4 ± 0.6*
Visceral fat (g)	26 ± 3	24 ± 1	64 ± 4*	56 ± 5*
Plasma glucose (mg/dl)	128.5 ± 13	128.1 ± 10	132.1 ± 7	138.4 ± 4
Plasma insulin (ng/ml)	3.9 ± 0.4	5.9 ± 1.0	8.3 ± 0.4*	4.5 ± 1.0†
HOMA index	51.0 ± 11.6	44.3 ± 12.1	98.7 ± 13.7*	56.3 ± 8.5†
Plasma glucose AUC (mg/dl×min×10^4)	2.1 ± 0.1	2.2 ± 0.2	2.9 ± 0.2*	2.2 ± 0.1†
Plasma total cholesterol (mg/dl)	72.3 ± 5.7	63.7 ± 5.3	93.8 ± 10.4*	76.5 ± 4.6†
Plasma total triglyceride (mg/dl)	87.9 ± 16.5	79.3 ± 10.8	108.5 ± 10.6	94.0 ± 14.7
Plasma HDL (mg/dl)	31.5 ± 1.1	30.5 ± 0.9	29.8 ± 0.7	31.2 ± 2.2
LDL cholesterol (mg/dl)	24.3 ± 2.6	27.6 ± 5.4	59.3 ± 9.2*	39.4 ± 5.2†

*$p<0.05$ in comparison with ND group, †$p<0.05$ in comparison with HFV group

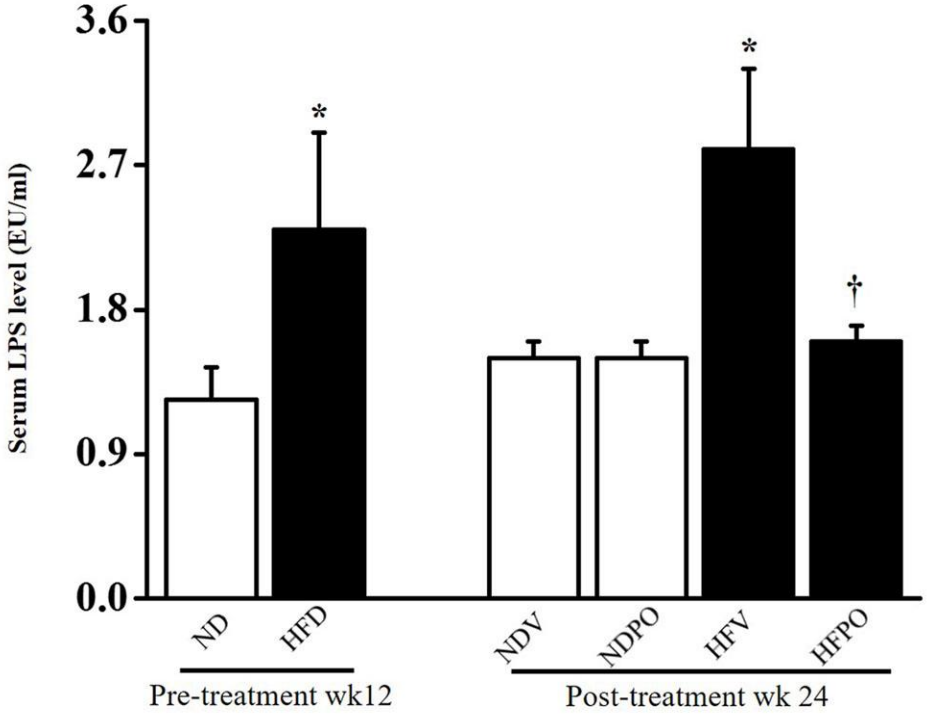

Figure 3.1 Effects of HFD consumption on metabolic endotoxemia at 12 weeks of HFD consumption and post-treatment. (A) Serum LPS level in ND and HFD rats at baseline, after 12 weeks of HFD consumption and post-treatment. *p<0.05 vs. ND. ND; Normal diet, HFD; High-fat diet, NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK12; week12, WK24; week 24, LPS; lipopolysaccharides.

3.2 Probiotics attenuated blood pressure in HFD-induced obese-insulin resistant rats.

At the baseline, all among group were not different in blood parameters including systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP). At week 12 of HFD consumption, SBP, DBP, and MAP were increased in HFD-fed rats, compared to ND-fed rats **(**Figure 3**.**2**).** In ND-fed rats, probiotics did not alter SBP, DBP, and MAP at weeks 4, 8, and 12 of the treatment period, compared to NDV (Figure 3.3). In the HFD group, HFV had higher SBP, DBP and MAP than NDV at weeks 4, 8, and 12 of the treatment period. We found that probiotics treatment reduced SBP, DBP and MAP in HFD-fed rats at weeks 4, 8, and 12

of the treatment period. Our data indicated that probiotics reduced BP in HFD-fed rats. However, a longer treatment period did not augment any significant effects on BP in HFD-fed rats, compared to HFV (Figure 3.3). In the ND group, probiotics treatment did not alter BP in ND-fed rats, compared to NDV (Figure 3.3).

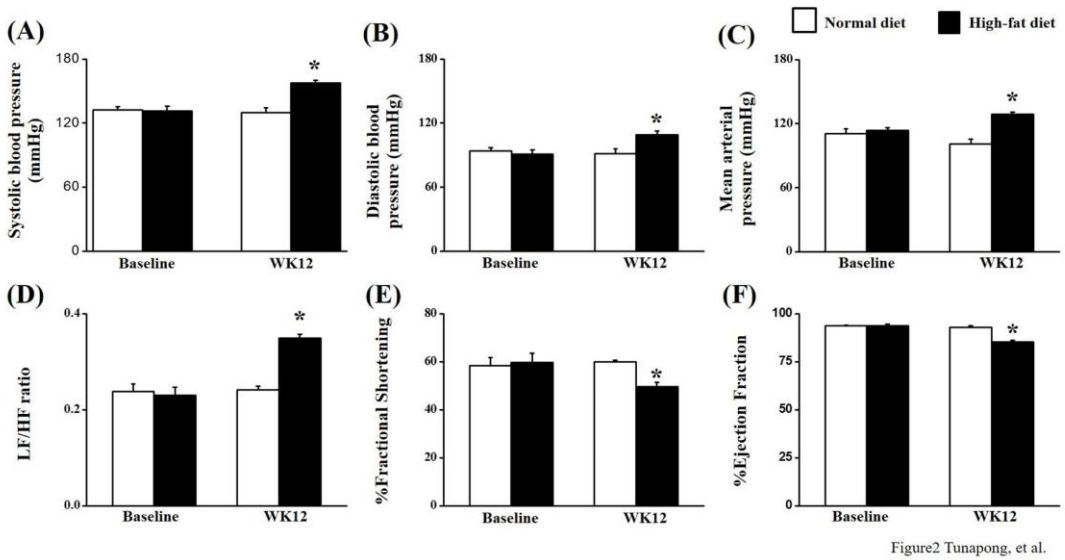

Figure 3.2 Effects of HFD consumption on blood pressure, heart rate variability and echocardiographic parameters at baseline and after 12 weeks of HFD consumption. (A) Systolic blood pressure, (B) Diastolic blood pressure, (C) Mean Arterial Pressure, (D) LF/HF ratio, (E) %Fractional shortening and (F) %Ejection fraction in ND and HFD rats at baseline and week 12. *$p<0.05$ vs ND. ND; Normal diet, HFD; High-fat diet, WK12; week12.

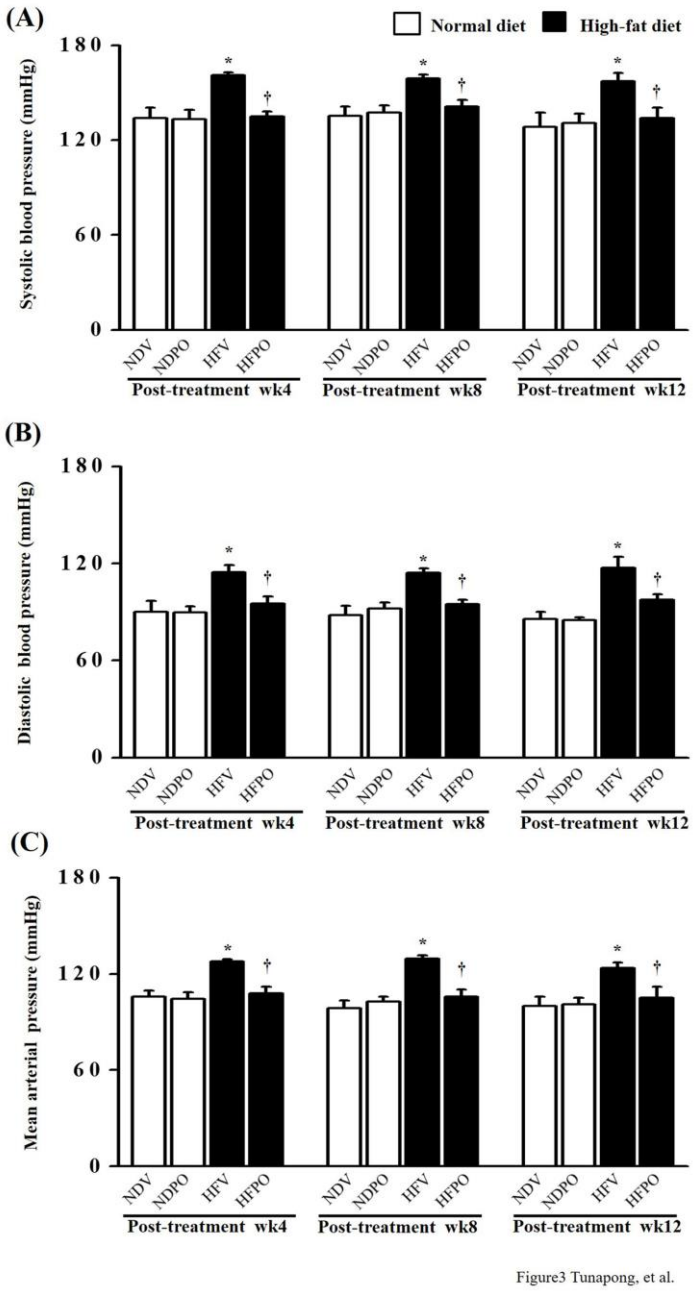

Figure 3.3 Effects of probiotics on blood pressure in ND and HFD-fed rats at 4, 8 and 12 weeks of the treatment period. (A) Systolic blood pressure, (B) Diastolic blood pressure, (C) Mean Arterial Pressure in ND and HFD rats at week 4, week 8 and week 12 of the treatment period.*$p<0.05$ vs. ND, $^{\dagger}p<0.05$ vs. HFV. NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK4; week4, WK8; week8, WK12; week12.

3.3 Probiotics improved heart rate variability in HFD-induced obese-insulin resistant rats.

LF/HF ratio of HRV was determined as an index of cardiac sympathovagal tone balance. At the baseline, all among group were not different in the LF/HF ratio, and the LF/HF ratio was increased after 12 weeks of HFD consumption (Figure 3.2). In HFD-fed rats, probiotics restored the LF/HF ratio back to the normal levels after 4 weeks after treatment, compared to HFV and NDV (Figure 3.4). However, a longer treatment period (week 8 and week 12) did not show a higher reduction of the LF/HF ratio in HFD-fed rats, compared to HFV and the LF/HF ratio at week 4 and week 8 (Figure 3.4). Furthermore, probiotics treatment did not alter the LF/HF ratio in ND-fed rats, compared to NDV (Figure 3.4).

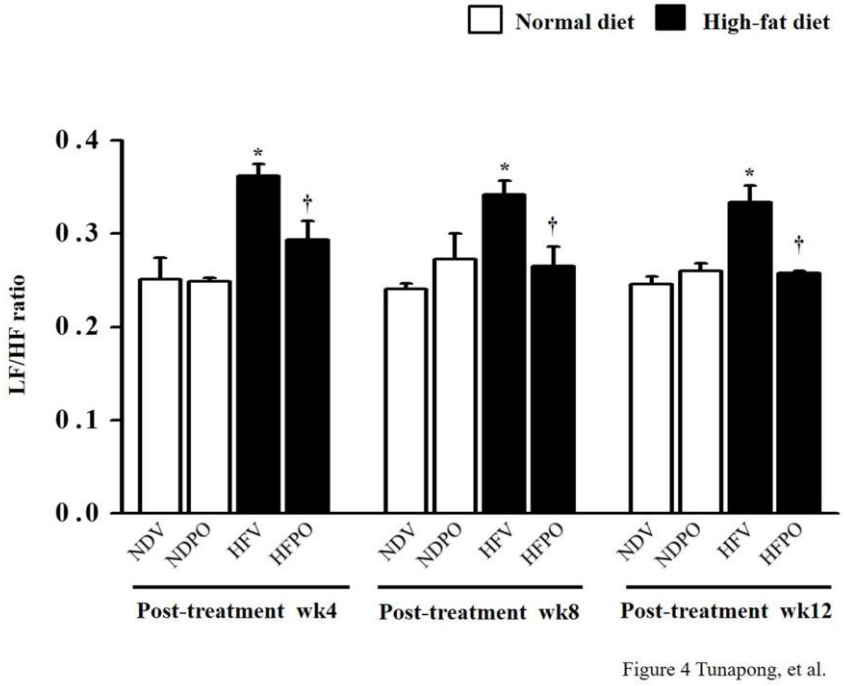

Figure 3.4 The effects of probiotics on LF/HF ratio in ND and HFD-fed rats at 4, 8 and 12 weeks of treatment period. *$p<0.05$ vs. ND, $^{\dagger}p<0.05$ vs. HFV. NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK4; week4, WK8; week8, WK12; week12.

3.4 Probiotics improved cardiac function in HFD-induced obese-insulin resistant rats.

The result from an echocardiogram showed that **%**FS and **%**EF were not different all among group at the baseline, and our results demonstrated that both **%**FS and **%**EF were decreased after 12 weeks of HFD consumption **(**Figure 3.2**)**. In HFD**-**fed rats, probiotics increased both **%**FS and **%**EF in HFD**-**fed rats, compared to HFV (Figure 3.6). Moreover, probiotics treatment returned both **%**FS and **%**EF in HFD**-**fed rats to the normal levels, compared to NDV (Figure 3.5. However, both **%**FS and **%**EF were not different among 4 weeks, 8 weeks, and 12 weeks of treatment **(**Figure 3.5**)**. In ND fed rats, probiotics did not change **%**FS and **%**EF, and were not different among groups, compared to NDV **(**Figure 3.5**)**.

P**-**V loop analysis was performed after 12 weeks of treatment periods, and we found that HR, ESP, EDP, dP/dtmax, dP/dtmin, ESV, EDV, and SV were not different among the ND group **(**Table 3**.**4**).** In HFD**-**fed rats, HFV significantly decreased ESP, dP/dt max, and SV, and increased EDP, dP/dt min and ESV, compared to NDV group **(**Table 3.3**)**. Probiotics improved ESP, EDP, dP/dt max, dP/dtmin, ESV and SV, compared to the HFV group **(**Table 3.3**)**.

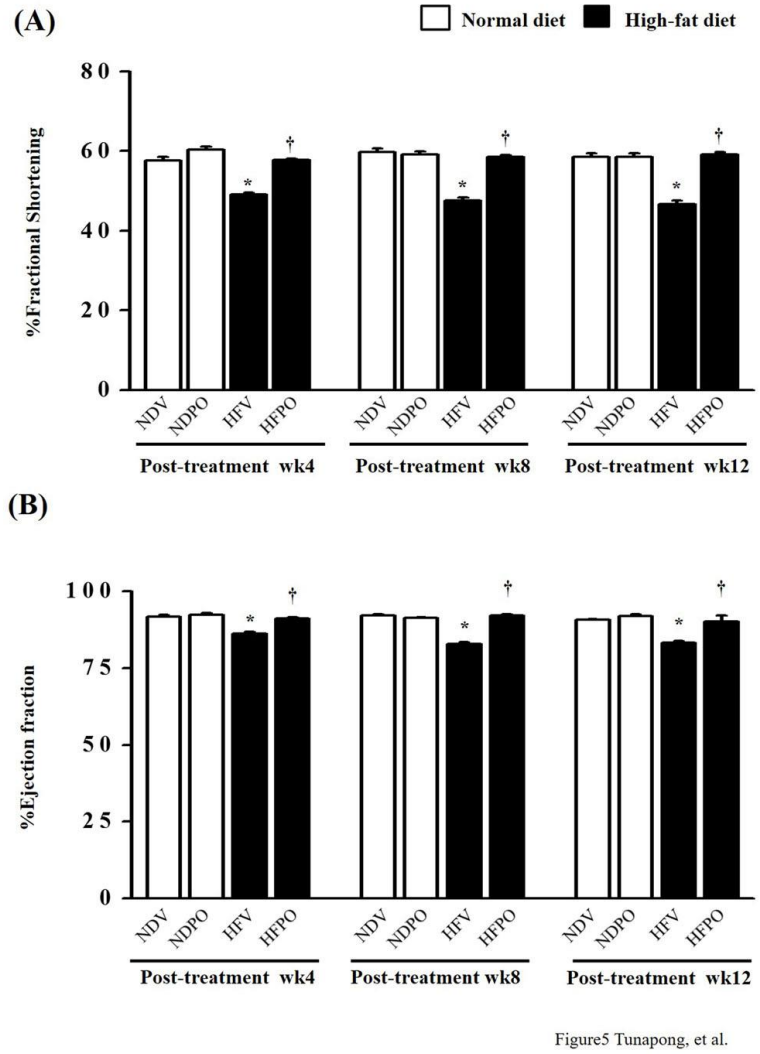

Figure 3.5 The effects of probiotics on echocardiography parameters in ND and HFD-fed rats at 4, 8 and 12 weeks of the treatment period.(A) %Fractional shortening and (B) %Ejection Fraction in ND and HFD rats at week 4, week 8 and week1 2 post-treatment.*p<0.05 vs. ND, †p<0.05 vs. HFV. NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK4; week4, WK8; week8, WK12; week12.

3.5 Probiotics attenuated cardiac mitochondrial dysfunction in HFD-induced obese-insulin resistant rats.

Table 3.3 The pressure-volume loop parameters after 12-weeks of vehicle and probiotics administration in ND-fed rats and HFD-fed rats. ND; normal diet, HF; high-fat diet, NDV; normal diet with vehicles, NDPO; normal diet with prebiotics, HFV; high-fat with vehicles, HFPO; high-fat diet with probiotics.

	NDV	NDPO	HFV	HFPO
HR (bpm)	302 ± 18	321 ± 38	290 ± 37	310 ±16
ESP (mmHg)	116 ± 4	110 ± 6	82 ± 5*	115 ± 10†
EDP (mmHg)	5 ± 1	4 ± 1	13 ± 1*	5 ± 1†
dP/dt max (mmHg/s)	8215 ± 568	8019 ± 274	5583 ± 292*	8117 ± 530†
dP/dt min (mmHg/s)	-5728 ± 702	-5726 ± 559	-3272 ± 429*	-5475 ± 492†
ESV (μl)	16 ± 1	16 ± 1	24 ± 2*	15 ± 2†
EDV (μl)	325 ± 40	304 ± 34	296 ± 72	360 ± 22
SV (μl/g BW)	0.6 ± 0.06	0.6 ± 0.08	0.3 ± 0.04*	0.6 ± 0.05†

$^{*}p<0.05$ in comparison with ND group, $^{\dagger}p<0.05$ in comparison with HFV group

In this study, cardiac mitochondrial function was assessed by cardiac mitochondrial ROS production, cardiac mitochondrial membrane depolarization, and cardiac mitochondrial swelling. In HFD rats, HFV significantly increased cardiac mitochondrial ROS levels, cardiac mitochondrial membrane depolarization, and mitochondrial swelling, compared to NDV (Figure 3.6). Probiotics treatment attenuated cardiac mitochondrial ROS levels, cardiac mitochondrial membrane depolarization, and cardiac mitochondrial swelling in HFD-fed rats, compared to HFV (Figure 3.6). In ND rats, we found that cardiac mitochondrial ROS production, cardiac mitochondrial membrane potential changes, cardiac mitochondrial swelling, and cardiac morphology were not different among ND-treated rats (Figure 3.6). We have confirmed our findings

by determining cardiac mitochondrial morphology, and we found an unfolded cristae and cardiac mitochondrial swelling in HFV, compared to NDV (Figure 3.5). Probiotics treatment restored the unfolded cristae and cardiac mitochondrial swelling in HFD-fed rats, compared to HFV (Figure3.6).

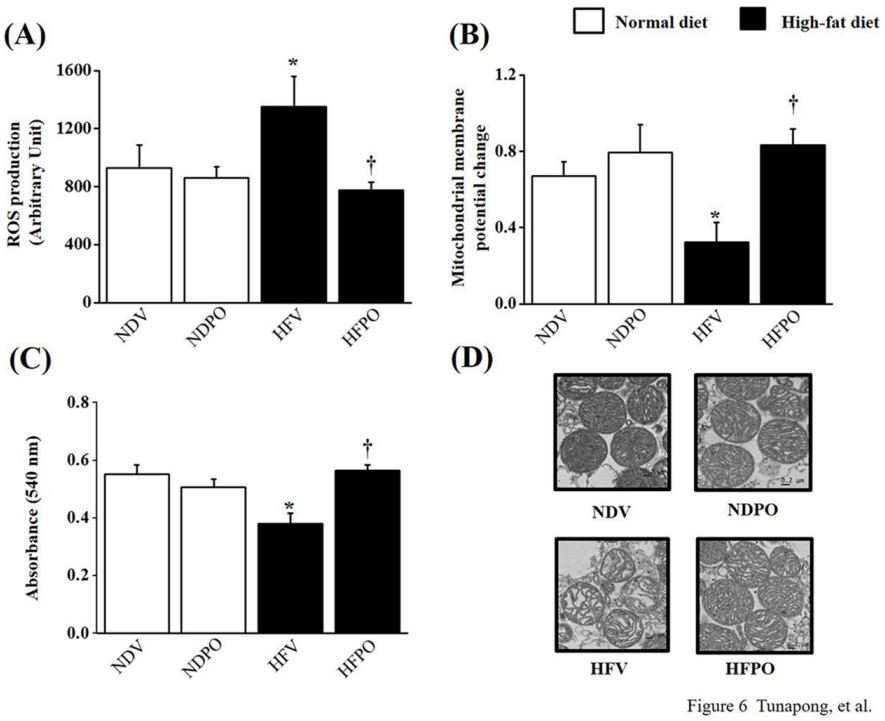

Figure 3.6 Effects of probiotics on cardiac mitochondrial function in ND and HFD-fed rats.(A) ROS production (B) Mitochondria membrane potential change (C) Absorbance at 540 nm (D) cardiac mitochondria morphology in ND and HFD fed-rats. *p<0.05 vs ND, †p<0.05 vs HFV. NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK4; week4, WK8; week8, WK12; week12.

3.6 Probiotics reduced oxidative stress but did not alter cardiac apoptosis in HFD-induced obese-insulin resistant rats.

MDA levels were used to represent the oxidative stress levels, cardiac MDA levels were determined as a tissue oxidative stress, and serum MDA levels were determined as a systemic oxidative stress. In HFD-fed rats, plasma and cardiac MDA levels were increased in HFV, compared to NDV (Figure 3.7). Probiotics reduced plasma and cardiac MDA levels in HFD-fed rats, compared to HFV (Figure 3.7). In

ND groups, our data showed that plasma and cardiac MDA levels were not different among groups (Figure 3.7). We also measured the expression of Bax and Bcl-2 as a marker of apoptosis. Our results showed that Bax and Bcl-2 expression were not different among groups (Figure 3.7).

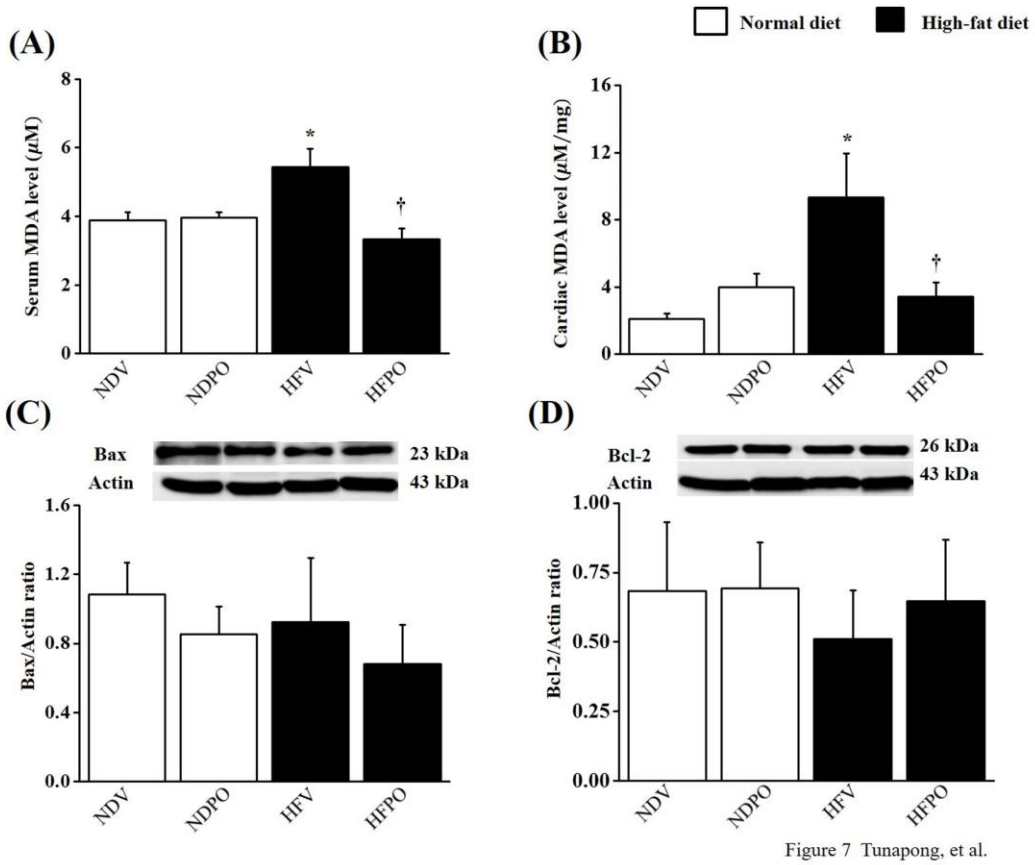

Figure 3.7 The effects of probiotics treatment on oxidative stress and apoptotic protein in ND and HFD-fed rats. (A) serum MDA level (B) cardiac MDA level (C) Bax/actin ratio (D) Bcl-2/actin ratio in ND and HFD fed-rats. *$p<0.05$ vs. ND, †$p<0.05$ vs. HFV. NDV; normal diet with vehicle, NDPO; normal diet with probiotics, HFV; high-fat diet with vehicles, HFPO; high-fat diet with probiotics, WK4; week4, WK8; week8, WK12; week12