RESEARCH IN METABOLIC-RELATED DISEASES BY USING MINIATURE PIG MODEL—DEVELOPMENT OF DIETARY INDUCED METABOLIC SYNDROME

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ABSTRACT

The prevalence of overweight and obesity in adults has been increasing globally. Obese adults (BMI = 30.0) are at increased risk of metabolic syndrome (MetS) including increased blood pressure, cholesterol, triglycerides, and insulin resistance. During the progression of MetS, cardiovascular diseases (CVD) appear clinically in many individuals and cause death. As a result, it is essential to setup an optimal animal model for study the mechanism of obesity and MetS leading to CVD. Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase that functions as a key metabolic/energy sensor and mediates homeostatic responses to nutrient availability. Accumulating evidence indicates that SIRT1 is a master regulator of the transcriptional networks that control hepatic lipid and carbohydrate metabolism. The objective of this study was to establish a miniature pig model of Western diet-induced MetS and investigate the role of SIRT1 played during the MetS development. Five-month-old Lee-Sung (LS) and Lanyu (LY) minipigs were randomly assigned to 2 groups individually: control diet (C) and Western diet (W), in a 6-month experimental period. The results indicated that Western diet caused obesity and higher back fat thickness in both minipig models. Compared with the CLS pigs, WLS pigs exhibited an elevated level of plasma triglyceride, LDL, HDL and total cholesterol. While OLY pigs maintained a similar plasma lipid profile as the CLY pigs did. WLS pigs had a lower antioxidant capacity and high triglyceride accumulation in the liver than CLS pigs did, while WLY pigs had similar hepatic antioxidant capacity and triglyceride accumulation as CLY pigs did. Higher mRNA expression of SIRT1, ACO and CPTI was found in the WLY pig liver than those in the CLY. WLS pigs had a higher CPT1 and lower SIRT1 expression in the liver than CLS pigs. No differences in the other lipid metabolism-related genes were found between WLS and CLS. To conclude, long-term feeding of Western diet to Lee-Sung miniature pigs not only caused obesity but also induced MetS and fatty liver, while Western diet induced obesity in Lanyu pigs without metabolic dysfunctions. SIRT1 and its downstream pathway might be one of the possible regulators for pathological obesity in Lee-Sung pigs. However, the related mechanisms need further studies.

Key words: Dietary-induced metabolic syndrome, Dyslipidemia, Lanyu miniature pig, LeeSung miniature pig, Western diet, SIRT1

INTRODUCTION

According the WHO report, the globe prevalence of overweight and obesity is 35 and 12 % in 2008. Obesity has been demonstrated to induce cardiovascular disease which is the first place of death causes in the world. The report also points out that Western diet is the main cause of obesity. As a result, to master the accurate relationship among Western diet, obesity and cardiovascular disease, and further to eliminate the probability of obesity- related metabolic dysfunction (metabolic syndrome, MetS) not only improves the public health, but also decreases the death caused by cardiovascular diseases.

Sirtuin 1 (SIRT1), a mammal silence information regulator 2 (Sir 2) homologue and a NAD dependent enzyme, is modulated according to the energy status of animal (Nemoto *et al*., 2004) (Coste *et al*., 2008). In addition, it is the key mediator of the glucose and lipid metabolism (Banks *et al*., 2008; Frescas *et al*., 2005; Rodgers *et al*., 2005); and several studies are focusing on its potential as a therapeutic target for treatment of metabolic diseases (Purushotham *et al*., 2012).

Rodent model has been widely used in MetS-related study; however, its physiological characteristics are not similar to human (Litten-Brown *et al*., 2010). Therefore, it is essential to setup a better representative animal model for MetS study. Miniature pigs maintain the highly similarity with human in anatomy and physiology (Litten-Brown *et al*., 2010). In addition, compared to the domestic pigs, the smaller size, low feeding cost and less space demands make them more suitable for MetS researches. In this study, we used two native miniature pigs in Taiwan (Lanyu and Lee-Sung) to setup a dietary-induced obesity (DIO) model. Lanyu miniature pig, a distinctive animal of Taiwan, is an indigenous pig breed from Lanyu (Orchid) Island off the south-east coast of Taiwan. Lee-Sung pigs are the crossbreed of Lanyu and Landrace pigs. These minipigs were fed with Western diet for 6 months to setup a MetS minipig model, and the physiological parameters and metabolic-related gene expression in the liver were monitored. In addition, the role of SIRT1 played during the MetS development was investigated.

MATERIALS AND METHODS

Animals and experiment diets

All animal care procedures used in this study were approved by the Institutional Animal Care and Use Committee of the National Taiwan University. Five-month-old Lanyu (LY) and LeeSung (LS) miniature pigs were randomly divided into two groups and fed the control diet (C) or Western diet (W) respectively for 6 months. Feed composition was provided in **Table 1**. The feed and water were provided *ad libitum.* Body weight was recorded monthly. At the end of experiment, pigs were anesthetized and blood samples were taken. Animals were then sacrificed and the tissues were excised, frozen in liquid nitrogen and stored at – 80℃ until analysis.

Blood analysis in tissue

Plasma lipid profiles were measured by using commercial kits: triglyceride (Fortress), total cholesterol (Fortress,) and high density lipoproteins (HDL) (Fortress). Values of low density lipoproteins (LDL) were calculated by total cholesterol minus HDL.

Table 1 Feed composition of control diet and Western diet

Dry matter basis	Control (C)	Western (W)
Ingredient (kg/ 1000 kg)		
Corn	355.0	427
Soybean meal	80.0	140
Alfalfa meal	207.0	0
Molasses	30.0	30
Wheat bran	160.0	0
Soybean hull	155.5	0
Lard	0.0	150
Sucrose	0.0	200
CaH2PO4	8.3	31.5
CaCO3	0.0	6.3
Mthionine	2.0	2.6
Lysine	1.3	3.1
NaCl	5.0	5.0
Premix	2.5	2.5
Choline	2.0	2.0
Total	999.9	1000.1
Chemical composition (calculated)		
ME (kcal/kg)	2436	3786
Crude protein (%)	14.5	9.8

Triglyceride content and oxygen radical absorbance capacity (ORAC) in tissue

Triglycerides content in tissue were extracted by 5% NP-40. After boiling and cooling to room temperature and centrifuged, the supernatant was used for the triglyceride assay (Fortress). Total antioxidant capacity was analyzed by the ORAC assay as described by Hsieh *et al*. (Hsieh *et al*., 2013).

Quantitative real-time polymerase chain reaction

Total RNA was extracted from liver and reverse transcripted into cDNA as described by Hsieh *et al.* (Hsieh *et al*., 2013). Each cDNA for individual genes was amplified by using SYBR Fast Master Mix (KAPA Biosystem, USA) and the StepOnePlus^{TM} Real-time PCR System (Applied Biosystem). The relative expression levels were calculated according to the formula

$2^{-\triangle CT}$ and normalized using the expression of the TATA-box binding protein (TBP) housekeeping gene in the same sample (Schmittgen and Livak, 2008).

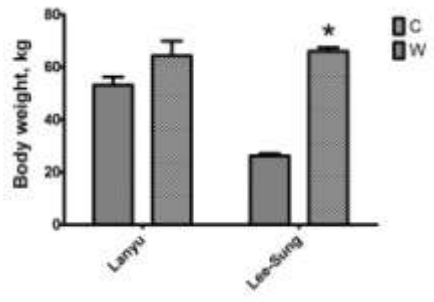
Statistical analysis

Data were expressed as mean ± standard error (SE). The results were analyzed by SAS 9.2. Statistical significance between different experimental groups was determined by Duncan's multiple range tests. $P < 0.1$ was considered pattern, and $P < 0.05$ was considered significant difference.

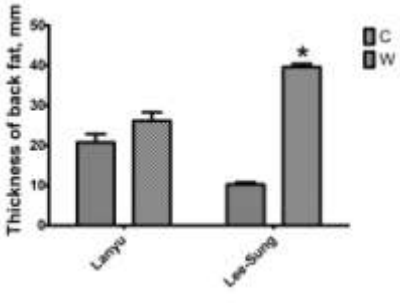
RESULTS AND DISCUSSION

Western diet caused obesity and higher back fat thickness in both minipig models (Figure 1 A and B). Compared with the CLS pigs, WLS pigs exhibited an elevated level of plasma triglyceride, LDL, HDL and total cholesterol (Figure 1 C and D). While OLY pigs maintained a similar plasma lipid profile as the CLY pigs did. These results demonstrated that WLS was induced dyslipidemia, where WLY did not. WLS pigs had a lower antioxidant capacity and high triglyceride accumulation in the liver than CLS pigs did, while WLY pigs had similar hepatic antioxidant capacity and triglyceride accumulation as CLY pigs did (Figure 2).

A



B



C

D

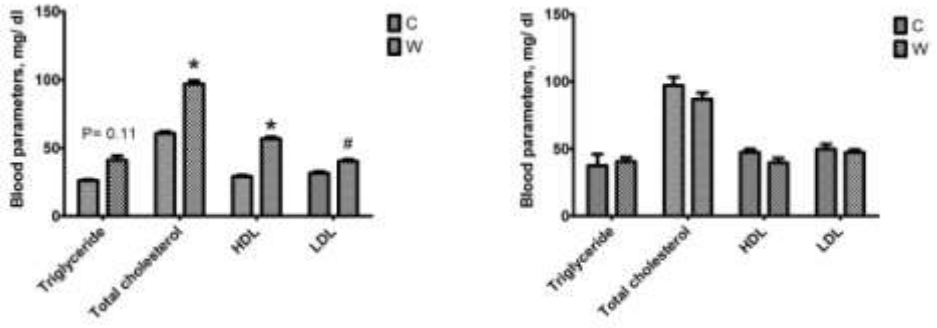


Figure 1. Body weight (A) and back fat thickness (B) of LeeSung and Lanyu pigs. Blood lipid profiles of LeeSung (C) and Lanyu (D) pigs. All results are expressed as mean ± SEM. * $p < 0.05$ vs. control group. # $p < 0.1$ vs. control group.

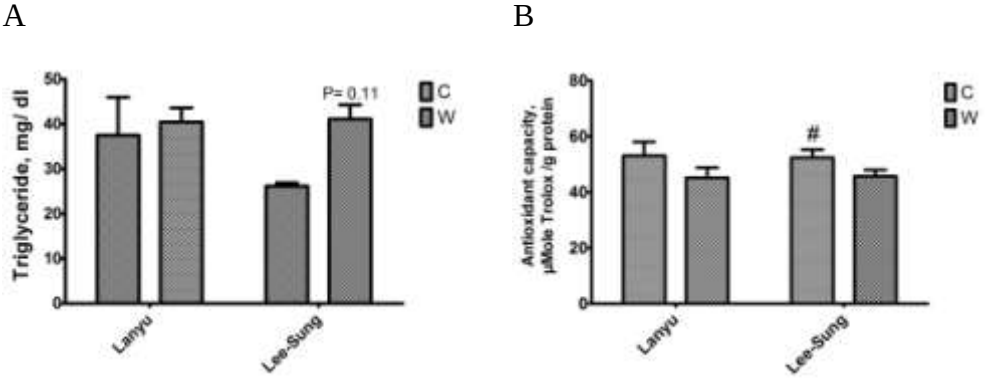


Figure 2. Triglyceride accumulation (A) and total antioxidant capacity (B) in the liver of LeeSung and Lanyupigs. All results are expressed as mean ± SEM. * $p < 0.05$ vs. control group. # $p < 0.1$ vs. control group.

Metabolic related genes were analyzed in two minipig models (Figure 3). Higher mRNA expression of SIRT1, ACO and CPTI was found in the WLY pig liver than those in the CLY. WLS pigs had a higher CPT1 and lower SIRT1 expression in the liver than CLS pigs. However, no differences in the other lipid metabolism-related genes were found between WLS and CLS. SIRT1 regulates a number of targets involved in lipid metabolism at different tissues (Schug and Li, 2011). Systemic SIRT1 insufficiency results in disruption of energy homeostasis (Purushotham *et al.*, 2012). In addition, deletion of SIRT1 in the

liver impairs PPARα signaling and decreases fatty acid β-oxidation, whereas over-expression of SIRT1 induces expression of PPARα target genes (Purushotham *et al*., 2009). When challenged with a high-fat diet, liver-specific SIRT1 knockout mice develop hepatic steatosis (Purushotham *et al*., 2009). We found a different pattern of hepatic SIRT expression in these two minpig models suggested a potential regulation role of SIRT1 on MetS, and LeeSung pig is an optimal disease animal model for MetS study, while Lanyu pig is a working model.

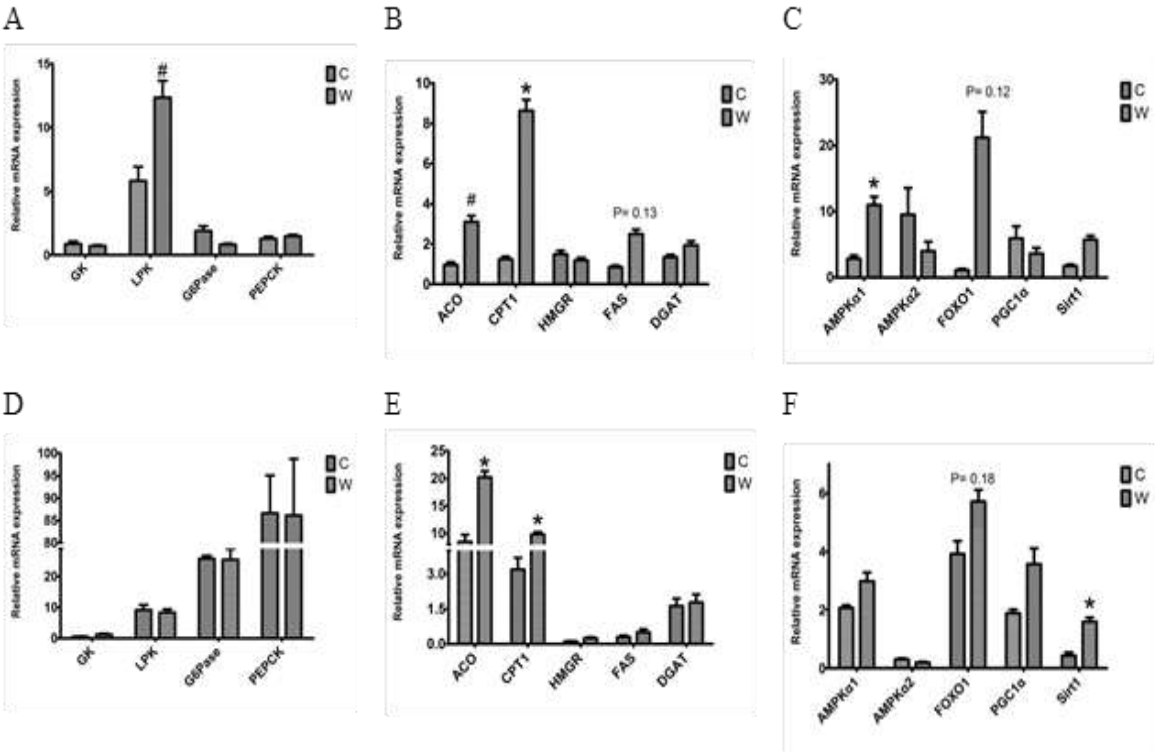


Figure 3. Hepatic gene expression of LeeSung (A, B, C) and Lanyu (D, E, F) pigs. A and D, hepatic carbohydrate metabolic mRNA expression. B and E, hepatic lipid metabolic mRNA expression. C and F, hepatic energy metabolic mRNA expression. Gene expression was normalized to reference gene (TBP) and expressed relative to C group. All results are expressed as mean ± SEM. * $p < 0.05$ vs. control group. # $p < 0.1$ vs. control group.

To conclude, in this study, we fed two native miniature pigs in Taiwan (Lanyu and LeeSung) with Western diet, and found two different responses to the diet. Lanyu pig induced obesity without pathological response, while LeeSung pigs induced obesity with dyslipidemia and fatty liver. These results demonstrated that we establish two animal

models for metabolic syndrome study, one working and one disease model, and SIRT1 might play a critical role in the regulation of physiological and pathological obesity.

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