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細菌素albusin B對於肥胖小鼠脂肪與碳水化合物利用之影響

Effect of albusin B on the lipid and carbohydrate utilization of obese mice

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中文摘要

肥胖被歸為二十一世紀全球性流行病且與代謝症候群相關，其增加第二型糖尿病與肝炎的風險，因此近年來逐漸備受重視。肥胖之盛行源於現代人生活型態的改變，隨著飲食習慣變遷為高糖高脂之西方飲食，導致人們普遍有肥胖問題。

Albusin B 為分子量 32 kDa 且由 *Ruminococcus albus* 7 所分泌之細菌素，藉由 *Saccharomyces cerevisiae* 表現系統進行量產。在先前研究發現，餵予健康小鼠 albusin B 會促進其脂肪代謝並降低小鼠體重，然而 albusin B 對於肥胖小鼠能量代謝之效應尚未被探討。故本試驗以 7 週齡 C57BL/6 公鼠為試驗模式，餵飼小鼠西方飼糧為期 20 週以誘發其肥胖。肥胖小鼠進而逢機分為 3 組，食鹽水組（WS），低濃度 albusin B 組 （0.125 μg albusin B /g 體重; WLA），與高濃度 albusin B 組（0.625 μg albusin B /g 體重; WHA）。小鼠予以灌食食鹽水或 albusin B 連續 4 週後犧牲。結果顯示，西方飼糧餵飼 20 週後之小鼠呈現病態肥胖，其血糖與血脂異常，且具有脂肪肝與脂肪細胞肥大現象。灌食低濃度 albusin B 顯著降低肥胖小鼠之體重、血液總膽固醇與低密度脂蛋白含量，並且減少肝臟脂肪堆積與脂肪細胞大小。高濃度 albusin B 無法調節肥胖小鼠之體重與血液生化值，然可降低肥胖小鼠之脂肪細胞大小。灌食 albusin B 亦降低小腸、肝臟與肌肉對於脂肪酸之吸收。相較於 WS 組，WLA 組小鼠具有較高肝臟與白色脂肪組織之脂質氧化作用，以及較低白色脂肪組織與肌肉之脂質合成作用。另外，albusin B 處理降低肥胖小鼠對果糖之

吸收且 WLA 組具有較高肝臟與肌肉之糖解作用。由呼吸商結果證實灌食 albusin B 可使肥胖小鼠之能量利用轉移至較多的碳水化合物。此外，灌食 albusin B 可增加抗氧化能力與盲腸內 *Bifidobacterium* 之含量。

綜觀上述，口服 0.125 μg /g 體重 albusin B 可降低肥胖小鼠之體重，促進其脂質代謝、碳水化合物之利用與抗氧化能力，並改善其盲腸菌相，進而有效改善飲食引起肥胖之小鼠健康。

關鍵字：Albusin B、飲食引起肥胖、脂質氧化作用、碳水化合物利用、腸道菌相

ABSTRACT

Obesity is one of the major worldwide epidemics and has became a public health problem of the 21st century because of its association with metabolic syndrome, which increases the risks of developing type 2 diabetes and hepatic steatosis. The expansive prevalence of obesity is caused by the dramatic changes of lifestyle. Western diet, made up of high-fat and high-sugars, is the major dietary concern for obesity prevalence.

Albusin B is a 32-kDa bacteriocin from the ruminal bacterium *Ruminococcus albus* 7 and mass-produced by *Saccharomyces cerevisiae* expression system. In the previous study, administration of albusin B caused a decrease in body weight (BW) and plasma triglycerides (TG) levels, and improved lipid metabolism of healthy BALB/C mice. However, the effect of albusin B on energy homeostasis of obese mice has not been elucidated. In this study, 7-week-old C57BL/6 male mice were fed with Western diet for 20 weeks to induce obesity. Then the obese mice (W) were randomly assigned to 3 groups: saline (WS), WLA [0.125 μg albusin B /g body weight (BW)], and WHA (0.625 μg albusin B /g BW). Saline / albusin B was orally administrated for extra 4 weeks then sacrificed. Results showed that Western diet induced morbid obesity in mice, including hyperglycemia, dyslipidemia, fatty liver, and hypertrophy of adipocytes. Oral administration of 0.125 μg albusin B/g BW significantly reduced BW, plasma levels of

total cholesterol (TC) and low density lipoprotein (LDL), hepatic lipid accumulation, and adipocyte size. High concentration of albusin B did not change BW and lipid profiles in plasma, but reduced adipocyte size. Administration of albusin B decreased fatty acid absorption in the ileum, liver, and muscle. Compared with WS group, WLA group had higher lipid oxidation rate in the liver and white adipose tissue (WAT) and lower lipid synthesis in the WAT and muscle. WHA group had a decrease of lipogenic gene expressions in the WAT as compared to WS group. Moreover, albusin B treatment suppressed hepatic fructose uptake and WLA mice had higher glycolytic gene expressions in the liver and muscle. Administration of albusin B increased the respiratory quotient of obese mice, demonstrated that a higher efficiency of carbohydrate utilization for energy expenditure. Albusin B treatments also promoted systemic antioxidant defense and increased caecal counts of *Bifidobacterium*.

Taken together, oral administration of 0.125 μg albusin B/g BW albusin B resulted in body weight loss and promoted lipid metabolism, carbohydrate utilization, and antioxidant capacity, and elevated caecal population of *Bifidobacterium* in diet-induced obese mice. These results therefore partially improve the health of obese mice.

Key Words: Albusin B, Diet-induced obesity, Lipid oxidation, Carbohydrate utilization, Gut microflora

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CHAPTER 1 Literature Review

I Obesity

Obesity is one of the major worldwide epidemics and has become a public health problem nowadays because of its association with metabolic syndrome, including insulin resistance, dyslipidemia, and hypertension, as well as an increase of risk of type 2 diabetes, hepatic steatosis, and cardiovascular diseases (Frayn, 1996; Barceló-Batllori and Gomis, 2009). According to the statistics of World Health Organization (WHO, 2008), the worldwide prevalence of obesity [body mass index (BMI) $\geq$ 30 kg/m^2 and > age 20 years] were 10% and 14% in men and women respectively, that is to say, approximately 205 million men and 297 million women were obese. The data showed that the worldwide prevalence of obesity in 2008 doubled that of 1980. Besides, the obesity prevalence of United States adults was 32.2% among men and 35.5% among woman according to the report of CDC (Centers for Disease Control and Prevention)'s National Health and Nutrition Examination Survey in 2007 to 2008. However, not only Western countries but also Asian populations are impacted by obesity. In many Asian countries, such as China, Japan, and India, the overweight and obesity rate are increased in recent years (Parizkova et al., 2007). According to the Nutrition and Health Surveys in Taiwan (NAHSIT) 1993-1996, the obesity prevalence (BMI $\geq$ 27 kg/m^2) was 10.1% and 12.7% in men and women respectively (Yeh et al., 2011). However, the report of

NAHSIT 2005-2008 showed the prevalence of obesity was 18.9% in men and 17.1% and in women (Yeh et al., 2011). The obesity prevalence of Taiwan was higher than several other Asian countries when obesity was defined as BMI≥ 30 kg/m^2 according to International Association for the Study of Obesity (IASO) in 2010. Due to the increased obesity prevalence, obesity-related diseases become a concerned issue in the last two decades.

The expansive obesity prevalence is caused by the dramatic changes of lifestyle, including lack of physical activities and the westernization of diet pattern (Kopelman, 2000). In the United States, Western diet has been identified. It is made up of high-fat and high-sugars mainly comprising processed meats, fast food, high-fat dairy products, refined carbohydrates, and low levels of vegetables and fruits (Michels and Schulze, 2005). Diet-induced obesity (DIO) is the most common consequence due to the prolonged intake of Western diet. Although Western diet has been identified, many studies have showed that diet effect varies distinctly depending on the dietary compositions, including the energy percentage from fat and carbohydrate, type of fat and carbohydrate, and compositions of fatty acids.

I.1 Effect of Dietary Fat

Table 1 shows the metabolic changes by different energy percentage level of fat in diet from several studies. It has been shown that treatment of fat-enriched diet for

Table 1 Dietary fat effects[1]

Rodent Model	Energy percentage from fat	Duration	vs.	Effects	Citation
Wistar rats	32%	8 weeks	Chow diet	BW↑, plasma TG↑, lipid peroxidation↑, and antioxidant enzyme activity↓	(Bełtowski et al., 2000)
C57BL/6J mice	40%	24 weeks	Chow diet	BW↑, fat pads weight↑, plasma TC↑, hepatic FFA↑, protein peroxidation↑, and insulin resistance	(Matsuzawa-Nagata et al., 2008)
C57BL/6J mice	42%	4 weeks	Chow diet	BW↑, plasma TC and LDL↑, plasma HDL↓, adipocyte hypertrophy, and hepatic steatosis	(Nascimento et al., 2010)
Wistar rats	45%	32-48 weeks	Chow diet	BW↑, plasma TG↑, and cardiac power↓	(Wilson et al., 2007)
C57BL/6J mice	60%	8 weeks	Chow diet	BW↑, fat pads weight↑, plasma Glc and FFA↑, and adipocyte size↑	(Gaidhu et al., 2010)
Wistar rats	60%	32-48 weeks	Chow diet	BW↑ and Plasma FFA↑	(Harmancey et al., 2010)
C57BL/6J mice	72%	16 weeks	Chow diet	Plasma glucose↑, hepatic steatosis and mitochondrial ROS↑	(Eccleston et al., 2011)

[1]Abbreviation: BW, body weight; TG, triglyceride; TC, total cholesterol; FFA, free fatty acids; LDL, low density lipoprotein; HDL, high density lipoprotein; ROS, reactive oxygen specie.

short-term or long-term, both induced an increase in BW (Wilson et al., 2007; Nascimento et al., 2010). However, consumption of diet with 30 to 45% energy from fat caused more adverse effects than consumption of diet with 60% to 75%, including dyslipidemia, insulin resistance, and oxidative stress (Bełtowski et al., 2000; Matsuzawa-Nagata et al., 2008; Nascimento et al., 2010). High fat diet induced dyslipidemia, hepatic steatosis, and adipocyte hypertrophy within 8 weeks in mice (Nascimento et al., 2010), then causes oxidative stress and insulin resistance (Matsuzawa-Nagata et al., 2008; Eccleston et al., 2011), and impaired the cardiac function (Wilson et al., 2007).

Abundant studies investigated the relationship between dietary fatty acid composition and metabolic disorders. Fatty acids have generally been classified into three groups: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Table 2 lists the metabolic changes by different FA composition of diet in several literatures. Compared to MUFA and PUFA, SFA-enriched diet appears to be more harmful to health. In SFA, stearic acid (18:0) might improve plasma lipid profiles (Grundy, 1997), but auric acid (12:0), myristic acid (14:0), and palmitic acid (16:0) caused hypercholestemia (Khosla and Sundram, 1996; Grundy, 1997). Furthermore, palmitic acid had been proposed that potentially harm to animals, including induction of hyperinsulinemia (Ikemoto et al., 1996) and an increase of

Table 2 Dietary fatty acids effects[1]

Model	FA type	FA	Supplementation	vs.	Effects	Citation
Guinea pig	SFA	12:0 + 14:0	Palmkernel oil diet	Palm oil diet	Plasma TC and LDL↑	(Khosla and Sundram, 1996)
Human	SFA	16:0	SFA-rich diet	PUFA-rich diet	Plasma LDL↑	(Grundy, 1997)
Human	SFA	16:0	Palm oil diet	Chow diet	Plasma HDL↓	(Mattson and Grundy, 1985)
Mice	SFA	16:0	Palm oil diet	Safflower oil diet	BW↑, glucose tolerance↓, and hyperinsulinemia	(Ikemoto et al., 1996)
Hepatocyte	SFA	16:0	Palmitate	Control	Cell viability↓	(Ji et al., 2005)
Hepatocyte	SFA	16:0	Palmitic acid	Control	Apoptosis↑ and autophagy↓	(Mei et al., 2011)
Human	SFA	18:0	Safflower oil diet	Palm oil diet	Plasma LDL/HDL↓	Grundy, 1997
Human	MUFA	16:1	High-palmitoleate safflower diet	Palm oil diet	Plasma LDL↓	(Mattson and Grundy, 1985)
β-cell	MUFA	16:1	Palmitoleate	Control	ER stress↓	(Diakogiannaki et al., 2008)
Human	MUFA	18:1	High-oleic safflower diet	Palm oil diet	Plasma TG, LDL, and HDL↓	(Shepherd et al., 1980)
Hepatocyte	MUFA	18:1	Oleic acid	Control	Apoptosis↓ and autophagy↑	(Mei et al., 2011)
Hepatocyte	MUFA	18:1	CLA (*t*-10,*c*-12)	Palmitic acid	Apoptosis↓ and ER stress↓	(Wei et al., 2007)

[1]Abbreviation: TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; ER, endoplasmic reticulum; CLA: conjugated linoleic acid.

Table 2 (continued) Dietary fatty acids effects[1]

Model	FA type	FA	Supplementation	vs.	Effects	Citation
Human	PUFA	18:2	High-linoleic safflower diet	Palm oil diet	Plasma LDL and HDL↓	(Mattson and Grundy, 1985)
Human	PUFA	18:2	meta-analysis	Oleic acid	Plasma LDL↓	(Mensink and Katan, 1992)
Human	PUFA	n-3 PUFA	Fish-oil + HFD	HFD	BW↓, fat pads↓, plasma TG↓ and HDL↑, adipocyte size↓, and hepatic lipid droplets↓	(Nascimento et al., 2010)
Human	*trans*-MUFA	*t*-18:1(n-9)	Elaidic acid-rich diet	Oleic acid-rich diet	Plasma LDL/HDL↑ and TC/HDL↑	(Khosla and Sundram, 1996)

[1]Abbreviation: TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; HFD, high fat diet.

cellular apoptosis and a decrease of cellular autophagy (Mei et al., 2011). Studies also showed that MUFA-enriched diet (both 16:1 and 18:1) improved lipid profiles of plasma (Shepherd et al., 1980), reduced apoptosis and ER stress to eliminate obesity-induced hepatic steatosis (Wei et al., 2007). PUFA-enriched diet is the most favorable to improve blood lipid profiles than SFA- and MUFA-enriched diet (Mattson and Grundy, 1985; Mensink and Katan, 1992) and n-3 PUFA might have anti-obesity effects (Nascimento et al., 2010). Dietary *trans*-MUFA (produced by chemical hydrogenation specially) was concerned with its adverse effects for many years. Actually, it has been indicated that *trans*-MUFA had adverse effects on blood lipid, including increasing total cholesterol and LDL, and decreasing HDL concentration (Grundy, 1997).

I.2 Effect of Dietary Carbohydrate

It has been conducted that DIO due to chronic exposure to high levels of FA and glucose results in insulin signal impairment and glucose intolerance (Garbarino and Sturley, 2009). Dietary carbohydrates, including polysaccharides (e.g. starch), disaccharides (e.g. sucrose), and monosaccharides (e.g. glucose and fructose), are absorbed in the small intestine as monosaccharide form. After ingestion and absorption of carbohydrates, glucose is transported to peripheral tissues as fuel sources or stored as

glycogen (Frayn, 1996). While, excessive glucose was converted into 16:0 and 18:0 SFA or MUFA and stored as TG by *de novo* lipogenesis (Jump, 2011). Major sources of carbohydrates in the diet include starch, sucrose, and fructose (Schaefer et al., 2009). In fructose metabolism, approximately 25% digested fructose is converted into lactate in the small intestine and liver, and was released to the circulation (Tappy et al., 2010). A large portion of fructose (about 50%) is converted into glucose in the liver and is released into the systemic circulation. A small portion of fructose is converted into fatty acids as TG storage or was stored as hepatic glycogen (Tappy et al., 2010).

As described above, hepatic metabolism of fructose is partially involved in *de novo* lipogenesis, suggesting that consumption of fructose might contribute to increases of blood lipid levels. Table 3 shows the metabolic changes by carbohydrate from various sources and levels in several studies. Fructose or sucrose treatment for short-term (within 10 weeks) induced dyslipidemia (Reiser et al., 1981; Swanson et al., 1992; Bantle et al., 2000; Rebolledo et al., 2008; Francini et al., 2010). However, prolonged consumption of fructose or sucrose caused insulin resistance (Chicco et al., 2003; Dell et al., 2012). Consumption of fructose or sucrose had adverse effects in health compared with consumption of an equal amount of starch or glucose (Swanson et al., 1992; Chicco et al., 2003; Stanhope and Havel, 2008). Stanhope et al. (2009) conducted that overweight subjects orally administrated with glucose had similar BW with those with

Table 3 Dietary carbohydrate effects[1]

Model	Carbohydrate type	Energy percentage from sugar	Duration	vs.	Effects	Citation
Human	Sucrose diet	5%, 18%, 33%	6 weeks	Starch diet	Plasma TG, TC, and LDL↑ in dose-dependent	(Reiser et al., 1981)
Wistar rats	Sucrose diet	63%	30 weeks	Starch diet	BW↑, plasma glucose and FFA↑, and insulin resistance	(Chicco et al., 2003)
Wistar rats	Fructose beverage	10%	3 weeks	Water	Plasma TG↑, GT↓, lipid peroxidation↑, and antioxidant enzyme activity↓	(Rebolledo et al., 2008)
Human	Fructose diet	17%	6 weeks	Glucose diet	Plasma TG↑	(Bantle et al., 2000)
Human	Fructose diet	20%	4 weeks	Starch diet	Plasma TC and LDL↑	(Swanson et al., 1992)
Human	Fructose beverage	25%	10 weeks	Glucose beverage	Plasma TG↑ and visceral adiposity↑	(Stanhope and Havel, 2008)
Sprague-Dawley rats	Fructose +SFA diet	62%	30 weeks	Starch diet	Hyperinsuliemia, dyslipidemia, and insulin resistance	(Dell et al., 2012)

[1]Abbreviation: BW, body weight; TG, triglyceride; TC, total cholesterol; FFA, free fatty acids; LDL, low density lipoprotein; GT, glucose tolerance; SFA, saturated fatty acids.

fructose, but only latter impaired glucose tolerance (Stanhope et al., 2009). In fact, it has been indicated that fructose is more lipogenic than glucose (Havel, 2005).Consumption of fructose along with glucose (e.g. sucrose form) enhances the fructose absorption (Havel, 2005). Besides, in contrast to glucose, fructose metabolism is not modulated by insulin, and fructose absorption is enhanced by dietary SFA (Perin et al., 1997). Dell *et al*. (2012) found that prolonged feeding of high fructose with SFA diet caused hyperinsulinemia, dyslipidemia, and insulin resistance, decreased palmitoleic acid content in plasma, and increased plasma arachidonic acid (20:4) levels, the most oxidation-prone fatty acid and may generated ROS (Dell et al., 2012). These characteristics of fructose appear to be a major factor to induce obesity and these results suggested that high fructose diet is associated with the development of metabolic syndrome.

I.3 Effect of Western diet

According to the definition of Western diet, in general, the range of energy percentage from fat is 35% to 45%, as for carbohydrate, is also ranged from 35% to 45% (Harmancey et al., 2010; Nascimento et al., 2010). That is to say, Western diet-induced obesity and metabolic disorders were contributed to dietary fat but also from dietary carbohydrates. Furthermore, Western diet frequently contains excessive SFA (especially

palmitic acid) and *trans*-MUFA (Cordain et al., 2005), and high fructose in the form of sucrose and high fructose corn syrup (42-55% energy as fructose and the reminder as glucose), a common sweetener used in the food industry (Rayssiguier et al., 2006). Besides, Western diet-induced lipotoxicity is correlated with development of morbid obesity, insulin resistance, cardiomyopathy, and hepatic steatosis (Boden and Shulman, 2002; Unger, 2002; Jump, 2011). Therefore, Western diet, enriched with high fat (palmitic acid-rich) and high sugar (fructose-rich), was shown adverse effects in previous studies, including morbid obesity, insulin resistance (Matsuzawa-Nagata et al., 2008), dyslipidemia (Nascimento et al., 2010), and oxidative stress (Bełtowski et al., 2000), known as diet-induced 'lipotoxicity'.

I.4 Lipotoxicity

Lipotoxicity is a term coined by Unger in 1994; it is indicated that increases in blood FFA concentrations concomitantly induces insulin hypersecretion and insulin resistance leading to abnormality of obesity-related β-cells before and at the onset of non-insulin-dependent diabetes mellitus (Lee et al., 1994). In addition to causing developments of insulin resistance and type 2 diabetes, lipotoxicity is correlated with morbid obesity, cardiomyopathy, and hepatic steatosis (Boden and Shulman, 2002; Unger, 2002; Jump, 2011). In a steady state, balance of energy intake and expenditure maintain energy homeostasis; however, excessive energy intake or decreases in energy

expenditure result in weight gain and fat deposition, further indicating obesity (Lelliott and Vidal-Puig, 2004). The surplus consumption of dietary fat or dietary carbohydrates is primarily stored as TG in white adipose tissue (WAT); hence it prevents excess energy accumulating in non-adipose tissues (e.g. liver and muscle), and further enhancing its catabolism so as to reduce ectopic fatty acid depositions (Unger et al., 2010). Nevertheless, most cells have limited capacity to store excess energy. If the impairment of fat deposition in WAT despite the elevated energy intake, excess energy inhibits glycolysis, further promotes *de novo* lipogenesis (Garbarino and Sturley, 2009). Because adipocytes are unable to store all of excess energy persistently and insulin resistance thus results in an increase of hormone sensitive lipase-mediated FA output from WAT, therefore non-adipose tissues suffer from free fatty acids (FFAs) overload from the circulation. These phenomena lead to 'lipotoxicity' ultimately.

In fact, lipotoxicity is associated with programmed cell death or apoptosis, resulting from mitochondrial dysfunction or endoplasmic reticulum (ER) stress (Malhi and Gores, 2008; Garbarino and Sturley, 2009). In metabolic pathways, oxidation of lipid and glucose concomitantly releases ROS and these free radicals can be eliminated by antioxidant defense (Rolo et al., 2012). However, chronic exposure to Western diet causes lipotoxicity, which induces massive formation of ROS, leading to damages of DNA, proteins, and lipids, whereas antioxidants are inadequate to diminish ROS

production, ultimately resulting in the oxidative stress (Mantena et al., 2008). Mitochondria are the primary site of cellular ROS formation. Mitochondrial dysfunction by surplus ROS, an increase of ceramide synthesis from palmitoyl-CoA, and a decrease of mitochondrial cardiolipin synthesis, leading to the induction of apoptosis via release of cytochrome *c* (Garbarino and Sturley, 2009; Wende and Abel, 2010). On the other hand, *in vitro* studies have shown that surplus lipid into the endoplasmic reticulum (ER) membrane and compromised the structure and integrity of ER, ultimately enhanced ER stress (Wende and Abel, 2010). The ER stress promoted the release of calcium into mitochondria and altered the function of proapoptotic BCL-2 family members, BAX and BAK, further leading to an increase of apoptosis (Wende and Abel, 2010).

I.4.1 Lipotoxicity and White Adipose Tissue

There are two types adipose tissue, white and brown. The main function of former is energy storage; brown adipose tissue provides heat instead (Bronk, 1999). Surplus glucose and fatty acids are converted into TG during the postprandial state, while WAT converts the stored TG into FFA as a metabolic substrate for requirements of other tissues, such as starvation and exercise (Girousse and Langin, 2012). TG droplets within an adipocyte come from two ways: dietary TG from the circulation and *de novo* lipogenesis, that is, the synthesis of TG from other substrates, particularly glucose (Frayn, 1996).

It has been shown that surplus lipid accumulation in WAT caused hypertrophy and hyperplasia of adipocytes, and induced mitochondrial oxidative stress and ER stress, therefore resulting in adipocytes dysfunction eventually (de Ferranti and Mozaffarian, 2008; Garbarino and Sturley, 2009). Dysfunctional adipocytes thus decrease lipogenesis and increased lipolysis, further leading to release of FFAs into the systemic circulation (Funaki, 2009). In addition, the secretion of adipocytokines was affected in dysfunctional adipocytes. Secretion of adiponectin decreases, while other adipokines, such as leptin, resistin, and visfatin increases, and to affect the metabolism of non-adipose tissues upon secretion into circulation (Wree et al., 2011). Adiponectin has been known to enhance insulin sensitivity and FA oxidation. Leptin regulates energy metabolism by increasing energy expenditure and decreasing energy intake. Resistin is associated with an increase in insulin resistance, and visfatin participates in glucose metabolism by stimulating glucose utilization (Vázquez-Vela et al., 2008). On the other hand, increases in the proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and monocyte chemotactic protein-1 (MCP-1), were observed in dysfunctional adipocytes due to overloading of FAs (Funaki, 2009). MCP-1 recruits marcrophages into the WAT, causing a chronic inflammatory response in WAT (Guilherme et al., 2008). Furthermore, FAs activate Toll-like receptor 4 on the surface of marcrophages and adipocytes, leading to activation of nuclear factor kappa B (NFκB)

and c-Jun N-terminal protein kinase (JNK) pathway, which are related with apoptosis, the inflammation and impairment of insulin signaling are further induced by FA overload through Toll-receptor pathway (Malhi and Gores, 2008).

I.4.2 Lipotoxicity and Non-adipose Tissue

As described above, ectopic lipid accumulation occurred when oxidative capacity of lipid in adipocyte becomes saturated. Liver plays a significant role in metabolism as both victim and culprit when lipotoxicity occurred (Ricchi et al., 2009). In fact, an increase of FFA influx into the liver with subsequent abnormal lipid accumulation may progress into development of fatty liver disease, such as nonalcoholic fatty liver disease (NAFLD) (Malhi and Gores, 2008). As the development of lipotoxicity in WAT, inflammatory cytokines mediate cytotoxicity in the liver. Surplus FAs induce production of TNF-α from adipocytes, resulting in an increase of plasma IL-6, IL-12, and NFκB and finally leading to the chronic inflammatory state (Trauner et al., 2010). On the other hand, elevated circulating levels of TNF-α due to inflammatory by adipocytes activate hepatic JNK-dependent pathway (van Herpen and Schrauwen-Hinderling, 2008), which inactivates Bcl-2, an anti-apoptosis member (Malhi and Gores, 2008), and directly activates Bax, leading to apoptosis (Wree et al., 2011). Besides, oxidative stress occurs due to massive ROS generation, which acts as a central role in causing lipotoxic liver injury and fatty liver disease (Neuschwander-Tetri, 2010; Wree et al., 2011).

Skeletal muscle is the major site responsible for glucose disposal upon insulin stimulation and is thought to be the early place in insulin resistance (Dulloo et al., 2004). In fact, a major contribution to whole-body insulin resistance results from the dysfunction in skeletal muscle (Defronzo et al., 1981). Excess FFAs result in accumulation of lipid in the skeletal muscle (called intramyocellular lipids, IMCL) and exerts a negative effect on insulin sensitivity (Schrauwen, 2007). On the other hand, the imbalance between fat availability and capacity of oxidation in skeletal muscle causes accumulation of lipid intermediates, including diglycerides (DG), ceramide, and acyl-CoA (van Herpen and Schrauwen-Hinderling, 2008). Cellular accumulation of ceramide causes cell dysfunction and apoptosis, and activation of DG signaling inhibits insulin receptor substrates / phosphatidylinositol 3-kinase pathway (IRS/PI3K), ultimately resulting in a decrease in translocation of glucose transporter 4 to membrane and glucose uptake in the muscle (Dulloo et al., 2004). In brief, prolonged consumption of Western diet causes lipotocixity in WAT and non-adipose tissues, leading to morbid obesity, even metabolic diseases.

I.5 Gut Microflora

Intestine is responsible to nutrition digestion and metabolic regulation. While there is the gut microbial community comprises trillions of microorganisms within the human gastrointestinal tract, collectively known as the gut microflora. Gut microflora is

dominated by anaerobic bacteria and includes 500 to 1000 species (Backhed et al., 2004). Though there are marked varieties in gut microflora compositions among individuals, counts of intestinal microbiota increases from 10^3 to 10^4 colony forming unit (CFU)/mL, 10^7 to 10^9 CFU/mL, 10^{10} to 10^{12} CFU/mL in the jejunum, the terminal ileum and the colon, respectively (DiBaise et al., 2008). Besides, the majority of phyla in the gut microflora belong to *Firmicutes*, *Bacteriodetes*, and *Actinobacteria* (Diamant et al., 2011). *Firmicutes*, includes *Lactobacillus*, *Clostridium*, *Bacillus*, and so on. The dominate genera of *Bacteriodetes* is *Bacteriodes*; in addition, one of the probiotics – *Bifidobacterium*, belongs to the order of *Actinobacteria* (Musso et al., 2011). Gut microflora can affect host metabolism, nutrition, and physiology. Hence it is regarded as a metabolic 'organ' (Backhed et al., 2004). Gut microflora can function to maintain host homeostasis, including fermentation of indigestible components from diet, defense of intestinal pathogens, synthesis of micronutrients (Rolfe, 1984; Macpherson and Harris, 2004; Backhed, 2005; Diamant et al., 2011).

In fact, a tight connection between obesity and gut microflora has been reported. Bäckhed *et al.* (2004) found conventionally raised mice had over 40% more fat pads compared with germ-free mice. Germ-free mice had smaller size of adipocytes and lower TG accumulation in the liver (Backhed et al., 2004). In addition, distractively different compositions of the gut microflora are observed between lean and obese

animals. A decrease of the proportion of *Bacteriodetes* relative to total bacteria was found in genetically obese leptin-deficient ob/ob mice as compared with wide-type mice (Murphy et al., 2010). DIO is correlated with the changes of gut microflora as well. Mice fed with Western diet harbored an increase of *Firmicutes* and reduction of *Bacteriodetes* in the cecal microbiota (Turnbaugh et al., 2008). Except for the two dominant populations, the contents of *Bifidobacterium* dramatically reduced in obese mice (Cani et al., 2007). Consistent with animal studies, the relative count of *Bacteriodetes* is decreased in obese adults (Ley et al., 2006). These data suggested that alteration of gut microflora may be able to modulate metabolic regulation of DIO.

II Becteriocin and Albusin B

Probiotics as a therapy to improve human health recent years (Isolauri et al., 2004), including suppressing plasma TG, TC, and LDL concentrations and promoting HDL levels (Bertazzoni Minelli et al., 2004; Ooi and Liong, 2010). However, it has been indicated that one of the beneficial effects by probiotics is throughout production of bacteriocins (Corr et al., 2007; Murphy et al., 2012) and some probiotics, such as lactic acid bacteria, actually produce bacteriocins to improve the health of host (Cleveland et al., 2001; O'sullivan et al., 2002).

II.1 Bacteriocin

Bacteriocins have been applied in food safety or additives (Cleveland et al., 2001),

and pathogens control in livestock (Diez-Gonzalez, 2007) in recent years. They are originally regarded as proteins which had lethal bioactivity, are further loosely defined as the substances produced by bacteria (Tagg et al., 1976). To date, bacteriocins, the naturally proteinaceous peptides from secretion of bacteria, have highly specific killing spectrum. Bacteria secrete bacteriocins to compete against closely related bacteria or bacteria of other species same as them, thus bacteriocins are distinct from traditional antibiotics (Klaenhammer, 1998; Lohans et al., 2012). Besides, antibiotics are known to be enzymatically synthesized compounds and act via metabolic inhibition, but bacteriocins are ribosomal synthesized gene-coded peptides (Sang and Blecha, 2008). Bacteriocins are found in almost all bacteria species, including gram-positive bacteria, gram-negative bacteria, and archaea. Bacteriocins have been classified into a variety of distinct types and classes in the term of size, microbial character, and inhibitory mechanism:

II.1.1 Bacteriocins from Gram-negative bacteria: colicins produced by *Escherichia coli* are probably most thoroughly established. At least 30% of all *E. coli* are able to produce at least one type of colicins, and these bacteriocins against other *E. coli* by destroying target DNA and inhibiting protein synthesis (Riley and Wertz, 2002a; Diez-Gonzalez, 2007).

II.1.2 Bacteriocins from Gram-positive bacteria: lactic acid bacteria (LAB) are

prolific in production of bacteriocins. According to the classification by Klaenhammer (1998), there are four classes of LAB bacteriocins.

II.1.2.1 Class I bacteriocins are the lantibiotics (< 5 kDa), which are post-translationally modified and contain lanthionine, β-methyllanthionine, and dehydrated amino acids, such as nisin. Type A lantibiotics depolarize the membrane of targets and type B lantibiotics kill targets by enzyme inhibition (Klaenhammer, 1998; Riley and Wertz, 2002b).

II.1.2.2 Class II bacteriocins are small and heat-stable, and have no post-translational modification (< 10 kDa). This class is divided into three subgroups. Class IIa bacteriocins are particularly inhibit *Listeria* species via the formation of pores in the membrane of target (Riley and Wertz, 2002b). Class IIb bacteriocins contain two different proteinaceous peptides for activity. The third bacteriocins (IIc) are circular (Lohans et al., 2012) and destroy targets by reduction of cycteine (Klaenhammer, 1998).

II.1.2.3 Class III are large heat-labile bacteriocins (generally >30 kDa). This class had not been studied well, and there are only six Class III bacteriocins were characterized so far. Acidophilucin A is produced by *Lactobacillus acidophilus* LAPT 1060 and against six strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* and six strains of *Lactobacillus helveticus* (Toba et al., 1991). Heveticin V–1829 is

a heat–labile (inactivated at 50℃ for 30 minutes) bacteriocin isolated from *Lactobacillus helveticus* 1829 (Vaughan et al., 1992). Heveticin J, a 37.5-kDa bacteriocin produced by *Lactobacillus helveticus* 481, has narrow antimicrobial spectrum (Venema et al., 1995). Millericin B is isolated from *Streptococcus milleri* NMSCC 061 and has antimicrobial activity with streptococci, listeriae, and lactococci (Nilsen et al., 2003). Zoocin A is a 29-kDa bacteriocin produced by *Streptococcus equi* subsp. *zooepidemicus* 4881 that is against other streptococcal species (Heath et al., 2004). Enterolysin A, a large and cell wall-degrading bacteriocin with anti-listerial activity, produced by *Enterococcus faecalis* LMG 2333 or *Enterococcus faecalis* II/1 (Nigutová et al., 2008). Class III bacteriocins have unique domain structure so their mode of action is different from other bacteriocins. They function directly on the cell wall of target by hydrolysis, leading to death of sensitive cells (Cotter et al., 2005).

II.1.2.4 Class IV bacteriocins require proteins plus lipids or carbohydrates for activity.

II.1.3 Archaea: these spices produce the unique bacteriocins-like antimicrobials, known as archaeocins (Riley and Wertz, 2002b).

II.2 Albusin B

Many bacteriocins have been investigated in ruminal microorganisms who are

involved in manipulating microflora of rumen, including *Clostridium* spp., *Bacteriodes* spp., *Streptococcus* spp., *Lactobacillus* spp., *Butyrivibrio* spp., *Staphylococcus* spp., and *Enterococcus* spp. (Kalmokoff et al., 1996; Kalmokoff and Teather, 1997; Mantovani and Russell, 2003). In addition, bacteriocins of the predominant ruminal cellulolytic bacteria have been readily surveyed. Odenyo et al. (1994) cocultured *Ruminococcus albus* 8 and *R. flavefaciens* FD-1 on cellobiose medium and found that *R. albus* 8 inhibited the growth of *R. flavefaciens* after 3 h of inoculation by production of heat stable substrates (Odenyo et al., 1994). Subsequent study indicated that *R. albus* 7 produced a heat-labile bacteriocin-like compound to inhibit the growth of *R. flavefaciens*, and the inhibitory substance was destroyed by protease (Chan and Dehority, 1999). Further studies purified and characterized the bacteriocin-like compound produced by *R. albus* 7 and named the 32-kDa protein as albusin B (Chen et al., 2004), which was conducted to be Class III bacteriocin as defined by Klaenhammer (1998).

To study the potential application of albusin B, albusin B was mass-produced by yeast system (Yang, 2008). Wang et al. (2011) suggested that broilers with supplementation of albusin B had better growth performance, intestinal absorption (Wang et al., 2011), and antioxidant defense (Wang et al., 2012). In addition, albusin B modulated the gut microflora by increasing caecal *Lactobacillus* counts and decreasing pathogenic populations (Chen et al., 2012). Furthermore, our laboratory also found that

oral administration of albusin B caused a decrease in BW, plasma TG and TC concentrations, and improved lipid metabolism in healthy mice (Hsieh, 2011). Except for *in vivo* study, Hsieh (2011) treated cardiomyocytes and colon cancer cells with albusin B under oxidative stress and found that albusin B increased the cell viability, lipid oxidation, and antioxidant capacity in both of two cell lines (Hsieh, 2011).

Albusin B regulates lipid metabolism on healthy mice, whereas the effect of albusin B on obese mice has not been elucidated. For this purpose, the effects of energy metabolism by albusin B in DIO mice was studied.

CHAPTER 2 Materials and methods

I Animal treatment

All animal procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan University. 5-week-old C57BL/6 male mice were obtained from the Laboratory Animal Center, National Taiwan University College of Medicine. After a 2-week acclimation period, mice were fed with chow diet (C) containing 13.5% fat (LabDiet® 5001 laboratory rodent diet, St. Louis, MO, USA) or Western diet (W) containing 45.7% fat (TestDiet® 58V8 DIO Rodent Purified Diet, Richmond, IN, USA) *ad libitum* for following 24 weeks. Then obese mice (W) were randomly assigned into 3 groups: W + saline (WS), W + LA [0.125 μg albusin B/g body weight (BW)], and W + HA (0.625 μg albusin B/g BW). Lean mice (C) were orally administrated with saline. Saline/albusin B were daily administrated by gavage in the last 4 weeks. Each treatment of 15 mice (5 mice / cage) was housed in a room at 25°C ± 1°C under a 12-hour light/12-hour dark cycle. Body weight and food intake were recorded weekly. At the end of experiment, blood sample and fresh caecal contents were collected. After mice were sacrificed by carbon dioxide, the heart, liver, epididymal adipose tissue, and perirenal adipose tissue were weighed. Samples from the liver, epididymal adipose tissue, ileum (3 to 6 cm upwards of the cecum), and muscle of

femurs were collected and stored at -80°C until analyzed.

II The preparation of albusin B

Mass-production of albusin B-expressing yeast was described previously (Yang, 2008). In brief, *alb*B gene was cloned into *Saccharomyces cerevisiae* and mass-produced the albusin B-expressing yeast by fermentation. Then yeast products were crushed by high pressure crusher and the supernatants were collected by centrifugation at 12000 × g, 4°C for 30 minutes. After obtainment of the concentrated albusin B, the protein concentration of albusin B was measured and calculated for the utilization of each albusin B treatments. The protein concentration was documented by Bradford method (B6916, Sigma, St. Louis, MO, USA).

III Blood biochemical parameters assay

Blood samples were obtained from the facial vein of mice in starved state before oral albusin B treatment and at the end of experiment. Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA; E5134, Sigma, St. Louis, MO, USA) was used as an anticoagulant. Plasma samples were collected by centrifugation at 2500 × g, 4°C for 10 minutes and stored at -80°C for use. Glucose concentration of plasma was measured within 12 hours of collection. After appropriate dilution of plasma samples, enzymatic assay kits were used for analysis of plasma glucose (GL2623, Randox, Antrim, UK), triglyceride (BXC0272C, Fortress, Antrim, UK), total cholesterol (BXC0261B, Fortress,

Antrim, UK), high density lipoprotein (BXC0442A, Fortress, Antrim, UK), and free fatty acids (K612, BioVision, Milpitas, CA, USA) were determined through enzymatic assay kits using the colorimetric method (Spectra max 190, Molecular Devices, CA, USA) according to the manufacturer's instructions.

IV Histological study

Liver and epididymal adipose tissue samples were rinsed by PBS (phosphate buffer saline; 8g/L NaCl, 0.2g/L KCl, 2.9 g/L $Na_2HPO_4 \cdot 12H_2O$, 0.2g/L KH_2PO_4, pH 7.4), then fixed in 10% neutral buffed formalin (Millinckrodt Baker, H121-08, Phillipsburg, NJ, USA) for one day and embedded in paraffin blocks. Paraffin tissue sections with 6 μm thickness were stained with hematoxylin and eosin. Slices were photoed under microscope (Olympus BX53, Tokyo, Japan) and the photomicrographs (original magnification x 200) were quantified by the ImageJ software (NIH Image, Bethesda, MD, USA). Liver sections were quantified according to color and shape of the images for proportion of lipid droplets in the whole field by the default tools of ImageJ. Images were adjusted to 8-bit and appropriate threshold, then the tool of analyze particles was executed with particle size = 80 - infinity and circularity = 0.55 - 1.00. Epididymal adipose tissue sections were quantified according to the shape of images to for the size of adipocytes in the whole field by the plugin of ImageJ, MRI Adipocyte Tools. After scale was set up (μm^2), images were analyzed by Simple

Adipocytes Segmentation with min. size = 100, max. size = 50000, and nr. of dilates = 10. All images were analyzed in 3 different fields.

V mRNA extraction and real-time polymerase chain reaction (PCR)

V.1 Homogenization: 0.1 – 0.2 g of the frozen tissue samples were homogenized in 1 mL of TRIsure (Bio-38033, Bioline, London, UK). Homogenized samples were centrifuge at 12000 × g, 4˚C for 10 minutes and the supernatants were collected.

V.2 Phase separation: 100 μL 1-bromo-3-chloropropane (BCP; BP151, Molecular research center, Cincinnati, OH, USA) was added to the supernatants. The mixtures were incubated for 15 minutes at 4˚C, then were centrifuged at 12000 × g, 4˚C for 10 minutes and upper aqueous phase was collected.

V.3 RNA precipitation: the aqueous phase and 1 mL isopropyl alcohol (0918, Amresco, Solon, OH, USA) were mixed thoroughly and stored at -20˚C for 1 hour, then the mixtures were centrifuged at 12000 × g, 4˚C for 10 minutes.

V.4 RNA washing: 1 mL 75% ethanol was added to RNA pellets for suspension. Samples were centrifuged at 12000 × g, 4˚C for 10 minutes.

V.5 RNA dissolving: the RNA pellets were air-dried on ice for 15 minutes. Adequate diethyl pyrocarbonate (DEPC; 1609-47-8, Bio Basic, Canada) -treated water was added to dissolve the RNA pellets at 65°C for 5 minutes.

RNA concentration was determined by the Nanodrop spectrophotometers (ND-1000, Thermo, Waltham, MA, USA).

V.6 DNA digestion: RNA samples were digested with Turbo DNase-*free*™ kit (AM1907, Ambion, Austin, TX, USA). The following reactions were mixed in a microfuge tube for each sample and incubated at 37°C for 30 minutes:

Reactions	Volume
7 - 10 μg RNA	x μL
10x TURBO DNase buffer	2 μL
TURBO DNase	1 μL
Nuclease-free water	17 - x μL
Total volume	20 μL

RNA samples were mixed with 2 μL inactivation reagent and incubated at room temperature for 5 minutes. Samples were centrifuged at 12000 × g, 4°C for 5 minutes and the supernatants were collected, then RNA concentration was determined.

V.7 Reverse transcription: RNA samples were reverse-transcribed to cDNA using High Capacity cDNA Reverse Transcription Kit (4368814, Applied Biosystem, Forter City, CA, USA). The following reactions were mixed in a microfuge tube for each sample:

Reacions	Volume
2 μg RNA	x μL
10x RT buffer	2 μL
10x RT primer	2 μL
25x dNTP (100 mM)	0.8 μL
Multiscribe™ Reverse Transcriptase	1 μL
RNaseOUT™ (10777-019, Invitrogen, Carlsbad, CA, USA)	1 μL
Nuclease-free water	13.2 - x μL
Total volume	20 μL

The reaction was incubated at 25°C for 10 minutes, at 37°C for 2 hours, and at 85°C for 10 minutes.

V.8 Real-time PCR: The cDNA samples were quantified by KAPA SYBR® Fast Master Mix (KK4603, KAPA Biosystems, Woburn, MA, USA) with StepOne™ Real-time PCR system (Applied Biosystem, Forter City, CA, USA) according to the manufacturer's recommendations. The real-time PCR condition is as the following:

95°C, 10 mins → 94°C, 30 secs (desaturation) → 62°C, 1 mins (annealing/extension)

40 cylces

All primer sequences used are listed in Table 4. Comparative Ct (threshold

cycle) method was used for relative quantification. The Ct value was performed with data from real-time PCR instruments. The gene expressions were normalized using β-actin expression (ΔCt) and expressed relative to the same value of control group ($\Delta\Delta$Ct). In brief, the gene expression was calculated by the following formula:

V.8.1 For each cDNA: ΔCt = Ct (target gene) - Ct (β-actin)

V8.2 Relative to control: $\Delta\Delta$Ct = ΔCt (tested cDNA) - ΔCt (control group)

V8.3 Target gene expression of tested cDNA related to control group: $2^{(-\Delta\Delta Ct)}$

VI Respiratory quotient (RQ) assay

Respiratory quotient (RQ) was determined prior administration of saline/albusin B at the end of experiment. Mice were placed in a metabolic chamber individually for the measurement of 24-hour oxygen (O_2) consumption and carbon dioxide (CO_2) production by the PhysioScan Metabolic System (AccuScan, Columbus, OH, USA). Water was free-accessed. Room air was pumped into chambers at a rate of 0.5 L/min. RQ was calculated as the ratio of VCO_2 to VO_2.

VII Oxygen radical absorbance capacity (ORAC) assay

Sample preparation: protein concentration of plasma samples were measured and diluted by 50-fold before ORAC assay.

After sample preparation, 3.78 mg/L β-phycoerythrin (BPE; P1286, Sigma, St.

Louis, MO, USA), 80 mM 2, 2'-Azobis (2-methylpropionamidine) dihydrochloride (AAPH; 440914, Sigma, St. Louis, MO, USA), and 20 μM Trolox (93510, Fluka, St. Louis, MO, USA) were freshly prepared. BPE and Trolox were avoided from light. Substances were added into individual wells as following: blank (100 μL of BPE + 30 μL of PB), PTC (100 μL of BPE + 30 μL of Trolox), and sample (100 μL of BPE + 30 μL of diluted samples). Then 30 μL of AAPH was added to each well and the fluorescence of plate was immediately measured by excitation at 515 nm, emission at 577 nm with fluorescent microplate reader (BioTeK, Synergy H1, Ashton Vale, BRS, USA). The plate was shake-incubated at 55°C for 5 minutes and the fluorescence was immediately measured at a 5-minutes interval. The assay was terminated when the fluorescence value was less than 10% of the initial value. All the samples were measured in triplication. The area under curve (AUC) for blank, PTC, and each sample were calculated and the antioxidant capacity (trolox μMole/g protein) was calculated by the following formula:

$$\frac{\left(\mathrm{AUC_{sample}} - \mathrm{AUC_{blank}}\right) \times (\text{the concentration of Trolox, } \mu\mathrm{M}) \times (\text{dilution of sample})}{\left(\mathrm{AUC_{PTC}} - \mathrm{AUC_{blank}}\right) \times \left(\text{the concentration of sample, } \frac{\mathrm{mg}}{\mathrm{mL}}\right)}$$

VIII Analysis of caecal microbial population

Anaerobic diluents were freshly prepared and stored at the anaerobic chamber before dilution of caecal samples. The formula of anaerobic diluents was listed in Table

5. Fresh caecal contents were weighed and mixed with 9-fold volume of sterile anaerobic diluents. After the diluted samples were homogenized, caecal microbial population were determined by serial dilution (10^{-2} - 10^{-6}) before inoculated onto plate of sterile agar. The plate media were Reinforced Clostridial agar (CM0151, Oxoid, Hampshire, England, UK) for *Clostridium*, *Eubacterium* Selective (ES) agar (Simpson et al., 2002) for *Eubacterium*, *Lactobacillus* selective media (7234A, Accumedia, Lansing, MI, USA) for *Lactobacillus*, Lombard-Dowell (LD) - esculin agar (Whaley et al., 1995) for *Bacteriodes*, *Bifidobacterium* iodoacetate medium 25 (Lin, 2007) for *Bifidobacterium*, Wilkins - Chalgren (WC) agar (7233A, Accumedia, Lansing, MI, USA) for total anaerobes. Total aerobes were determined by 3M Petrifilm™ (3M, St. Paul, MN, USA). The formulas of plate media and individual incubation time were listed in Table 6 to 11. 3M Petrifilm™ was incubated aerobically for 48 hours. The colony-forming units (CFU) of plate were measured at least 1 mm in diameter by colony counter (aCOLyte SuperCount, Symbiosis, Frederick, MD, USA).

IX Statistical analysis

All experimental data were analyzed by SAS® 9.2 (SAS Institute, Cary, NC, USA) with completely randomized design using GLM procedures. The Duncan's multiple range test was used to assess the differences of variance among groups. The Student's *t*-test was used to analyze the differences of variance between two groups. Results were

expressed as mean ± standard error (SE). *P* values < 0.05 were considered significant and *P* values < 0.1 were considered significant in gene expression results.

Table 4 Primer sequences of genes for real-time PCR

Gene	Access number	Primer sequences	Products (bp)	Annealed Temperature (℃)
ACC1 (Acetyl-CoA carboxylase 1)	NM_133360.2	Forward:5'-TAATGGGCTGCTTCTGTGACTC-3' Reverse: 3'-CTCAATATCGCCATCAGTCTT-5'	146	62
ACC2 (Acetyl-CoA carboxylase 2)	NM_133904.2	Forward:5'-ACCCTAGTGCCGGCTCCTTCC-3' Reverse:3'-TCCAGACATGCTGGGCCTCATAGT-5'	137	62
ACO (Acyl-CoA oxidase)	NM_015729.2	Forward: 5'-GACCCACAAGCCCTTGCCAGG-3' Reverse: 3'-CCATCAGGCTTCACCTGGGCGT-5'	150	62
ATGL (Adipose triglyceride lipase)	NM_001163689.1	Forward: 5'-TATCCGGTGGATGAAAGAGC-3' Reverse: 3'-CAGTTCCACCTGCTCAGACA-5'	112	62
β-actin	NM_007393.3	Forward: 5'-TGTTACCAACTGGGACGACA-3' Reverse: 3'-CTTTTCACGGTTGGCCTTAG-5'	130	62
CPT1α (Carnitine palmitoyltransferase 1α)	NM_013495.2	Forward: 5'-GGTCTCAAGTAATGGGTGC-3' Reverse: 3'-GAATACCAAACGGAGTTGC-5'	102	62
CPT1β (Carnitine palmitoyltransferase 1β)	NM_009948.2	Forward: 5'-GCTTTGGTCCCGTGGCGGAT-3' Reverse: 3'-TCCCAAAGCGCTGGGCGTTC-5'	123	62
DGAT (Diacylglycerol acyltransferase)	NM_010046.2	Forward: 5'-TGGGTGGCCAGGACAGGAGTAT-3' Reverse: 3'-CCAGTGGGACCTGAGCCATCATG-5'	121	62
FABP1 (Fatty acid binding protein 1)	NM_017399.4	Forward: 5'-TCGGTCTGCCGGAAGAGCTCA-3' Reverse: 3'-TGGACCCAGCGGTGATGGTGA-5'	105	62
FABP2 (Fatty acid binding protein 2)	NM_007980.2	Forward: 5'-GGAGCTCACTGGGGCCTGGA-3' Reverse: 3'-TCGCTTGGCCTCAACTCCTTCA-5'	151	62
FABP3 (Fatty acid binding protein 3)	NM_010174.1	Forward: 5'-GTGGGCTTTGCCACCAGGCA-3' Reverse: 3'-CGTCCAGCGTCACCAGTGACTTG-5'	190	62
FAS (Fatty acid synthase)	NM_007988.3	Forward: 5'-GGAGGTGGTGATAGCCGGTAT-3' Reverse: 3'-TGGGTAATCCATAGAGCCCAG-5'	140	62

Table 4 (continued) Primer sequences of genes for real-time PCR

Gene	Access number	Primer sequences	Products (bp)	Annealed Temperature, (℃)
GK (Glucose kinase)	NM_010292.4	Forward: 5'-TGTCGCAGGTGGAGAGCGACT-3' Reverse: 3'-TCACAGGCACGGCGCACAAT-5'	112	62
GLUT2 (Glucose transporter 2)	NM_031197.2	Forward: 5'-GCCTGTGTATGCAACCATTG-3' Reverse: 3'-GAAGATGGCAGTCATGCTCA-5'	205	62
GLUT4 (Glucose transporter 4)	NM_009204.2	Forward: 5'-CCACCAGACCCGCCCTTTGC-3' Reverse: 3'-GGGGTTCCCCATCGTCAGAGC-5'	174	62
GLUT5 (Glucose transporter 5)	NM_019741.3	Forward: 5'-CAGCGCAGGCGTGAAAAGCG-3' Reverse: 3'-TGGTGTTCTGCAGCGCCAGT-5'	191	62
HK2 (Hexokinase 2)	NM_013820.3	Forward: 5'-GGGCATGAAGGGCGTGTCCC-3' Reverse: 3'-CCAGGTCAAACTCCTCTCGCCG-5'	182	62
HSL (Hormone sensitive lipase)	NM_010719.5	Forward: 5'-ATGGAGCCGGCCGTGGAATC-3' Reverse: 3'-AACGCTGAGGCTTTGATCTTGCC-5'	119	62
PEPCK (Phosphoenolpyruvate carboxykinase)	NM_028994.2	Forward: 5'-TTGGAGAGAATGCTCGTGTG-3' Reverse: 3'-TGGAGAACAGCTGACTGGTG-5'	150	62
PKLR (Pyruvate kinase: liver/RBC)	NM_013631.2	Forward: 5'-ACATGCGATTGCCCGGGAGG-3' Reverse: 3'-GACCTCGGTTGGGTCACGGC-5'	196	62
PKm (Pyruvate kinase: muscle)	NM_011099.3	Forward: 5'-GCACCTGATTGCCCGAGAGGC-3' Reverse: 3'-GGCAGCTTCTGTGGGGTCGC-5'	103	62

Table 5 The formula of anaerobic diluent

Composition	Content (per 100 mL)
Gelatin (G2500, Sigma, St. Louis, MO, USA)	0.2 g
Distilled water	50 mL
Salt solution[1]	50 mL
25% resazurin solution (R2127, Sigma, St. Louis, MO, USA)	0.4 mL
Cysteine hydrochloride (C1276, Sigma, St. Louis, MO, USA)[2]	0.05 g

[1] Salt solution (per 250 mL distilled water): 10 g $MgSO_4 \cdot 7H_2O$, 0.5 g $FeSO_4 \cdot 7H_2O$, 0.4 g $MnSO_4 \cdot 2H_2O$, and 0.5g NaCl.

[2] Cysteine hydrochloride was added after the mixture was boiled. After mixing, the mixture was autoclaved at 121°C for 15 minutes.

Table 6 The formula of Reinforced Clostridial agar (commercial formula)[1, 2]

Composition	Content (g/L)
Yeast extract	3
Lab-Lemco Powder	10
Peptone	10
Glucose	5
Soluble starch	1
Sodium chloride	5
Sodium acetate	3
Cysteine hydrochloride	0.5
Agar	15

[1] After mixing and boiling to dissolve completely, the mixture was autoclaved at 121˚C for 15 minutes.

[2] Reinforced Clostridial agar was incubated anaerobically at 37˚C for 72 hours.

Table 7 The formula of *Eubacterium* Selective (ES) agar (Simpson et al., 2002) [4]

Composition	Content (g/L)
Base medium[1]	
Columbia agar base (CM331, Oxoid, Hampshire, England, UK)	39
Agar (152, Gibco, Paisley, UK)	10
Cysteine hydrochloride (C1276, Sigma, St. Louis, MO, USA)	5
Glucose (G5767, Sigma, St. Louis, MO, USA)[2]	5
Antibiotics[3]	
4% Streptomycin (S6501, Sigma, St. Louis, MO, USA)	5 mL
0.02% Polymyxin B (81334, Fluka, St. Louis, MO, USA)	5 mL

[1] Base medium was mixed and boiled to dissolve completely, then autoclaved at 121˚C for 15 minutes.

[2] Glucose was autoclaved at 121˚C for 15 minutes.

[3] 0.22 μm membrane-filtered antibiotics and sterile glucose were added to base medium at 45 - 50˚C.

[4] ES agar was incubated anaerobically at 37˚C for 72 hours.

Table 8 The formula of *Lactobacillus* selective agar (commercial formula)[3]

Composition	Content (g/L)
Commercial medium[1]	
Enzymatic digest of casein	10
Yeast extract	5
Monopotassium phosphate	6
Ammonium citrate	2
Dextrose	20
Sodium acetate hydrate	25
Magnesium sulfate	0.575
Manganese sulfate	0.12
Ferrous sulfate	0.034
Polysorbate 80	1
Agar	15
Glacial acetic acid[2]	1.32 mL

[1] Commercial medium was mixed and boiled to dissolve completely. Do not autoclave.

[2] Glacial acetic acid was added to the mixture at 45 - 50˚C.

[3] *Lactobacillus* selective agar was incubated anaerobically at 37˚C for 48 hours.

Table 9 The formula of Lombard-Dowell (LD) - esculin agar (Whaley et al., 1995) [1, 3]

Composition	Content (g/L)
Casein (211610, Difco, Le Pout de Claix, France)	5
Yeast extract (L21, Oxoid, Hampshire, England, UK)	5
L-tryptophan (T0254, Sigma, St. Louis, MO, USA)	0.2
Sodium chloride (7647-14-5, Bio Basic, Canada)	2.5
Esculin (A11624, Alfa Aesar, Ward Hill, MA, USA)	1
Ferric citrate (195181, Wako pure chemical industries, Osaka, Japan)	0.5
Agar (152, Gibco, Paisley, UK)	20
L-cycteine[2] (C1276, Sigma, St. Louis, MO, USA)	0.4
Hemin[2] (H2250, Sigma, St. Louis, MO, USA)	0.01
Vitamin K_1[2] (95271, Sigma, St. Louis, MO, USA)	0.001

[1] After mixing and boiling to dissolve completely, the mixture was autoclaved at 121°C for 15 minutes.

[2] L-cycteine and hemin were dissolved in 1N sodium hydroxide and vitamin K_1 was dissolved in absolute ethanol before adding them to the medium.

[3] LD - esculin agar was incubated anaerobically at 37°C for 72 hours.

Table 10 The *Bifidobacterium* iodoacetate medium 25 agar (Lin, 2007) [4]

Composition	Content (g/L)
Base medium[1]	
Reinforced Clostridial agar base (CM0151, Oxoid, Hampshire, England, UK)	52.5
Iodoacetic acid (I4386, Sigma, St. Louis, MO, USA)	0.0025
2, 3, 5 - triphenyltetrazolium chloride (A10870, Alfa Aesar, Ward Hill, MA, USA)	0.025
Agar (152, Gibco, Paisley, UK)	10
Antibiotics[3]	
Nalidixic acid (70162, Fluka, St. Louis, MO, USA)[2]	0.02
Polymyxin B (81334, Fluka, St. Louis, MO, USA)	0.0085
Kanamycin (K4000, Sigma, St. Louis, MO, USA)	0.05

[1] Base medium was mixed and boiled to dissolve completely, then autoclaved at 121˚C for 15 minutes.

[2] Nalidixic acid was dissolved in the 2N sodium hydroxide.

[3] 0.22 μm membrane-filtered antibiotics were added to the base medium at 45 - 50˚C.

[4] ES agar was incubated anaerobically at 37˚C for 72 hours.

Table 11 The formula of Wilkins - Chalgren (WC) agar [2]

Composition	Content (g/L)
WC broth (commercial formula)	33
Enzymatic Digest of Casein	10
Enzymatic Digest of Gelatin	10
Yeast Extract	5
Sodium Chloride	5
Dextrose	1
L-Arginine	1
Sodium Pyruvate	1
Hemin	0.005
Vitamin K	0.0005
Agar (152, Gibco, Paisley, UK)[1]	15

[1] WC broth and agar were mixed and boiled to dissolve completely, then autoclaved at 121˚C for 15 minutes.

[2] WC agar was incubated anaerobically at 37˚C for 72 hours.

CHAPTER 3 Results

I Western diet induced morbid obesity in mice

After 20 weeks of Western diet feeding, mice in W group displayed an average 62% greater BW than mice in C group (Fig. 1A). Consistent with the BW, mice fed with Western diet had heavier weight of heart, liver, epididymal fat, and perirenal fat than C group (Fig. 1B). The results of blood biochemical parameters exhibited that mice fed with Western diet had higher plasma levels of glucose, TG, and total cholesterol (1.4-, 1.2- , and 2.5-fold, respectively) when compared with control mice (Fig. 2). A significant increase of hepatic lipid accumulation in histology section of W group was observed (Fig. 3A-C). Moreover, compared with C group, mice fed with Western diet had larger adipocyte size in the epididymal fat (Fig. 3D-F). These results indicated that prolong feeding of Western diet caused obesity-associated dysregulation in mice, including the fatty liver, hypertrophy of adipocyte, hyperglycemia, and dyslipidemia.

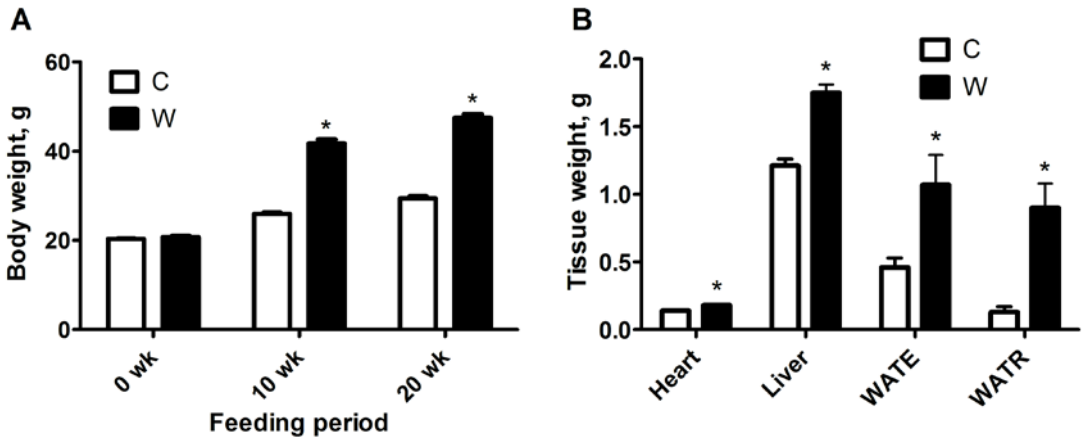

Fig. 1 Body weight (A) and organ weights (B) of mice in response to Western diet at the end of experiment. Values for each group are means ± SE (n=15). Asterisk indicates statistical significance between C and W group, $P<0.05$. C: chow diet. W: Western diet. WATE, epididymal white adipose tissue. WATR, perirenal white adipose tissue.

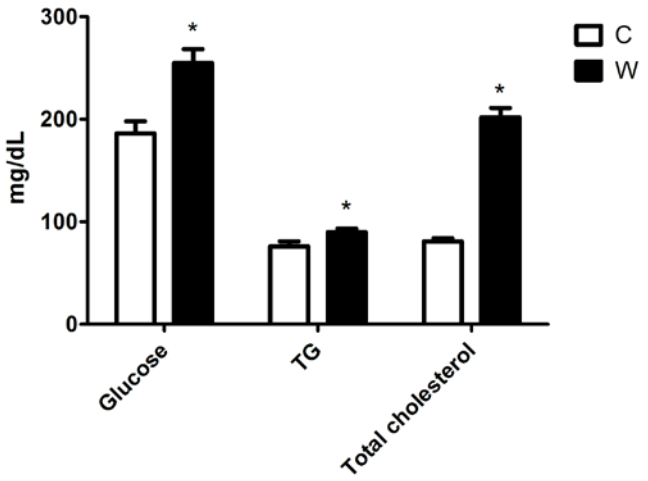

Fig. 2 Blood biochemical parameters of mice in response to Western diet after 20 weeks of Western diet feeding. Values for each group are means ± SE (n=15). Asterisk indicates statistical significance between C and W group, $P<0.05$. TG: triglycerides. C: chow diet. W: Western diet.

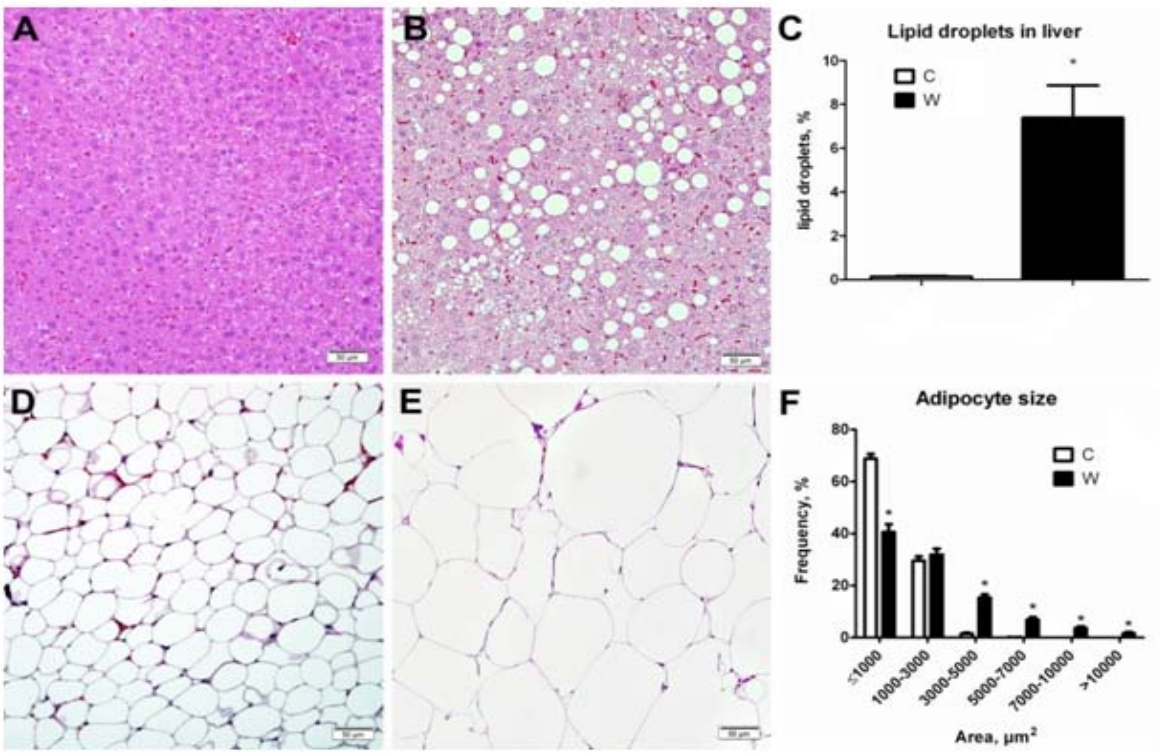

Fig. 3 Hepatic lipid accumulation (A and B) and morphology of adipocyte (D and E) of mice in response to Western diet. Histology sections (A, B, D, and E; 200 x magnification) were stained with hemetoxylin and eosin. (C) The contents of hepatic lipid droplets and (F) frequency of epididymal adipocytes distribution. Values for each group are means ± SE (n=6). Asterisk indicates statistical significance between C and W group, $P<0.05$. C: chow diet. W: Western diet.

II Effect of albusin B on physiological parameters of DIO mice

After oral administration of albusin B for 4 weeks, WLA mice had a significant decrease in BW when compared with WS mice (Fig. 4). WHA mice and WS mice exhibited a similar BW (Fig. 4A). There was no significant difference in the average energy intake among the three groups (Fig. 4B). WLA mice had lowest weight of perirenal fat among three groups, but WLA and WHA mice had similar heart, liver, and epididymal fat weight as WS group did (Fig. 5). There was no significant difference in plasma level of glucose, TG, and FFA among the three groups (Fig. 6A-C). Mice orally administrated with lower concentration of albusin B had a significant decrease in plasma levels of total cholesterol and LDL as compared to WS mice, but WHA mice had similar plasma level of cholesterol as WS mice did (Fig. 6D).

WLA mice had lowest hepatic lipid accumulation among three groups (Fig. 7A). Compared to WS mice, WHA mice had higher hepatic lipid accumulation. In the morphology study of WAT, mice administrated with albusin B had smaller size of adipocytes than WS mice (Fig. 7B). Compared with WS mice, WLA mice had higher percentages in adipocyte size smaller than 1000 μm^2 and lower percentages in larger adipocytes size (5000-10000 μm^2) (Fig. 7B). In brief, oral administration of 0.125 μg albusin B/g BW reduced BW, plasma LDL, and TG contents in liver and visceral fat of the DIO mice.

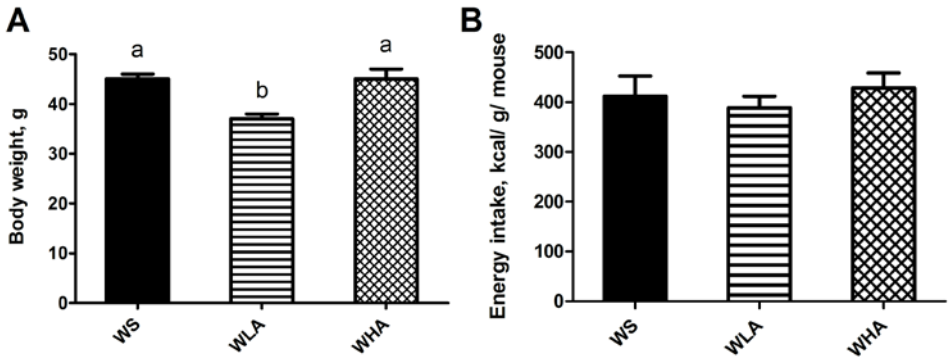

Fig. 4 Body weight (A) and average energy intake (B) of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments (P<0.05). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

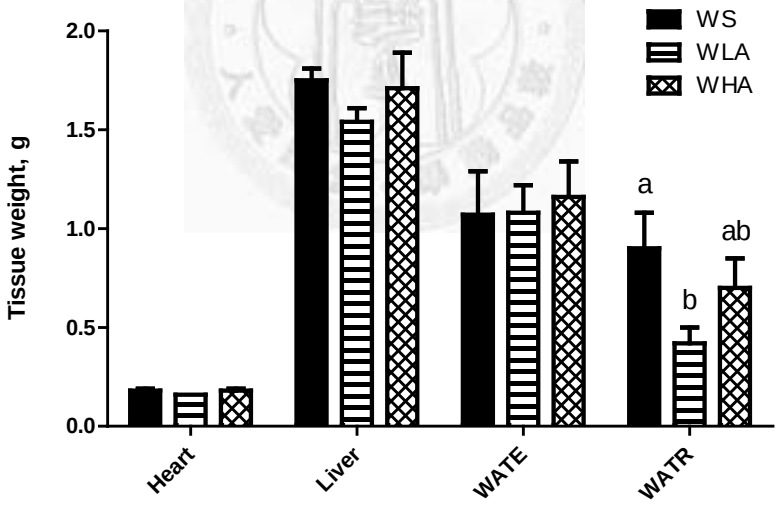

Fig. 5 Tissue weights of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments (P<0.05). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW. WATE, the epididymal white adipose tissue. WATR, the perirenal white adipose tissue.

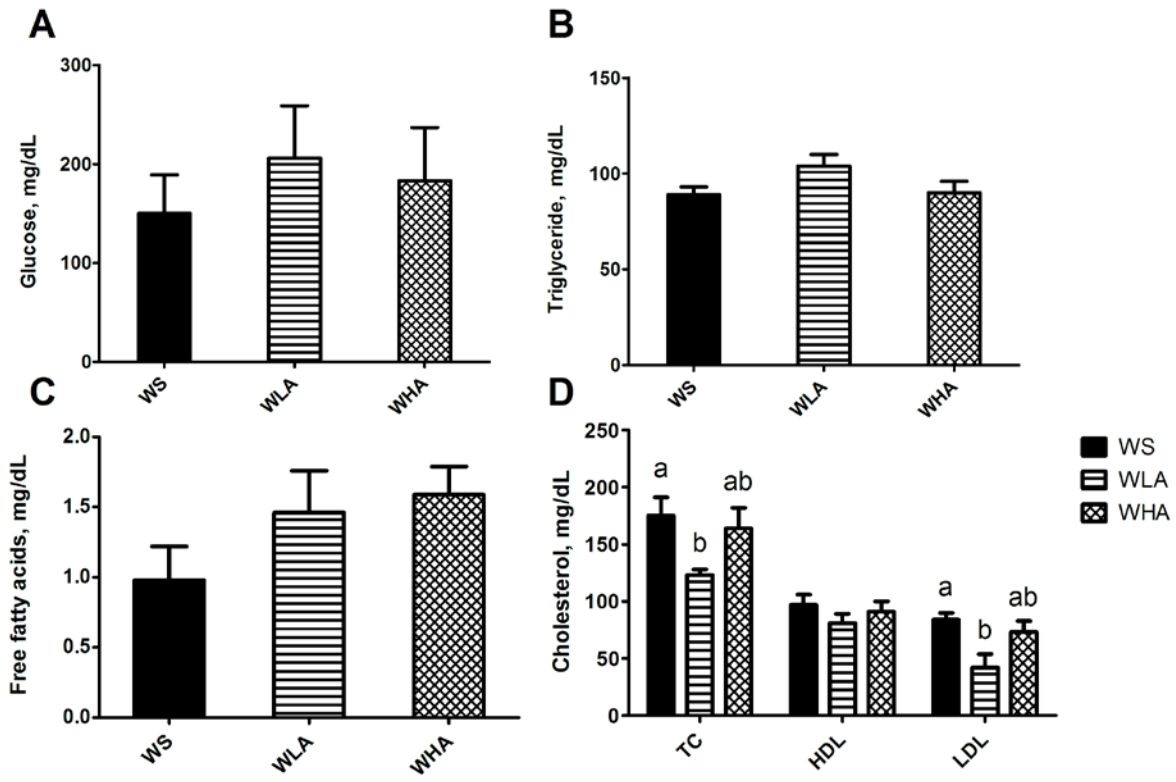

Fig. 6 Plasma parameters of DIO mice in response to oral administration of albusin B. (A) glucose (B) triglyceride (C) free fatty acids (D) cholesterol, including total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL). Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments ($P<0.05$). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

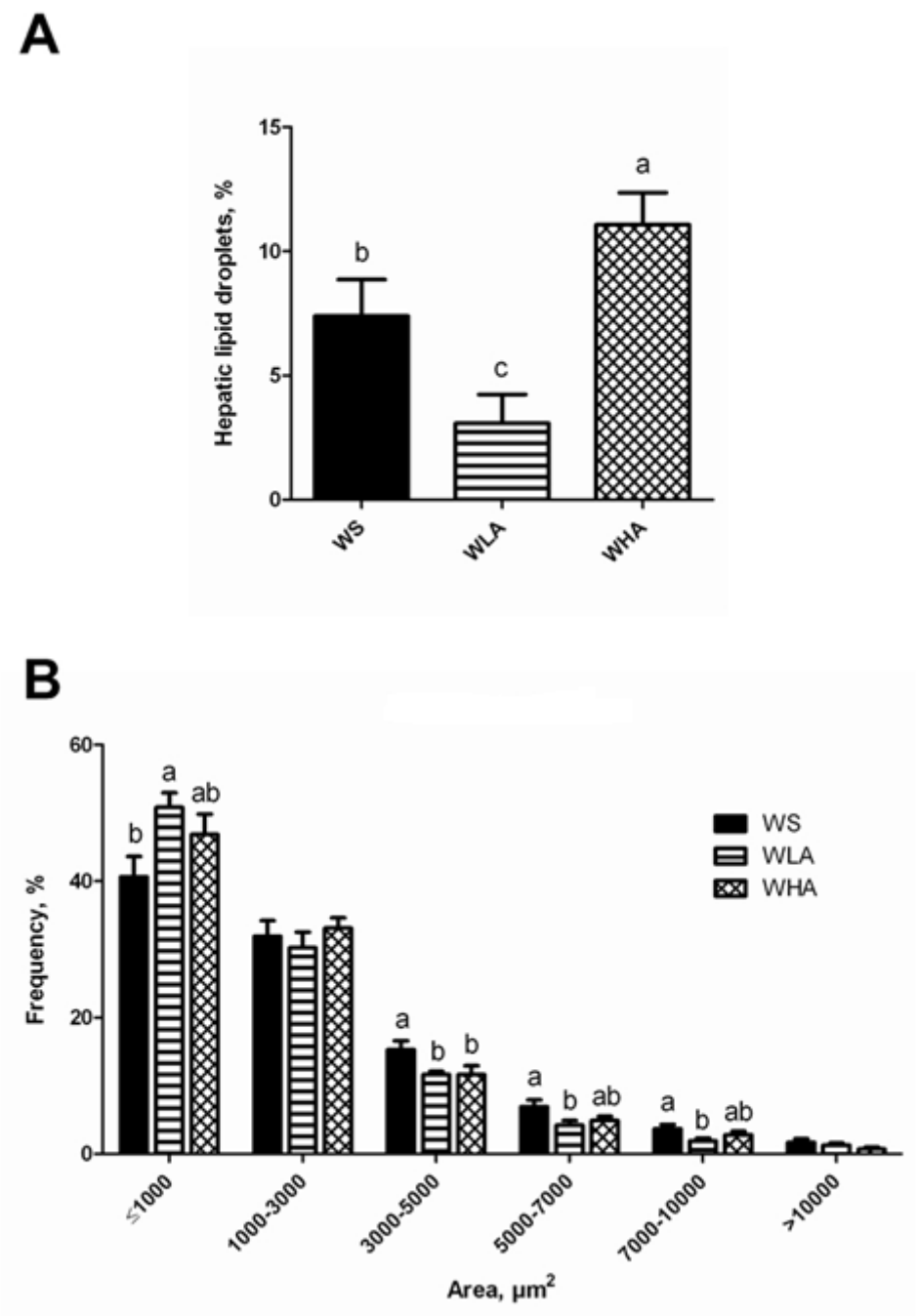

Fig. 7 Contents of hepatic lipid droplets (A) and frequency of epididymal adipocytes distribution (B) of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments (P<0.05). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

III Effect of albusin B on lipid metabolism of DIO mice

Since plasma lipid profile and tissue lipid accumulation were modulated by albusin B, to explore the mechanism underlying action of albusin B, genes associated with lipid metabolism in liver, WAT, and muscle were determined. In contrast to WS group, the low concentration of albusin B decreased the ileal fatty acid binding protein 2 (FABP2) expression (Fig. 8A).

Administration of albusin B also decreased hepatic gene expression of FABP1 (Fig. 8B). Compared with WS group, WLA mice had higher transcript abundance of fatty acid synthase (FAS) and HMG-CoA reductase (HMGCR) (Fig. 9A). The low concentration of albusin B significantly increased hepatic mRNA level of acyl-CoA oxidase (ACO) (Fig. 9B). No significant difference in hepatic gene expression of lipid synthesis and oxidation were observed between WS and WHA mice (Fig. 9A-B).

Since a decrease in larger adipocyte size in WAT was found in albusin B feeding groups, gene expression associated with TG metabolism were further determined. Albusin B treatments suppresed mRNA level of diglyceride acyltransferase 1 (DGAT1) in epididymal adipose tissue as compared to WS mice (Fig. 10A). WLA mice had lower hormone sensitive lipase (HSL) but higher adipose triglyceride lipase (ATGL) expression in epididymal adipose tissue than WS mice (Fig. 10A). WHA and WS mice had siminar lipolytic mRNA expression in epididymal adipose tissue (Fig. 10A).

Compared with WS group, both WLA and WHA group had a lower FABP3 transcript level in the skeletal muscle (Fig. 10B). WLA mice had a lowest mRNA expression of acetyl-CoA carboxylase 2 (ACC2) in skeletal muscle among three groups (Fig. 10B). No significant difference in FA synthesis and oxidation related gene expressions were found between WHA and WS group.

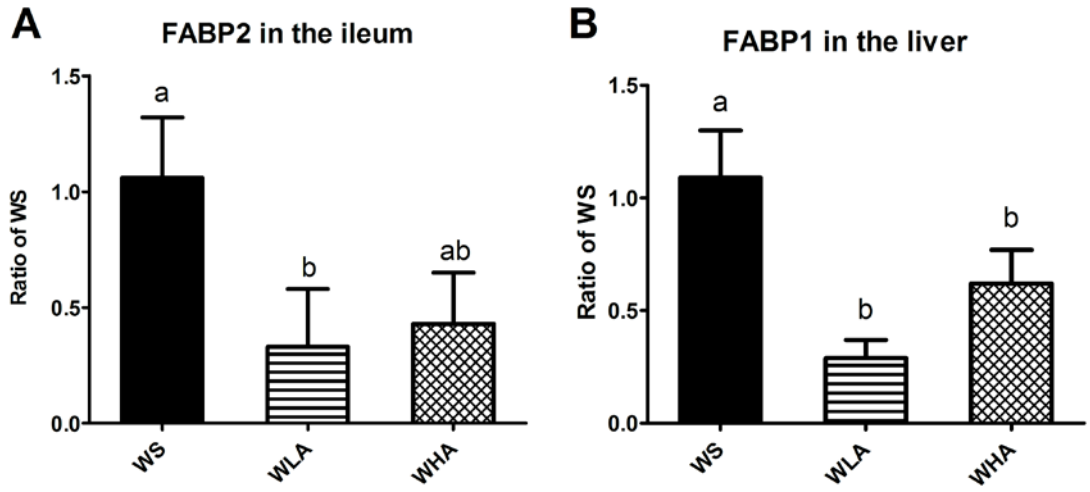

Fig. 8 Ileal (A) and hepatic (B) fatty acid transporter expression of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments (P<0.1). FABP2: fatty acid binding protein 2. FABP1: fatty acid binding protein 1. WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

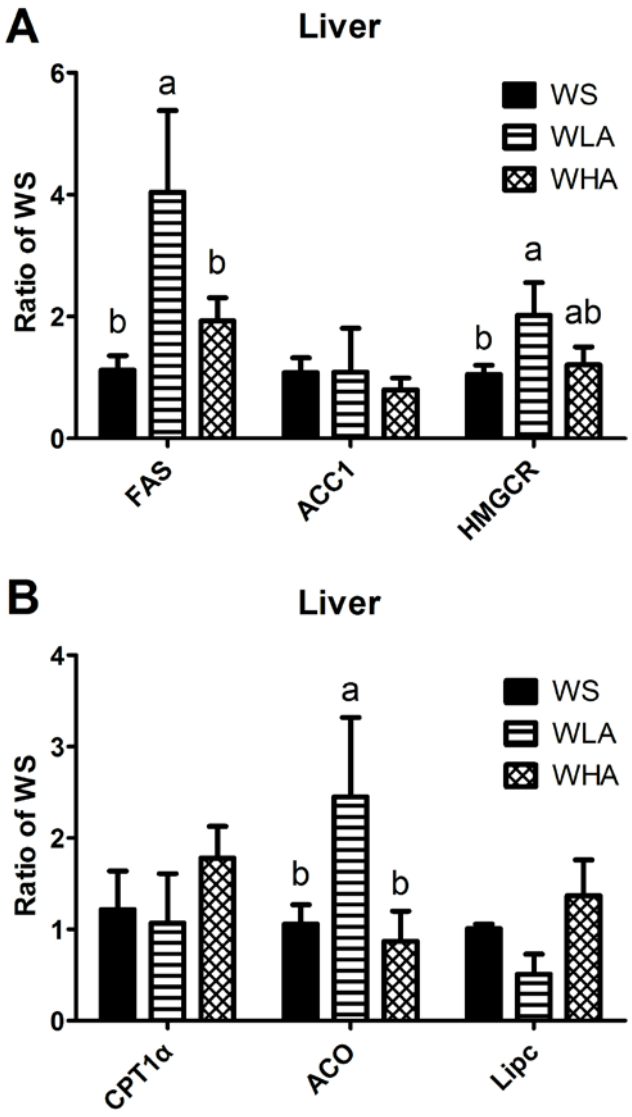

Fig. 9 Hepatic lipid synthesis (A) and oxidation (B) related gene expression of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=6). Different letters represent significant difference among treatments (P<0.1). FAS: fatty acid synthase. ACC1: acetyl-CoA carboxylase 1. HMGCR: HMG-CoA reductase. CPT1α: carnitine palmitoyltransferase I-α. ACO: acyl-CoA oxidase. Lipc: hepatic lipase. WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

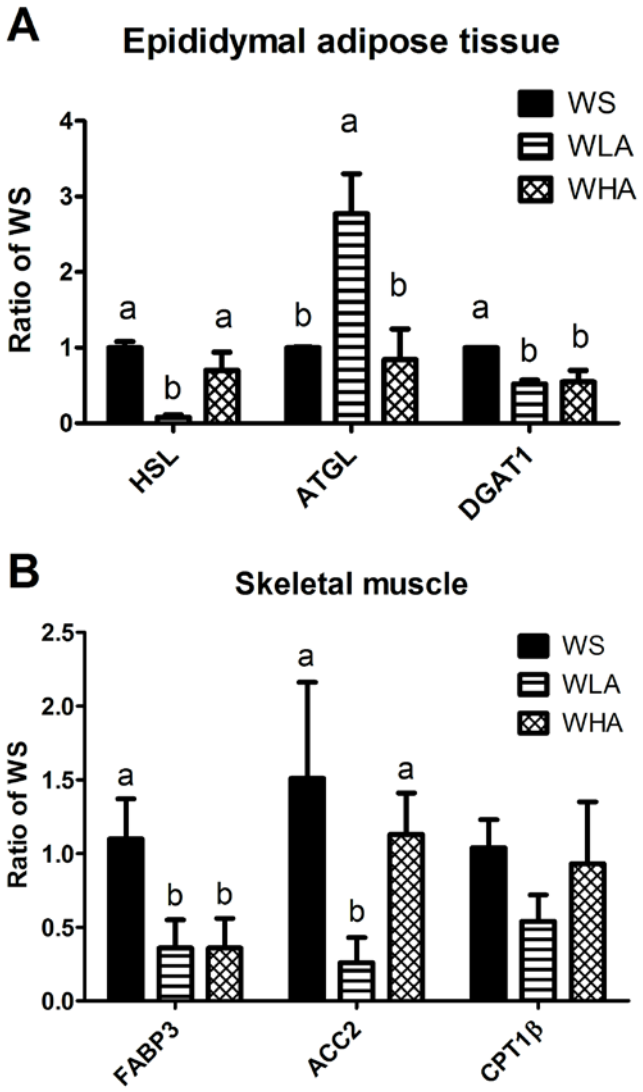

Fig. 10 Lipid metabolism related gene expression in the epididymal adipose tissue (A) and skeletal muscle (B) of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=5). Different letters represent significant difference among treatments (P<0.1). HSL: hormone sensitive lipase. ATGL: adipose triglyceride lipase. DGAT1: diglyceride acyltransferase 1. FABP3: fatty acid binding protein 3. ACC2: acetyl-CoA carboxylase 2. CPT1β: carnitine palmitoyltransferase I-β. WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

IV Effect of albusin B on carbohydrate metabolism of DIO mice

Carbohydrate metabolism of obese mice administrated with albusin B was further studied. Compared with WS mice, WLA mice exhibited no difference in ileal glucose transporter 2 (GLUT2) and GLUT5 expression, while WHA mice had higher ileal GLUT5 expression (Fig. 11A).

Administration of albusin B decreased hepatic GLUT5 expression as compared to WS group (Fig. 11B). WLA had higher mRNA expression of glucose kinase (GK), but exhibited no change of mRNA expression related to hepatic gluconeogenesis (Fig. 11C). No significant differences hepatic glycolysis and gluconeogenesis gene expressions were found between WHA and WS group (Fig. 11C).

Gene expression of glucose uptake and glycolysis in skeletal muscle was also determined. Results showed that WLA group exhibited no significant difference in GLUT4 expression but had higher gene expression of hexokinase (HK) in skeletal muscle than WS group (Fig. 12). WHA mice had the lowest mRNA level of GLUT4 in skeletal muscle among three groups (Fig. 12). These results suggested that oral administration of 0.125 μg albusin B/g BW decreased hepatic fructose uptake, but increased glucose utilization of the liver in DIO mice.

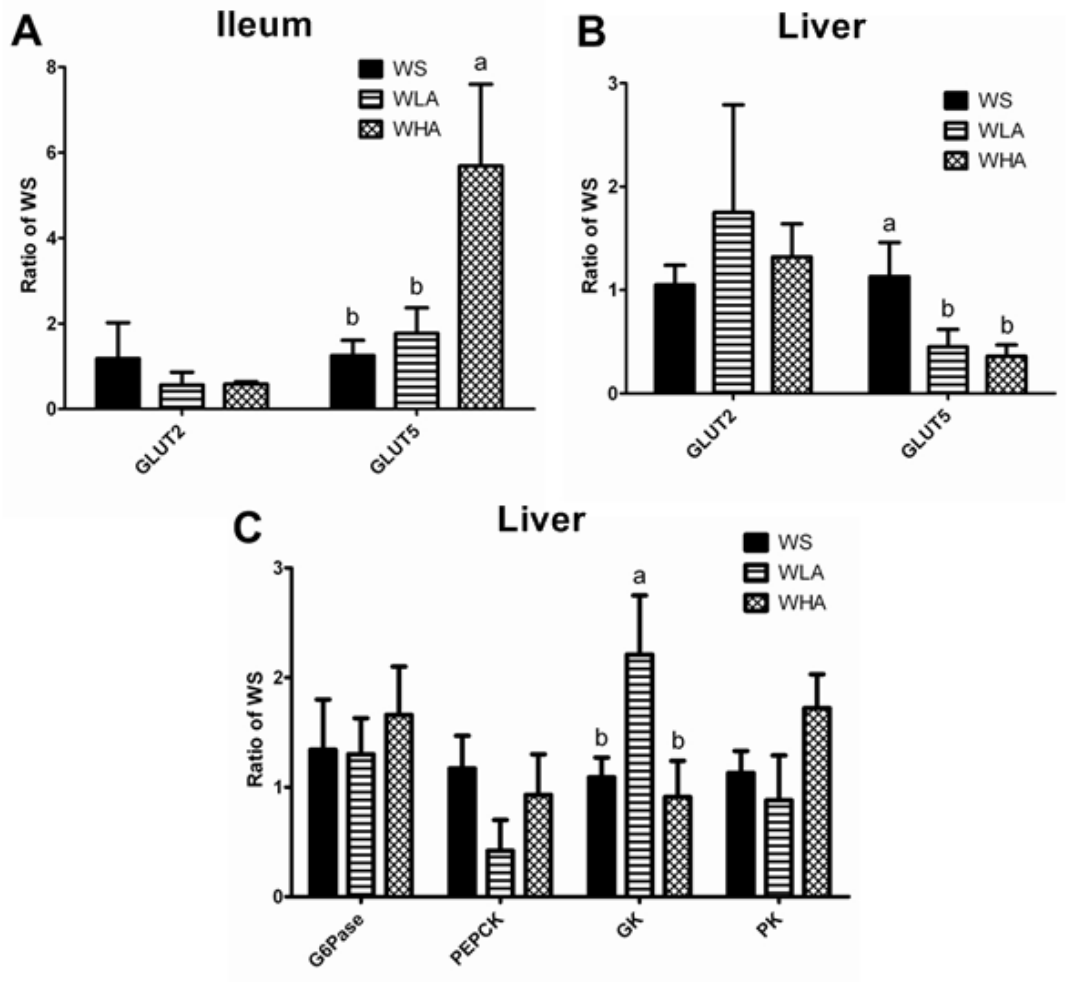

Fig. 11 Ileal (A) and hepatic (B and C) carbohydrate metabolism related gene expression of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=5). Different letters represent significant difference among treatments (P<0.1). GLUT2: glucose transporter 2. GLUT5: glucose transporter 5. G6Pase: glucose-6-phosphatase. PEPCK: phosphoenolpyruvate carboxylase. GK: glucose kinase. PK: pyruvate kinase. WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

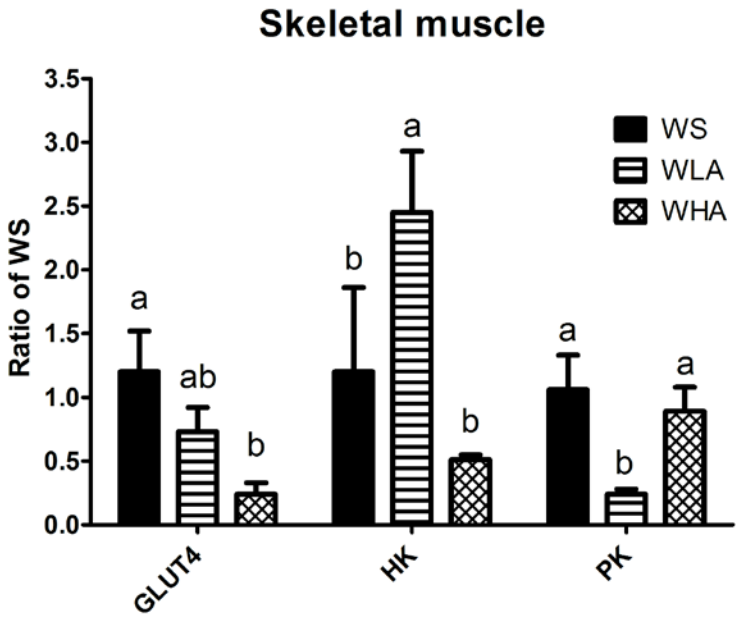

Fig. 12 Glucose metabolism related gene expression in the skeletal muscle of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=5). Different letters represent significant difference among treatments (P<0.1). GLUT4: glucose transporter 4. HK: hexokinase. PK: pyruvate kinase. WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

V Albusin B increased the carbohydrate utilization of DIO mice

To investigate the effect of albusin B on systemic energy utilization in obese mice, the respiratory quotient (RQ) of mice prior to administration of saline/albusin B and at the end of experiment were determined. After administration of albusin B for 4weeks, the RQ value of 24-h period was significantly shifted from 0.73 to 0.8, indicating that both WLA and WHA mice used more carbohydrates as energy source compared with WS mice (Fig. 13A-B).

The heat production of mice at the end of experiment was analyzed for investigating the energy expenditure of obese mice by albusin B. Results showed that mice administrated with albusin B had similar heat production as WS mice did (Fig. 14).

VI Effect of albusin B on systemic antioxidant capacity of DIO mice

Total antioxidant capacity of obese mice was measured by oxygen radical absorbance capacity assay (ORAC). Results indicated that obese mice administrated with low concentration of albusin B for 4 weeks had approximately more 30% increase in systemic antioxidant capacity than WS mice (Fig. 15). WHA mice had siminar antioxidant defense in plasma as WS mice did (Fig. 15).

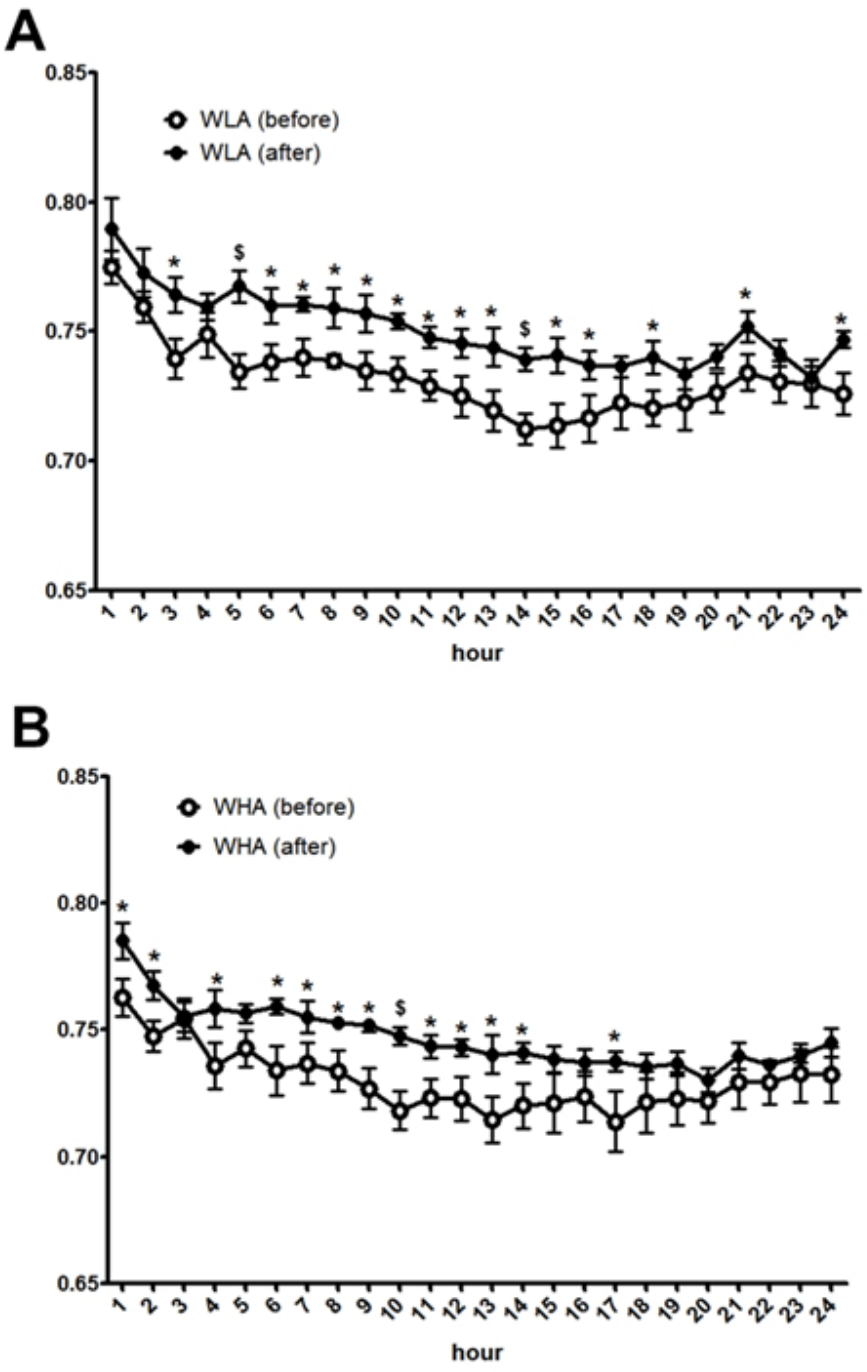

Fig. 13 24-hour respiratory quotient (RQ) of DIO mice prior to administration of albusin B and at the end of experiment. (A) RQ of WLA mice. (B) RQ of WHA mice. Values for each group are means ± SE (n=6). * and $ indicate statistical significance difference (P<0.1 and P<0.001, respectively). WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

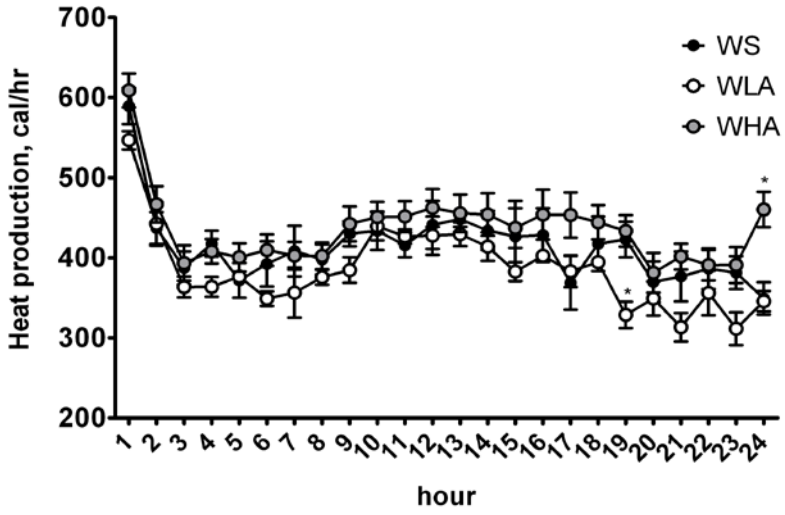

Fig. 14 Heat production of mice of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=3). Asterisk indicates statistical significance compared with WS group at the same timing (P<0.05). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

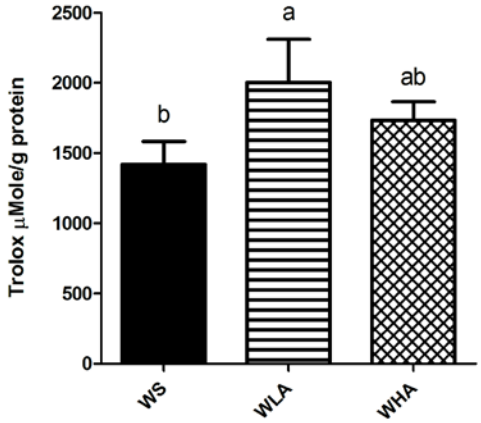

Fig. 15 Total antioxidant capacity in the plasma of DIO mice in response to oral administration of albusin B. Values for each group are means ± SE (n=5). Different letters represent significant difference among treatments (P<0.1). WS: Western diet + saline. WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

VII Effect of albusin B on gut microflora of DIO mice

The results of caecal bacterial populations in mice were shown in Table 12. The major phyla of the gut microflora, such as *Firmicutes*, *Bacteriodetes*, and *Actinobacteria* were determined. The caecal counts of *Clostridium* and *Bifidobacterium* were higher in WLA group than WS group. Mice administrated with albusin B had no significant difference in count of *Eubacterium*, *Lactobacillus*, and *Bacteroides* as compared to WS mice. The ratio of *Firmicutes* to total anaerobes and *Bacteriodetes* to *Firmicutes* were further analyzed. Compared with WS group, albusin B treatments did not change the ratio of *Firmicutes* to total anaerobes, while WLA mice had higher ratio of *Bacteroidetes* to *Firmicutes* in cecum of obese mice. These results demonstrated that oral administration of 0.125 μg albusin B/g BW increased the caecal counts of *Clostridium* and *Bifidobacterium*, and the ratio of *Bacteroidetes* to *Firmicutes* in obese mice.

Table 12 Caecal microbial populations (log CFU[1]/g cecum content) of DIO mice in response to oral administration of albusin B[2]

Type	**WS**	**WLA**	**WHA**
Total aerobes	7.70 ± 0.78	8.46 ± 0.99	9.74 ± 0.54
Total anaerobes	7.63 ± 1.19	9.12 ± 0.25	8.03 ± 0.87
Firmicutes			
Clostridium	5.13 ± 1.24 ^{b}	8.47 ± 0.10 ^{a}	7.55 ± 0.77 ^{ab}
Eubacterium	7.57 ± 1.09	8.34 ± 0.32	6.68 ± 1.01
Lactobacillus	7.96 ± 1.40	9.06 ± 0.25	8.29 ± 0.91
Bacteroidetes			
Bacteroides	3.51 ± 1.75	5.44 ± 1.23	4.24 ± 1.24
Actinobacteria			
Bifidobacterium	5.30 ± 1.31 ^{b}	8.41 ± 0.26 ^{a}	7.54 ± 0.79 ^{ab}
***Firmicutes*/total anaerobes**	1.07 ± 0.02	1.03 ± 0.02	1.06 ± 0.02
Bacteroidetes/Firmicutes	0.06 ± 0.06 ^{b}	0.24 ± 0.04 ^{a}	0.20 ± 0.05 ^{ab}

[1] CFU: colony-forming unit.

[2] Values for each group are means ± SE (n=6). Different letters represent significant difference in the same row (P<0.05). WLA: Western diet + 0.125 μg albusin B/g BW. WHA: Western diet + 0.625 μg albusin B/g BW.

CHAPTER 4 Discussion

To investigate the effect of albusin B on energy metabolism of obesity, we established a murine DIO model based on a 24-weeks feeding of Western diet with which mice displayed a significant increase of BW, hyperglycemia, dyslipidemia, fatty liver, and hypertrophy of adipocyte. While administration of 0.125 μg albusin B/g BW for 4 weeks decreased BW, plasma TC levels, hepatic lipid accumulation, and the number of larger adipocyte, but increased lipid oxidation, glucose utilization, antioxidant capacity, and caecal counts of *Bifidobacterium* in DIO mice, indicating that albusin B improved the health of DIO mice.

I Body weight-lowering effect

Several possible mechanisms of BW-lowering effect have been proposed to elucidate probiotic and prebiotic actions, including (1) inhibiting lipid absorption (Kadooka et al., 2010); (2) increasing the thermogenic responses (Tanida et al., 2008); (3) reducing energy intake via suppressing ghrelin (Parnell and Reimer, 2009), glucagon-like-peptide 1 (Delmée et al., 2006), and enhancing peptide YY (Parnell and Reimer, 2009); (4) decreasing lipogenesis (Delzenne and Kok, 1999; Daubioul et al., 2000); (5) increasing the lipolytic response (Tanida et al., 2008). Hsieh (2011) suggested that albusin B caused body weight loss in healthy mice by promoting lipid

oxidation and ATP production, and suppressing lipid synthesis (Hsieh, 2011). In our study, we found that the same dosage of albusin B enhanced lipid oxidation, and suppressed FA absorption and lipogenesis therefore reduced BW in DIO mice rather than reducing energy intake or increasing the systemic heat production.

It has been documented that gut microflora is associated with anti-obesity effect. The relative abundance of *Bacteroidetes* was increased in DIO mice which had lower BW (Murphy et al., 2012). Besides, DIO mice administrated with *Bifidobacterium* in the gut exhibited significant reduction of BW, fat mass, and hepatic lipid deposition (Cani et al., 2007; Kondo et al., 2010; Yin, 2010). In fact, short chain FA (SCFA) produced by gut bacteria, such as acetate and propionate, had antagonistic effects on lipid metabolism to modulate BW. The former was a lipogenic substrate and the latter was an effective inhibitor of lipid synthesis (Demigné et al., 1995). Our results showed that WLA decreased BW and increased the caecal ratio of *Bacteroidetes* to *Firmicutes* and the caecal counts of *Bifidobacterium* in DIO mice. However, further determination of SCFA in the gut or feces to clarify the effect of SCFA on reduction of BW in DIO mice are needed. Taken together, these results provide evidences that albusin B induced weight loss of DIO mice might operate suppressing lipid synthesis and FA uptake, enhancing lipid oxidation, and modulating the gut microflora.

II Cholesterol-lowering effect

In the present study, we found that low concentrations of albusin B decreased plasma level of TC and LDL in DIO mice, consistent with other *in vivo* studies. Pigs fed with live probiotics for 4 weeks exhibited lower blood levels of TC and LDL than control pigs, and there was no change in blood HDL concentration (de Smet et al., 1998). Human with hyperlipidemia consuming inulin-containing foods resulted in decreases of serum TC and LDL without changing serum HDL and TG as well (Davidson et al., 1998).

The mechanisms for plasma cholesterol reduction could be: (1) decreasing cholesterol absorption from the gut; (2) enhancing plasma cholesterol clearance; (3) increasing bile acids production and greater cholesterol excretion from body in the form of bile acids (Parnell and Reimer, 2009). Besides, several possible mechanisms of LDL-lowering effect have been proposed, including a decreased synthesis of apoLDL, a modification of lipid profile in LDL, and an increased excretion of bile acids (Shepherd et al., 1980). In fact, it has been suggested that deconjugation of bile acids was the possible mechanism for the reduction of serum cholesterol (Pereira and Gibson, 2002). Deconjugated bile acids are not well absorbed by gut mucosa and thus most of deconjugated bile acids are excreted through the feces, leading to removal of cholesterol. The excretion of bile acids increased the demand of cholesterol for the *de novo* bile acid

synthesis to replace the loss in feces (Begley et al., 2006). In our study, DIO mice fed with 0.125 μg albusin B/g BW displayed a higher hepatic expression of HMG-CoA reductase, while neither plasma TC nor plasma HDL were increased, and plasma LDL was decreased, suggesting that albusin B reduced bile acids uptake from the gut, and thus decreased hepatic exposure to bile salts, thereby stimulating HMG-CoA reductase expression to synthesize cholesterol (Parnell and Reimer, 2009).

It has been indicated that probiotics had cholesterol-lowering effect through enhancing bacterial bile salt hydrolase (BSH) activity (de Smet et al., 1998). In fact, some bacteria, such as *Bacteroides* spp., bifidobacteria, clostridia, and lactobacilli, have been suggested to suppressed cholesterol absorption from the gut by deconjugating bile salts which in turn affect the cholesterol metabolism or directly assimilated cholesterol (Hylemon et al., 1983; Begley et al., 2006). Present study showed a low concentration of albusin B increased the caecal *Clostridium* and *Bifidobacterium* counts of DIO mice and these bacteria had been demonstrated with BSH activity to assimilation of cholesterol (Klaver and Meer, 1993; Begley et al., 2006). Therefore, we postulated that albusin B increased cholesterol excretion as the form of bile acids, which might be modulated by BSH activity of caecal *Clostridium* and *Bifidobacterium*, resulting in an increase of hepatic cholesterol synthesis and decreases of plasma cholesterol and LDL levels.

III Albusin B promotes lipid oxidation and suppresses lipid synthesis

Long-term surplus FA accumulation caused hypertrophy and hyperplasia of adipocytes, resulting in mitochondrial oxidative stress and adipocytes dysfunction eventually (de Ferranti and Mozaffarian, 2008; Garbarino and Sturley, 2009). Consumption of prebiotics or probiotics inhibits hypertrophy and hyperplasia of adipocytes, which are characterized by a decrease in the number of larger adipocytes and an increase in the number of smaller adipocytes in visceral adipose (Sato et al., 2008; Kadooka et al., 2011). The mechanisms might be attributed to inhibition of intestinal lipid absorption (Mizote et al., 2009) and lipoprotein lipase activity via increasing intestinal expression of angiopoietin-like 4 (Aronsson et al., 2010). In this study, 0.125 μg albusin B/g BW significantly decreased the adipocyte size of visceral fat in DIO mice, and the might be due to suppressing FA uptake from gut and lipogenesis in WAT, or enhancing lipolysis ofWAT.

It had been indicated that administration of probiotics or prebiotics improved the ectopic lipid disorder in DIO animals through increasing the hepatic expression of peroxisome proliferators-activated receptor (Esposito et al., 2009) or suppressing the elevation plasma levels of lipopolysaccharide-binding protein (Naito et al., 2011) and hepatic FAS activity (Agheli et al., 1998). The present study showed a decrease in FA absorption and fructose absorption, but an increase in FA oxidation might account for

the reduction of hepatic TG accumulation. Besides, WLA suppressed FA utilization and FA synthesis in the skeletal muscle may be able to decrease the ectopic lipid accumulation. These results suggested that WLA caused reduction of hepatic lipid accumulation might be through modulating FA absorption and FA metabolism in the liver. These changes in lipid accumulation by albusin B might account in part for the weight loss in DIO mice. In contrast to low concentration of albusin B, high concentration of albusin B did not modulated BW and lipid metabolism of DIO mice.

IV Albusin B enhances carbohydrate utilization

We found DIO mice used more carbohydrate as main energy source after oral administration of albusin B for 4 weeks by RQ analysis, together with increases of glucose utilization in liver and skeletal muscle, and decreases of FA uptake in liver, muscle, and ileum. These changes by albusin B indicated the ability of albusin B in enhancing the glucose utilization of DIO mice. Consumption of surplus FA for long-term caused the saturation of storage capacity in adipocyte, excess FA further promotes *de novo* lipogenesis (Unger, 2002), lipid peroxidation and massive generation of ROS (Nascimento et al., 2010). Utilization of carbohydrate (glucose especially) as energy source caused a decreased production of ROS than FA did and reduced circulating glucose, therefore decreased insulin resistance (Unger, 2002).

V Albusin B increases systemic antioxidant defense

Free radicals diffused intracellularly and resulted in the damage of mitochondrial DNA, proteins, and lipids, further impaired cellular functions (Bonnefont-Rousselot et al., 2000). Hsieh (2011) and Wang et al. (2012) have been proposed that supplementation of albusin B increased antioxidant defense in broilers and healthy mice. Consistent with their studies, DIO mice orally administrated with lower concentration of albusin B had higher systemic antioxidant capacity as compared WS mice.

Taken together, oral administration of 0.125 μg albusin B/g BW promotes the lipid metabolism, the utilization of carbohydrate, and antioxidant capacity and elevates the caecal population of *Bifidobacterium* in DIO mice. Therefore, albusin B has beneficial effects on energy metabolism and gut microflora in DIO mice.

CHAPTER 5 Reference

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