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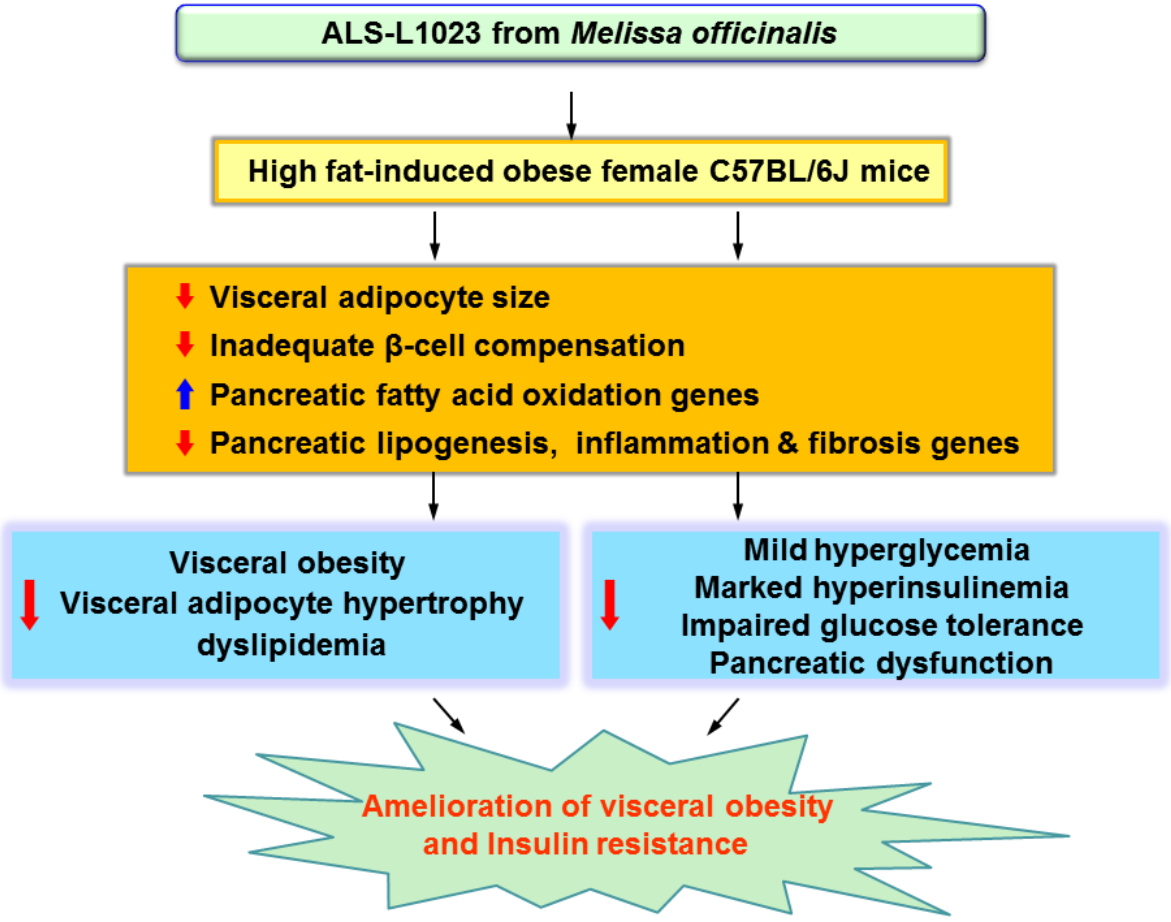
ALS-L1023 from *Melissa officinalis*
High fat-induced obese female C57BL/6J mice
Visceral adipocyte size
Inadequate β-cell compensation
Pancreatic fatty acid oxidation genes
Pancreatic lipogenesis, inflammation & fibrosis genes
Visceral obesity
Visceral adipocyte hypertrophy
dyslipidemia
Mild hyperglycemia
Marked hyperinsulinemia
Impaired glucose tolerance
Pancreatic dysfunction
Amelioration of visceral obesity
and Insulin resistance

The herbal extract ALS-L1023 from *Melissa officinalis* alleviates visceral obesity and insulin resistance in obese female C57BL/6J mice

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Running Head: Regulation of female obesity and insulin resistance by *Melissa officinalis*

Abstract

*Ethnopharmacological relevance***:** *Melissa officinalis* L. (Labiatae; lemon balm) has traditionally been used as a medicinal herb to treat stress, anxiety, and insomnia. Current reports suggest that not only chronic stress stimulates angiogenesis, but angiogenesis also regulates adipogenesis and obesity. Because the herbal extract ALS-L1023 from *Melissa officinalis* inhibits angiogenesis, we hypothesized that ALS-L1023 could suppress visceral obesity and insulin resistance in obese female C57BL/6J mice, a mouse model of obese premenopausal women.

Materials and methods: The mice were grouped and fed for 16 weeks as follows: 1) low-fat diet (LFD), 2) high-fat diet (HFD), or 3) HFD supplemented with 0.4 or 0.8% ALS-L1023. Variables and determinants of visceral obesity, insulin resistance, and pancreatic dysfunction were then assessed via blood analysis, histology, immunohistochemistry, and real-time polymerase chain reaction.

Results: ALS-L1023 decreased weight gain, visceral adipocyte size, and serum lipid levels in HFD-fed obese mice. ALS-L1023 also normalized hyperglycemia and hyperinsulinemia and concomitantly reduced blood glucose levels during oral glucose tolerance tests. The pancreatic islet size and insulin-positive β-cell area were significantly reduced in ALS-L1023-treated mice compared with untreated obese controls, reaching a level similar to that of LFD-fed lean mice. ALS-L1023 suppressed pancreatic lipid accumulation, infiltration of inflammatory cells, and collagen levels. ALS-L1023 treatment altered the pancreatic expression of genes involved in steatosis, inflammation, and fibrosis.

Conclusions: Our findings indicate that the herbal extract ALS-L1023 from *Melissa officinalis* not only inhibits visceral obesity, but also attenuates the increased fasting blood glucose, impaired glucose tolerance, and pancreatic dysfunction seen in female obese mice. These results suggest that ALS-L1023 may be effective in the prevention of visceral obesity and insulin resistance in obese premenopausal women.

Keywords: female obesity, glucose tolerance, insulin resistance, *Melissa officinalis*, lemon balm, pancreatic dysfunction

Abbreviations: α-SMA, α-smooth muscle actin; AMPK, AMP-activated protein kinase; C/EBPα, CCAAT/enhancer-binding-protein α; CPT-1, carnitine palmitoyltransferase I; FAS, fatty acid synthase; HFD, high-fat diet; H&E, hematoxylin and eosin; HOMA-IR, homeostasis model assessment-estimated insulin resistance; LFD, low-fat diet; MCAD, medium-chain acyl-CoA dehydrogenase; MCP-1, monocyte chemoattractant protein 1; PCR, polymerase chain reaction; QUICKI, quantitative insulin sensitivity check index; SD, standard deviation; SCD1, stearoyl-CoA desaturase 1; SREBP-1c, sterol regulatory element-binding protein 1c; T2D, type 2 diabetes; TGFβ1, transforming growth factor β1; TNFα, tumor necrosis factor α

1. Introduction

Insulin resistance is a premonitory condition that is considered the strongest marker of subsequent type 2 diabetes (T2D) (Sung et al., 2012). Normoglycemia is maintained under insulin-resistant conditions by a process known as β-cell compensation, in which pancreatic islets secrete more insulin (Ahrén and Pacini, 2002; Kahn, 2003; Prentki and Nolan, 2006). Over time, however, this compensatory response fails, leading to a progressive decline in β-cell function, diminishing tolerance to glucose, and ultimately T2D. Insulin resistance is also associated with pancreatic β-cell expansion in a mouse model of obesity (Ahrén et al., 2010), suggesting that β cells and pancreatic islets are key in the development of insulin resistance and T2D.

C57BL/6J mice fed a high-fat diet (HFD) develop obesity, insulin resistance, and dyslipidemia (Reuter, 2007; Jeong and Yoon, 2009; Fraulob et al., 2010). An adaptive increase in insulin secretion during insulin resistance has been reported in the model of HFD-fed female C57BL/6J mice (Winzell and Ahrén, 2004; Ahrén et al., 2010). Marked glucose intolerance and early T2D are also observed in these female mice. In addition, insulin resistance accompanying HFD feeding in mice is followed by progressive β-cell expansion, including both increased β-cell volume and total number of β cells.

Males and females differ in their regulation of body weight and fat (Shi and Clegg, 2009). Men and postmenopausal women accumulate more visceral fat than do premenopausal women, and therefore have a greater risk of metabolic complications related to obesity (Geer and Shen, 2009).

Although the incidence of metabolic risks is relatively low in premenopausal women compared with men and postmenopausal women, abdominal obesity in premenopausal women also increases the risk of metabolic disorders such as insulin resistance, hyperglycemia, and dyslipidemia (Ross et al., 2002).

Melissa officinalis L. (Labiatae), commonly known as lemon balm, has been used as a medicinal plant for more than 2000 years ((Vogl et al., 2013; Shakeri et al., 2016). The leaves of *Melissa officinalis* are known to exhibit many pharmacological effects on stress, anxiety, insomnia, learning and memory (Blumenthal et al., 2000; Kennedy et al., 2002; Scholey et al., 2014; Shakeri et al., 2016). Current results suggest that not only chronic stress increases angiogenesis (Thaker et al., 2006), but angiogenesis also promotes adipogenesis and obesity (Rupnick et al., 2002; Cao, 2007). We prepared ALS-L1023 by a two-step organic solvent fractionation from *Melissa* leaves and tested the extract in cell culture for inhibition of angiogenesis (kim et al., 2006). Our previous studies demonstrate that ALS-L1023 decreases blood vessel density in adipose tissues and inhibits obesity in obese mice (Park et al., 2015; Woo et al., 2016; Kim et al., 2017a, 2017b). Based on the suggestion in traditional medicine that *Melissa officinalis* ameliorates stress and anxiety as well as the documented role of *Melissa officinalis* in the regulation of angiogenesis and obesity, we therefore evaluated the effects of ALS-L1023 on visceral obesity and insulin resistance in HFD-fed obese female C57BL/6J mice, a mouse model of obese premenopausal women. Our results indicate that the herbal extract ALS-L1023 from *Melissa officinalis* regulates visceral obesity and islet β-cell mass and also attenuates impaired glucose metabolism in this mouse model.

2. Materials and methods

2.1. Preparation of ALS-L1023

ALS-L1023 was manufactured by activity-guided fractionation from *Melissa officinalis* L. leaves that were purchased from Alfred Galke GmbH, (Harz, Germany). Briefly, the dried *Melissa* leaves were extracted with aqueous ethanol and the extract was filtered and concentrated. The concentrated ethanol extract was further fractionated with ethyl acetate, concentrated and dried to obtain ALS-L1023 in a dried powder form. ALS-L1023 was standardized with two reference compounds of rosmarinic acid and caffeic acid by high-performance liquid chromatography (Woo et al., 2016).

2.2. Animal studies

Eight-week-old female wild-type C57BL/6J mice (n = 8/group) were housed and bred at the Mokwon University under pathogen-free conditions with a standard 12-h light/dark cycle. Prior to the administration of special diets, mice were fed standard rodent chow and water *ad libitum*. Mice were randomly assigned to one of four treatment groups. Each group of mice was fed one of four diets for 16 weeks: a low fat diet [LFD; 10% kcal fat (5.6% kcal unsaturated and 4.4% kcal saturated fat), 70% kcal carbohydrate, 20% kcal protein, Research Diets, New Brunswick, NJ, USA], an HFD [HFD-Con, 45% kcal fat (5.6% kcal unsaturated and 39.4% kcal saturated fat), 35% kcal carbohydrate, 20% kcal protein, Research Diets], or an HFD supplemented with 0.4% or 0.8% (w/w) ALS-L1023 [HFD-ALS (0.4%) or HFD-ALS (0.8%), respectively]. Four grams or 8 g ALS-L1023 powder was

mixed with 1 kg of the HFD. The body weight of each animal was measured three times a week by a person blinded to the treatments. Food intake was determined by measuring the amounts of food consumed by the mice throughout the treatment period. Blood was collected from the retro-orbital sinus into tubes, and the plasma was separated and stored at -80°C until analysis. Tissues were also harvested and were weighed, snap-frozen in liquid nitrogen, and stored at -80°C. Additional tissue sections were prepared for histological studies. All animal experiments were approved by the Institutional Animal Care and Use Committees of Mokwon University (permit number: NVRGS AEC-9), and followed National Research Council Guidelines.

2.3. Blood analysis

Plasma levels of triglycerides and free fatty acids were measured using an automatic blood chemical analyzer (CIBA Corning, Oberlin, OH). Levels of blood glucose and HbA1c were measured using the Accu-Chek Performa System (Roche, Basel, Switzerland) and NycoCard Reader II (Alere/Axis-Shield, Oslo, Norway), respectively. Oral glucose (2 g/kg body weight) tolerance tests were performed to determine blood glucose levels at selected time intervals. Quantitative insulin sensitivity check index (QUICKI) values were calculated as: 1/(log [fasting insulin μU/ml] + log [fasting glucose mg/dl]). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated via an online Oxford HOMA calculator (available at: www.dtu.ox.ac.uk) using the formula: (fasting insulin μU/ml × fasting glucose mg/dl)/405.

2.4. Histological analyses

Tissue specimens were fixed in 10% phosphate-buffered formalin for 1 day and processed in a routine manner to obtain paraffin sections. To quantify the sizes of visceral adipocytes, the hematoxylin and eosin (H&E) -stained sections (5 μm thick) were analyzed using an Image-Pro Plus analysis system (Media Cybernetics, Bethesda, MD, USA). To analyze pancreatic steatosis, inflammation and fibrosis, pancreas sections (5 μm) were cut and stained with H&E, toluidine blue and Masson's trichrome, respectively. Insulin-secreting β-cells were detected using a monoclonal mouse anti-insulin antibody (I2018; Sigma-Aldrich, St Louis, MO, USA). Sections of pancreas tissue (3 μm thick) were irradiated in a microwave oven for epitope retrieval. The sections were then incubated with the primary insulin antibody (1:1,400 dilution) and an anti-mouse IgG biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) using diaminobenzidine (Vector Laboratories) as the chromogen. Immunostained β-cell areas were analyzed using ImageJ software and relative insulin-positive areas were expressed as percentages of the total surveyed pancreatic area occupied by β cells.

2.5. Quantitative real-time polymerase chain reaction (PCR)

Total cellular RNA from pancreas tissues was prepared using Trizol reagent (Gibco-BRL, Grand Island, NY, USA). Total cellular RNA (2 μg) was reverse transcribed to generate an antisense cDNA template. The genes of interest were amplified from the synthesized cDNA using *Accu*Power® GreenStar™ qPCR PreMix (Bioneer, Deajeon, Korea) on an Excycler™ 96 Real Time Quantitative Thermal Block machine (Bioneer). The PCR primers used for gene expression analysis are shown in

Supplementary Table 1. The relative expression levels were calculated as the ratio of target gene cDNA to β-actin cDNA.

2.6. Statistical analysis

Values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using analysis of variance followed by Turkey's post-hoc tests. Statistical significance was defined as a *p*-value <0.05.

3. Results

3.1. ALS-L1023 regulates visceral obesity and adipocyte hypertrophy in obese female mice

To determine whether ALS-L1023 regulates visceral obesity in HFD-fed obese female C57BL/6J mice, we measured body weight, visceral adipose tissue mass, and adipocyte size. Mice fed the HFD supplemented with 0.4% and 0.8% ALS-L1023 had lower body weights and body weight gains compared with HFD-Con mice fed the HFD alone after 16 weeks of treatment (Fig. 1A and B). Treatment with 0.8% ALS-L1023 also significantly decreased total and visceral fat mass in the HFD-fed mice (Fig. 1C and D). The average size of the visceral adipocytes was also reduced by both 0.4% and 0.8% ALS-L1023 (Fig. 1E and F). However, there was not a significant difference in food intake between the HFD-Con and HFD-ALS mice (data not shown).

3.2. ALS-L1023 alleviates impaired glucose metabolism in obese female mice

Consistent with the observed weight loss, 0.8% ALS-L1023 treatment decreased plasma triglycerides and free fatty acids in obese HFD-Con mice compared to HFD-Con mice (Fig. 2A and B). Hypoglycemic effects of 0.8% ALS-L1023 were demonstrated by a 31% reduction in fasting glucose levels (Fig. 2C) and a 67% reduction in circulating insulin levels compared to obese controls (Fig. 2D).

Insulin sensitivity, indicated by the well-known QUICKI assessment, was increased in the 0.8% ALS-L1023-treated mice compared with the obese HFD-Con mice (Fig. 3A). Treatment with 0.8%

ALS-L1023 resulted in lower HOMA-IR scores in the obese mice (Fig. 3B). Similarly, ALS-L1023 treatment resulted in significantly reduced blood glucose levels in a glucose tolerance test in the obese mice (Fig. 3C and D).

3.3. ALS-L1023 normalizes islet β-cell area in obese female mice

Examination of H&E-stained pancreas sections revealed pancreatic islet hypertrophy in the obese HFD-Con mice (Fig. 4A). However, ALS-L1023 treatment resulted in decreased islet size compared to the HFD-Con mice; this smaller size was similar to the size found in the LFD mice. HFD intake also caused an increase in β-cell mass, as indicated by the insulin-positive β-cell area (Fig. 4B and C). Consistent with the decreased insulin secretion, the β-cell area in the 0.8% ALS-L1023-treated mice was reduced to a level similar to that of mice fed the LFD.

3.4. ALS-L1023 regulates pancreatic steatosis, inflammation, and fibrosis in obese female mice

Pancreatic steatosis was increased in HFD-Con mice compared with LFD mice, as indicated by an increase in lipid droplets (Fig. 5A). However, ALS-L0123 treatment decreased the triglyceride contents in the HFD-fed mice. The infiltration of mast cells into the pancreas, measured by staining sections with toluidine blue, was increased in the HFD-fed mice (Fig. 5B). However, ALS-L1023 decreased the numbers of mast cells. Masson's trichrome staining of pancreas sections showed that collagen levels were increased in HFD-fed mice but decreased by ALS-L1023 treatment (Fig. 5C).

3.5. ALS-L1023 modulates pancreatic expression of genes involved in lipid metabolism, inflammation,

and fibrosis in obese female mice

To evaluate whether the inhibitory effects of ALS-L1023 on pancreatic steatosis in obese mice were associated with alterations of the expression of genes involved in fatty acid β-oxidation, we measured the mRNA levels of carnitine palmitoyltransferase 1 (CPT-1), medium-chain acyl-coenzyme A dehydrogenase (MCAD), AMP-activated protein kinase α1 (AMPK α1), and AMPK α2. The expression of these genes was higher in the ALS-L1023-treated mice than in the untreated HFD-Con mice (Figure 6A). We also analyzed the effects of ALS-L0123 on the mRNA levels of genes involved in lipogenesis, such as fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), CCAAT/enhancer-binding-protein α, and sterol regulatory element-binding protein 1c (SREBP-1c). The FAS, SCD1, and SREBP-1c mRNA levels were reduced in the treated mice compared with those in the untreated obese mice (Fig. 6B). Pancreatic inflammation is increased by the expression of inflammatory genes in hepatocytes including tumor necrosis factor α, CD68, and monocyte chemoattractant protein 1 (MCP-1). ALS-L0123 decreased the mRNA levels of CD68 and MCP-1 in the pancreas of HFD-fed mice (Figure 6C). Pancreatic fibrosis is associated with the increased expression of fibrogenic and fibrotic molecules, such as transforming growth factor β1 (TGFβ1), α-smooth muscle actin (α-SMA), and collagen α1. ALS-L0123 reduced the mRNA levels of TGFβ1, α-SMA, and collagen α1 compared with those in HFD-fed untreated mice (Fig. 6D).

4. Discussion

Our results demonstrate that administration of the lemon balm extract ALS-L1023 to HFD-fed obese female C57BL/6J mice not only decreased weight gain and visceral adipocyte size, but concomitantly alleviated insulin resistance and pancreatic dysfunction. Body weight and visceral fat mass were significantly decreased by ALS-L1023 treatment, although these levels did not reach those observed in LFD-fed mice. Histological examination of visceral adipose tissue revealed that 0.8% ALS-L1023 decreased the average size of adipocytes by 37% compared with untreated counterparts, suggesting that ALS-L1023 prevented adipocyte hypertrophy. Because visceral obesity due to adipocyte hypertrophy is closely associated with insulin resistance (Okuno et al., 1998, Jeong and Yoon, 2009), ALS-L1023 might attenuate visceral obesity-induced insulin resistance in female mice. Our data are supported by other results which indicate that visceral adipose tissue is strongly associated with insulin resistance in abdominally obese premenopausal women (Ross et al., 2002), although the prevalence of obesity is higher in postmenopausal than in premenopausal women.

Consistent with weight loss, ALS-L1023 administration suppressed severe hypertriglyceridemia and elevated circulating free fatty acids in HFD-fed female C57BL/6J mice. The release of free fatty acids and triglycerides into the circulation may be decreased in part due to increased fatty acid β-oxidation and decreased lipogenesis in pancreatic tissue. ALS-L1023 treatment upregulated and downregulated pancreatic mRNA expression of fatty acid-metabolizing enzymes and lipogenic genes, respectively, in ALS-L1023-treated mice compared with untreated obese mice. Consistent with

changes in these expression levels, circulating free fatty acids and triglycerides were decreased following ALS-L1023 treatment in obese mice. Given the known role of circulating free fatty acids in the inhibition of glucose uptake and utilization by muscle, our results suggest that ALS-L1023-induced reduction in circulating free fatty acids contributes to the decrease in skeletal muscle insulin resistance observed in obese animals (Boden et al., 1994; Roden et al., 1996).

HFD consumption by C57BL/6J mice is a potent model of impaired glucose tolerance and early T2D (Winzell and Ahrén, 2004). These animals have insulin resistance, as evidenced by intravenous glucose tolerance tests, and insufficient compensatory islet responsiveness (Ahrén and Pacini, 2002). Expectedly, obese HFD-Con mice exhibited elevated fasting glucose levels and plasma insulin levels. However, the elevated fasting blood glucose concentrations were significantly lower in the ALS-L1023-treated than in untreated obese mice. The markedly increased levels of serum insulin were also significantly decreased by ALS-L1023 treatment, which attests to the glucose-lowering effects of ALS-L1023 in obese, insulin-resistant mice. These results suggest that ALS-L1023 inhibits hyperglycemia and hyperinsulinemia during 16 weeks of HFD intake in this mouse model. Administration of ALS-L1023 also reduced circulating glucose levels during the oral glucose tolerance test, showing that it ameliorated the impaired glucose tolerance of the HFD-fed mice. QUICKI and HOMA-IR are used for assessment of insulin sensitivity and resistance, respectively. The HOMA-IR index quantifies insulin resistance based on the amount of fasting plasma glucose and insulin, whereas QUICK is a useful and accurate index of insulin sensitivity (Quon, 2002; Antuna-Puente et al., 2011). In this study, ALS-L1023 raised QUICKI and lowered HOMA-IR values. These

results suggest that ALS-L1023 supplementation enhances insulin sensitivity and inhibits insulin resistance in HFD-fed obese female mice.

Insulin resistance is closely associated with expansion of β-cell mass in obese rodents (Bock et al., 2003; Butler et al., 2003). Expanded β-cell mass is an important adaptation to insulin resistance that may be critical for maintenance of normal or near normal glycemia, but inappropriate adaptation can lead to glucose intolerance. Pancreatic islet hypertrophy and expansion of β-cell mass have previously been reported to occur in HFD-fed C57BL/6J mice (Ahrén et al., 2010). Consistent with the decreased circulating insulin levels induced by ALS-L1023, treatment of HFD-fed C57BL/6J mice with ALS-L1023 decreased the mean size of islets and the insulin-positive β-cell area to levels similar to those in LFD-fed mice. This result suggests that ALS-L1023 treatment in obese female mice normalizes the β-cell mass that is increased by insulin resistance, restoring the mass to the level of LFD mice. It is likely, therefore, that insulin-affected tissues (e.g., liver, skeletal muscle, and adipose tissue) become insulin sensitive in the ALS-L1023-treated female mice.

During obesity, ectopic triglycerides and fatty acids accumulate in non-adipose tissues (e.g., liver, muscle, heart, and pancreatic gland), and this ectopic fat contributes to the development of insulin resistance, T2D, and cardiovascular disease (van Herpen and Schrauwen-Hinderling, 2008; Pezzilli and Calculli, 2014; Zhou et al., 2016). Our histological analyses revealed that ALS-L1023 decreased lipid accumulation in the pancreas. Consistent with the histological data, ALS-L1023 increased and decreased the pancreatic expression of genes involved in fatty acid β-oxidation and lipogenesis, respectively. ALS-L1023 treatment reduced mast-cell infiltration and collagen accumulation, and

concomitantly decreased mRNA levels of genes related to inflammation and fibrosis in the pancreas of obese mice. These results suggest that ALS-L1023 inhibits obesity-induced pancreatic steatosis, inflammation, and fibrosis, potentially by regulating the expression of genes responsible for lipid metabolism, inflammation, and fibrosis. It has been shown that excess fat infiltration into pancreatic islets can trigger apoptosis of islet β-cells (Unger, 2003; Unger et al., 2010). In addition, long-term HFD feeding induces fat accumulation, inflammatory cell infiltration, and fibrosis in the pancreas, leading to islet damage, β-cell loss, and dysfunction (Shimabukuro et al., 1998; Kharroubi et al., 2004; Zhang et al., 2008). In contrast to these results, our results show that HFD feeding induced the expansion of β-cell mass rather than the loss of β-cell mass in female mice. This difference may be due to a greater demand for β-cell compensation to insulin resistance than increased β-cell apoptosis caused by steatosis.

Phytochemical studies on *Melissa officinalis* have revealed that *Melissa officinalis* possesses various phytochemicals including terpenes and phenolic compounds (Shakeri et al., 2016). Some pharmacological actions of *Melissa officinalis* have been attributed to several phenolic compounds, such as gallic acid, chlorogenic acid, syringic acid, caffeic acid, ferulic acid, and rosmarinic acid. These phenolic compounds produce anti-inflammatory and antioxidant effects. The biologically active components of *Melissa officinalis*, caffeic acid and rosmarinic acid have been shown to prevent diet-induced hyperlipidemia, obesity, and insulin resistance (Liao et al., 2013; Alam et al, 2016; Seyedan et al., 2016), suggesting that the inhibitory actions of ALS-L1023 on visceral

obesity and insulin resistance may be due to its active constituents, such as caffeic acid and rosmarinic acid.

With regard to the average daily doses of each compound, the crude extract of *Melissa officinalis* contains phenolic acids in relatively high concentrations. However, the quantities of caffeic acid and rosmarinic acid are significantly variable under the different manufacturing conditions. For example, the levels of caffeic acid and rosmarinic acid are 0.047-0.705 and 0.158-48.608 mg/g of dry weight, respectively (Arceusz and Wesolowski, 2013). Actually, we did not quantitate the concentrations of caffeic acid and rosmarinic acid in the leaf extract of *Melissa officinalis* and the daily doses of these compounds were not measured in this study. Quantitation of pharmacologically active compounds of *Melissa officinalis* should be determined under the same manufacturing conditions.

5. Conclusion

In conclusion, these results demonstrate that the herbal extract ALS-L1023 from *Melissa officinalis* not only inhibits obesity and dyslipidemia, but also alleviates hyperglycemia, glucose intolerance, and islet hypertrophy in obese, insulin-resistant C57BL/6J female mice. Overall, these data suggest that lemon balm may be a promising target for the prevention of obesity and insulin resistance in female obesity. Further studies are necessary to determine the mechanism by which ALS-L1023 regulates insulin resistance, to identify and characterize active components for their effects on obesity and insulin resistance and to develop potential therapeutic uses of ALS-L1023 for premenopausal women with obesity and hyperglycemia.

Conflict of interest

None declared.

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Author contributions

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Figure legends

Fig 1. Regulation of body weight, adipose tissue mass, and visceral adipocyte size by ALS-L1023. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. (A) Body weights, (B) body weight gain, (C) total adipose tissue mass, and (D) visceral adipose tissue mass. (E) Histological analysis of visceral adipose tissue and (F) visceral adipocyte size. All values are expressed as the mean ± SD. $^{\#}p<0.05$ compared to LFD. *p<0.05 compared to HFD-Con.

Fig 2. Circulating levels of lipids, glucose and insulin following ALS-L1023 treatment. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. Plasma levels of (A) triglycerides and (B) free fatty acids. (C) Fasting blood glucose levels. (D) Plasma insulin levels. All values are expressed as the mean ± SD. $^{\#}p<0.05$ compared to LFD. *p<0.05 compared to HFD-Con. $^{@}p<0.05$ compared to HFD-ALS (0.4%).

Fig 3. QUICKI, HOMA-IR, and OGTT after ALS-L1023 treatment. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. (A) QUICK and (B) HOMA-IR. (C) Oral glucose tolerance test (OGTT) and (D) OGTT area under the curve (AUC). All values are expressed as the mean ± SD. $^{\#}p<0.05$ compared to LFD. *p<0.05 compared to HFD-Con. $^{@}p<0.05$ compared to HFD-ALS (0.4%).

Fig 4. Pancreatic morphology and insulin-secreting β-cell area after ALS-L1023 treatment. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. (A) H&E-stained pancreatic sections (original magnification, ×100). (B) Pancreas sections stained with an anti-insulin antibody (original magnification, ×100). (C) Relative insulin-positive β-cell area. All values are expressed as the mean ± SD. $^{\#}p<0.05$ compared to LFD. *$p<0.05$ compared to HFD-Con. $^{@}p<0.05$ compared to HFD-ALS (0.4%).

Fig 5. Inhibition of pancreatic steatosis, inflammation, and fibrosis by ALS-L1023. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. (A) H&E-stained pancreas sections (original magnification ×100). Arrows indicate lipid droplets. (B) Toluidine blue-stained sections of pancreas tissues (original magnification × 400). (E) Masson's trichrome-stained sections of pancreas tissues (original magnification × 100).

Fig 6. Effects of ALS-L1023 on the mRNA expression of genes involved in lipid metabolism, inflammation, and fibrosis in the pancreas. Female C57BL/6J mice (n = 8/group) were fed an LFD, an HFD or an HFD supplemented with 0.4 or 0.8% ALS-L1023 for 16 weeks. Expression of (A) fatty acid oxidation and (B) lipogenic genes. Expression of (C) inflammatory and (D) fibrogenic genes. All values are expressed as the mean ± SD. $^{\#}p<0.05$ compared to LFD. *$p<0.05$ compared to HFD-Con. $^{@}p<0.05$ compared to HFD-ALS (0.4%).

Fig. 1.

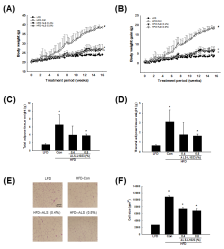
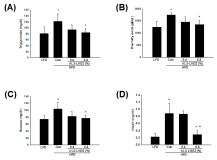


Fig. 2.



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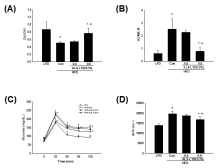

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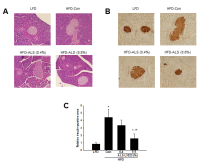

Fig. 5.

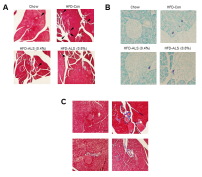

Fig. 6.