Effects of Root Extract of *Morinda officinalis* in Mice with High-Fat-Diet/Streptozotocin-Induced Diabetes and C2C12 Myoblast Differentiation

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**ABSTRACT:** Type 2 diabetes is the most common type of diabetes and causes a decline in muscle quality. In this study, we investigated the effects of the root extract of *Morinda officinalis* (MORE) on skeletal muscle damage in mice with high-fat-diet (HFD)/streptozotocin (STZ)-induced diabetes and the expression of myogenic and biogenesis regulatory proteins in C2C12 myoblast differentiation. An in vivo model comprised C57BL/6N mice fed HFD for 8 weeks, followed by a single injection of STZ at 120 mg/kg. MORE was administered at 100 and 200 mg/kg once daily (p.o.) for 4 weeks. The changes in body weight, calorie intake, and serum levels of glucose, insulin, total cholesterol (TCHO), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), aspartate transaminase (AST), and alanine aminotransferase (ALT) were investigated in diabetic mice. The histological changes in the gastrocnemius muscle were observed by H&E staining, and then the myofiber size was measured. The expression of the myogenic (MHC, myogenin, and MyoD) and biogenesis (PGC-1α, SIRT1, NRF1, and TFAM) regulatory proteins was examined in the muscle tissues and differentiated C2C12 myoblasts by Western blot, respectively. The administration of MORE at 200 mg/kg in mice with HFD/STZ-induced diabetes significantly reduced weight gains, calorie intake, insulin resistance, and serum levels of glucose, TCHO, LDL-C, AST, and ALT. MORE administration at 100 and 200 mg/kg significantly increased serum insulin and HDL-C levels in diabetic mice. In addition, MORE significantly increased the expression of MHC, myogenin, MyoD, PGC-1α, SIRT1, NRF1, and TFAM in muscle tissues as well as increased the myofiber size in diabetic mice. In C2C12 myoblast differentiation, MORE treatment at 0.5, 1, and 2 mg/mL significantly increased the expression of myogenic and biogenesis regulatory proteins in a dose-dependent manner. MORE improves diabetes symptoms in mice with HFD/STZ-induced diabetes by improving muscle function. This suggests that MORE could be used to prevent or treat diabetes along with muscle disorders.

**INTRODUCTION**

Metabolic syndromes with abdominal obesity, high blood pressure, high blood glucose, and high serum triglyceride levels seriously affect the regulation of insulin in glucose, fat, and protein metabolism and ultimately promote the occurrence and development of type 2 diabetes mellitus (T2DM). The incidence of T2DM with insulin resistance and deficits in the islet cell function and quantity increases with age, leading to a progressive decline in skeletal muscle mass and function from increasing proteolysis and decreasing protein synthesis. Therefore, T2DM is an important factor in the poor quality of life of older people with many complications.

The skeletal muscle is responsible for 80% of the glucose uptake in the whole body. A decline in muscle quality leads to decreased muscle capacity, insulin sensitivity, and peripheral glucose management ability that increases the risk of diabetes. Therefore, it has been implicated as both a cause and consequence of diabetes; a bidirectional relationship exists between muscle atrophy and T2DM. T2DM is mediated mainly through diet and oral hypoglycemia medications, such as metformin and other antidiabetes drugs. Unfortunately, there is no broadly effective treatment for delaying diabetes progression. Over the past decade, the benefits of many herbal medicines used traditionally as diabetes therapies have been demonstrated from in vivo studies and clinical trials. Traditional Chinese medicine (TCM) and Korean medicine (TKM) are becoming increasingly popular because of their effectiveness in treating...
**Table 1. Effects of MORE on the Physiological Changes in Mice with HFD/STZ-Induced Diabetes**

| group       | body weight (g) | calorie intake (kcal) | serum glucose (mg/dL) | serum insulin (μU/mL) | HOMA-IR index |
|-------------|-----------------|-----------------------|-----------------------|-----------------------|---------------|
| Nor         | 28.00 ± 1.00    | 74.40 ± 3.10          | 94.60 ± 16.89         | 4.78 ± 0.42           | 0.96 ± 0.09   |
| control     | 32.33 ± 2.08*** | 99.56 ± 5.24***       | 516.20 ± 33.16***     | 3.19 ± 0.38***        | 3.31 ± 0.31***|
| MORE100     | 28.67 ± 2.08**  | 85.59 ± 3.02***       | 461.60 ± 35.23*       | 4.11 ± 0.61          | 2.61 ± 0.47*  |
| MORE200     | 28.33 ± 2.51*** | 90.83 ± 8.01*         | 403.40 ± 29.05***     | 4.45 ± 1.05*         | 2.60 ± 0.73*  |
| Met         | 26.33 ± 1.52*** | 76.85 ± 8.00***       | 286.00 ± 37.58***     | 4.68 ± 1.08***       | 2.43 ± 0.67** |

The data are expressed as the mean ± S.D. (n = 5). Nor, normal group; Control, group with HFD/STZ-induced diabetes; MORE100, mice administered 100 mg/kg MORE; mice administered 200 mg/kg MORE; and Met, group administered 250 mg/kg metformin. *p < 0.01, **p < 0.001 vs Nor; *p < 0.05, **p < 0.01, ***p < 0.001 vs control.

**Figure 1.** Effects of MORE on the serological changes in mice with HFD/STZ-induced diabetes. The levels of (A) total cholesterol (TCHO), (B) LDL-cholesterol (LDL-C), (C) HDL-cholesterol (HDL-C), (D) AST, and (E) ALT were measured in the sera of mice. The data are expressed as the mean ± S.D. (n = 5). Nor, normal group; Control, group with HFD/STZ-induced diabetes; MORE100, group administered 100 mg/kg MORE; MORE200, group administered 200 mg/kg MORE; and Met, group administered 250 mg/kg metformin. *p < 0.05 and **p < 0.001 vs Nor; *p < 0.05, **p < 0.01, ***p < 0.001 vs Control.

**RESULTS AND DISCUSSION**

**Effects of MORE on the Physiological Changes in Diabetic Mice.** The effects of MORE on diabetes symptoms were examined by measuring the changes in the physiological markers, body weight, calorie intake, serum levels of glucose, insulin, and HOMA-IR in mice with HFD/STZ-induced diabetes.

As shown in Table 1, the body weight, calorie intake, and glucose level were increased significantly (p < 0.001, respectively) in diabetic mice and the insulin level was decreased significantly (p < 0.01) compared to that in the normal (Nor) group. The administration of MORE at 100 mg/kg (p < 0.01) and 200 mg/kg (p < 0.01) and metformin (Met, p < 0.001) to the diabetic mice decreased their body weights significantly. The calorie intake was significantly lower (p < 0.001, p < 0.05, and p < 0.001) in the MORE100 (p < 0.001) and MORE200 (p < 0.05) and the Met (p < 0.001) groups compared to that in the control group.

The glucose level in the serum was decreased significantly in the MORE100 (p < 0.05), MORE200 (p < 0.001), and Met (p < 0.001) groups. The insulin level was significantly higher in...
the MORE200 ($p < 0.05$) and Met ($p < 0.01$) groups than that in the control. On the other hand, there was no significant difference between the control group and the MORE100 group.

The HOMA-IR index showed that the insulin resistance score was significantly ($p < 0.001$) increased in the control group compared with that in the normal group. The insulin resistance was significantly decreased in the MORE100 and MORE200 ($p < 0.05$, respectively) and met ($p < 0.01$) groups compared with that in the control group.

**Effects of MORE on the Changes in Lipid Metabolites in Diabetic Mice.** The effects of MORE on the change in the lipid profile were examined by measuring the serum levels of lipid metabolites in mice with HFD/STZ-induced diabetes. As shown in Figure 1, the total cholesterol (TCHO; Figure 1A) and LDL-cholesterol (LDL-C; Figure 1B) levels were higher in the control group ($p < 0.001$, respectively). The MORE100 ($p < 0.01$) and MORE200 ($p < 0.05$) or Met ($p < 0.01$) groups had significantly decreased levels of total cholesterol (TCHO) and LDL-cholesterol (LDL-C). The HDL-cholesterol (HDL-C; Figure 1C) level in the control group was lower than that in the normal group ($p < 0.05$), and the levels increased after treatment with MORE or metformin ($p < 0.001$, respectively). The levels of AST (Figure 1D) and ALT (Figure 1E) were measured as markers of liver damage. As a result, they were increased significantly in the control group ($p < 0.001$, respectively) and reduced significantly in MORE100 and MORE200 or Met groups ($p < 0.001$, respectively).

**Figure 2. Effects of MORE on the histological changes in the skeletal muscle tissues in diabetic mice.** Cross sections of gastrocnemius tissues were stained with H&E stain; the nucleus stained blue, and the cytoplasm stained red under a microscope. (A) Representative pictures of tissue staining in each group (200×). The photomicrograph of the organization of the muscle fibers (MFs) into fascicles or bundles and the space between the muscle bundles (P). (B) Cross-sectional areas of myofibers in the gastrocnemius muscle were measured and expressed as mean ± S.D. ($n = 5$). Nor, normal group; Control, mice with HFD/STZ-induced diabetes; MORE100, group administered 100 mg/kg MORE; MORE200, group administered 200 mg/kg MORE; and Met, group administered 250 mg/kg metformin. ***$p < 0.001$ vs Nor; ****$p < 0.001$ vs Control.

Nor, normal group; Control, mice with HFD/STZ-induced diabetes; MORE100, group administered 100 mg/kg MORE; MORE200, group administered 200 mg/kg MORE; and Met, group administered 250 mg/kg metformin. ***$p < 0.001$ vs Nor; ****$p < 0.001$ vs Control.

The morphological changes in the muscle tissues in mice with HFD/STZ-induced diabetes were observed to investigate the effects of MORE on muscle weakness. The main histological feature of skeletal muscle atrophy is a reduction in muscle fiber diameter and areas with a loose arrangement of muscle fibers (MFs). The muscle fibers of the gastrocnemius tissues in the normal group had no degradation or necrosis according to H&E staining (Figure 2A). In the diabetic mice, each bundle of muscle fibers was separated farther from others, and there was considerable space between the muscle bundles. The myofibers were often rounded to angular. On the other hand, the MORE100 and
MORE200 or Met mice showed a normal structure with a significant ($p < 0.001$, respectively) increase in the fibers (Figure 2B).

**Effects of MORE on the Muscle Tissue in Mice with HFD/STZ-Induced Diabetes.** The effects of MORE on the skeletal muscle were examined by measuring the expression of various regulatory proteins, such as myogenetic (MyoD, myogenin, and MHC), biogenetic (PGC-1α), and atrophy (Atrogin1) in mice with HFD/STZ-induced diabetes.

As the result (Figure 3), compared to the normal group, the diabetic mice showed a significant decrease in the expressions of MHC ($p < 0.05$, Figure 3B), MyoD ($p < 0.05$, Figure 3C), myogenin ($p < 0.001$, Figure 3D), and PGC-1α ($p < 0.001$, Figure 3E) and an increase in Atrogin1 ($p < 0.001$, Figure 3F) in skeletal muscle tissues. The MORE100 and MORE200 mice showed a significantly increased expressions of MHC ($p < 0.01$), MyoD ($p < 0.001$), myogenin ($p < 0.05$), and PGC-1α ($p < 0.05$) in the muscle tissues but significantly decreased Atrogin1 expression ($p < 0.001$).

**Effects of MORE on the Expression of Myogenic Proteins in C2C12 Myoblasts.** The effects of MORE on myoblast differentiation were investigated by measuring the expressions of MHC, myogenin, and MyoD.

The expressions of myogenin (Figure 4B) and MyoD (Figure 4C) were increased significantly ($p < 0.001$, respectively) by treatment with 2 mg/mL MORE. MORE induced the differentiation of myoblasts into myotubes with elongated and widened cylindrical shapes and multiple nuclei (Figure 4F) under microscopic observation. MORE also increased MHC (Figure 4E) expression significantly at 0.5 mg/mL ($p < 0.05$), 1 mg/mL ($p < 0.01$), and 2 mg/mL ($p < 0.01$) in C2C12 myoblasts. The Met-treated cells showed a significant increase in myogenin, MyoD, and MHC in C2C12 cells ($p < 0.01$, respectively).

**Effects of MORE on the Expression of Biogenetic Factors in C2C12 Myoblasts.** The effects of MORE on biogenesis in muscle cells were examined by measuring the expression of biogenetic factors PGC-1α, NRF1, SIRT1, and TFAM in C2C12 myotubes by Western blotting.

As shown in Figure 5, the treatment with 0.5 mg/mL MORE ($p < 0.05$), 1 mg/mL MORE ($p < 0.01$), and 2 mg/mL MORE ($p < 0.001$) significantly increased the expressions of PGC-1α (Figure 5B), NRF1 ($p < 0.05$ for 1 and 2 mg/mL; Figure 5C), SIRT1 ($p < 0.05$ for 0.5 and 2 mg/mL; $p < 0.01$ for 1 mg/mL; Figure 5D), and TFAM ($p < 0.05$ for 0.5 mg/mL and $p < 0.01$ for 1 and 2 mg/mL; Figure 5E). Met also increased the expressions of PGC-1α ($p < 0.05$), NRF1 ($p < 0.01$), SIRT1 ($p < 0.05$), and TFAM ($p < 0.01$) in C2C12 myotubes.

Type 2 diabetes (T2DM) is the most common type of diabetes characterized by hyperglycemia and urine sugar resulting from resistance to insulin action and an inadequate compensatory insulin secretory response. Approximately 90% of individuals with obesity and the elderly have a high incidence of diabetes, and the disease is growing rapidly in developing countries. Medications for diabetes patients include various drugs, such as metformin, insulin-releasing pills, starch blockers, amylin analogues, and incretin-based therapeutics for lower blood sugar. In the concepts of traditional medicines, the human body includes two aspects, blood (Yin) and Qi (Yang). The Yin-blood is the material basis for motivation and Yang-qi is the power of the human body. Type 2 diabetes is thought to be caused by deficiency.
syndromes, which mainly include Qi deficiency, Yin deficiency, and Yang deficiency, and dietary irregularities, emotional disharmony, and phlegm wet constitution (obesity), which lead to three main diabetes symptoms of polydipsia, polyuria, and polyphagia. Type 2 diabetes involves insulin resistance, which could stimulate glucose uptake, severely change energy metabolism, and negatively affect skeletal muscle quality through protein degradation and mitochondrial dysfunction. Thus, insulin resistance is regarded as an important contributor to decreased muscle growth and development and induced fiber atrophy in diabetes myopathy. Skeletal muscle also is a key factor in the development of insulin resistance because quantitatively it is the principal tissue that contributes to whole-body insulin-mediated glucose disposal. Therefore, it is believed that diabetes is associated with excessive muscle loss (atrophy) that contributes to functional abnormalities of muscle. Recently, it has been reported that sarcopenia, a type of muscle atrophy occurring in aging, is induced by poor glucose disposal and a decreased metabolic rate and physical activity that is considered to increase the risk of developing T2DM in the elderly. At this point, it is being studied that antidiabetes drugs such as metformin may alter the balance between protein synthesis and degradation through various regulation mechanisms of muscle mass and improve muscle function by controlling various regulatory factors on myogenesis and mitochondrial biogenesis. However, the prevention of muscle loss and dysfunction is viewed as being crucial in terms of treating T2DM. In our study, we investigated the effects of MO, a herbal medicine used for strengthening the muscles and increasing energy in the elderly, on muscle function in mice with HFD/STZ-induced diabetes. A high-calorie diet and obesity are the main risk factors for T2DM. In the present study, HFD-fed mice showed an increase in body weight, induction of both insulin resistance, and glucose tolerance that may mimic the human lifestyle. Streptozotocin is selectively toxic to the insulin-producing β cells of the pancreas in mammals and has been long injected in experimental animals for the reproduction of diabetes pathology in humans. Multiple methods of STZ dosing depend on the type and the severity of diabetes in mice, and the dose range also varies largely, so they are applied in various ways from 100 to 200 mg/kg in a single high dose. The mice with HFD/STZ-induced diabetes are a useful experimental animal model for screening hypoglycemic drugs and examining the corresponding mechanisms. In this study, we produced a
diabetes mouse model with an HFD for 12 weeks, and a single injection of STZ at 120 mg/kg, a single moderately sized dose could induce diabetes symptoms. Our diabetes model could not observe dead mice during drug administration. We also used two different dosages of MORE at 100 and 200 mg/kg according to the previous study.18,20

The main characteristics of human T2DM are high blood glucose, increase in body weight, more food/calorie intake, and lipid metabolism disorders in a clinical setting.46,47 Our in vivo model exhibited these symptoms, which were improved by MORE administration for 4 weeks. This suggests that MORE may improve the symptoms of diabetes such as obesity, insulin resistance, and T2DM, which have been associated with muscle atrophy. Although there are few studies of MORE efficacy in T2DM or muscle function, it is reported that the ethanol extract of MO at 150 mg/kg significantly reduced fasting serum glucose levels in rats with STZ-induced diabetes;20 interestingly, the stem bark extract of M. lucida, the sister species of MO, has reducing effects at 500 mg/kg on increases in weight loss, water and food consumption, lipid peroxidation, and cholesterol, triglycerides, and glucose in rats with STZ-induced diabetes.48 In this study, we compared the efficacies of MORE and metformin as the first medicine to treat T2DM, which decreases hepatic glucose production and increases the insulin sensitivity of target cells. Administration of metformin at 250 mg/kg in mice with HFD/STZ-induced diabetes was shown to have similar effects on physiological and serological changes as MORE.

In our study, an increase in serum levels of TCHO, LDL-C, AST, and ALT and a low level of HDL-C were observed in the mice with HFD/STZ-induced diabetes compared to the normal group. These levels were reduced in the MORE100 and MORE200 groups, suggesting that MORE can improve dyslipidemia in diabetes. Our finding that the TCHO level is reduced is consistent with previous reports.20

Previous studies explained that dyslipidemia leads to the apoptosis and atrophy of skeletal muscle.50 The present study observed pathological changes to the gastrocnemius muscle tissues of mice. As a result, the diabetic mice showed histological changes in the muscle tissues, such as a reduction in myofiber size with a loose arrangement and decreasing numbers. On the other hand, the diabetic mice administered MORE at low and high doses showed similar structures to the normal group. This result indicates that MORE can decrease the glucose level, reduce abnormal lipid metabolism, and decrease muscle atrophy finally in the mice with HFD/STZ-induced diabetes.

Differentiation of the skeletal muscle is a dynamic multistep process that involves multiple myogenic regulatory factors, such as MyoD, myogenin, and MHC.51 MyoD plays a crucial role in the differentiation of satellite cells into myoblasts.52 Myogenin controls the differentiation of myoblasts to myotubes53 and MHC is a differentiation marker in neoformed myotubes.54,55 In our study, it was observed that the decreased expression of myogenic proteins in muscle tissues of diabetic mice which were increased by MORE administration. One of the features of diabetes is an altered mitochondrial function in skeletal muscle. Thus, impaired mitochondrial glycolytic capacity and altered β-oxidation in the muscle of T2DM patients have been reported.56 The AMPK/SIRT1/
PGC-1α signaling pathways on energy homeostasis in muscle under diabetes conditions have been studied. The mitochondria are essential organelles for ATP production and regulate the lipid and glucose metabolism in the skeletal muscle. PGC-1α, a metabolic mediator has a crucial role in the regulation of mitochondrial biogenesis by co-activating NRF1, which subsequently increases the expression of TFAM. SIRT1 is another crucial factor of glucose and lipid metabolism and stimulates mitochondrial biogenesis via the PGC-1α/AMPK pathway. Therefore, muscle differentiation and mitochondrial biogenesis are associated with an increase in muscle mass and strength. In our study, MORE significantly increased the expression of myogenic proteins and biogenesis regulatory proteins in muscle tissues of not only diabetic mice but also in C2C12 myotubes. Moreover, a comparison of the effects of MORE at 2 mg/mL and metformin at 2.5 mM showed that MORE was more effective in the expression of MHC, MyoD, myogenin, and PGC-1α than metformin. This suggests that MORE can be more effective in enhancing muscle function than metformin.

MORE is contains various ingredients such as oligosaccharides, polysaccharides, iridoid glucosides, plant sterols, anthraquinones, flavonoids, vitamin C, and other ingredients, but their effects on obesity, diabetes, and muscle wasting. However, the components of MORE need to be considered in a future study.

### CONCLUSIONS

The administration of MORE at 100 and 200 mg/kg for 4 weeks in mice with HFD/STZ-induced diabetes resulted in decreased body weight, calorie intake, high glucose and lipid levels, and increased insulin levels. MORE improved the mass loss of the skeletal muscle by enhancing the expression of myogenetic proteins, MyoD, myogenin, MHC and biogenetic proteins, PGC-1α, NRF1, SIRT1, and TFAM in the skeletal muscle tissues of diabetic mice and C2C12 myoblasts differentiation. These results suggest that MORE can be a preventative and therapeutic natural drug for T2DM patients showing muscle loss.

### EXPERIMENTAL SECTION

**Preparation of MORE.** The dried root of *M. officinalis* (Morinidae Radix) was purchased from a medicinal materials company (Kwangmyungdang Herbal Company, Ulsan, Korea) and identified by a botanical expert, Dr. Y.-K. Park, College of Korean Medicine, Dongguk University, Korea. Approximately 200 g of small pieces of *M. officinalis* was boiled with 2000 mL of distilled water in a round-bottom flask under reflux and extracted twice for 3 h. The crude extract was filtrated through Whatman Filter Paper (GE Healthcare UK Limited, Buckinghamshire, U.K.), concentrated under a vacuum rotary evaporator (Eyela Co. Ltd., Tokyo, Japan) at 60 °C, and then lyophilized in a freeze-dryer (ILShin Lab Co., Yangju, Korea) at −80 °C under 5 mTorr. The powder of the MORE (yield = 58%) was stored at −20 °C until needed in in vivo and in vitro study.

**Preparation of In Vivo Model.** Male C57BL/6N mice (Koatech, Pyeongtaek, Korea) were used in this study. The Institutional Animal Care and Use Committee of the Dongguk University (IACUC-2018-11) approved the animal care protocol and experimental procedures. The mice were acclimated to the feeding protocol 1 week before the experiment. The mice were housed in cages at 22 ± 3 °C and relative humidity of 60 ± 5% and a 12/12 h light/dark cycle. They were given access to food and water ad libitum. The mice were divided randomly into two groups: a normal group (Nor) with standard chow [3.10 kcal/g, 18% kcal from fat, 58% kcal from carbohydrates, and 24% kcal from protein (Cat. 2018S, Envigo, IN)] and a group with a high-fat diet (HFD, 5.24 kcal/g, 60% kcal from fat, and 20% kcal from carbohydrates, 20% kcal from protein) (Cat. D12492, Research diets, NJ) for 8 weeks. After feeding, the HFD mice were single injected intraperitoneally (i.p.) with STZ at 120 mg/kg body weight (b.w.), which was dissolved in citrate buffer (pH 4.5). To confirm the induction of diabetes, the fasting blood glucose (FBG) levels were measured in the whole blood of the tails using an Accu-Chek Inform II Glucose Meter (Roche, Basel, Swiss). Mice with a blood glucose concentration of more than 300 mg/dL were used.

The mice with HFD/STZ-induced diabetes were divided randomly into four groups (n = 5 per each group): the control group with HFD/STZ-induced diabetes (control), group administered MORE at 100 mg/kg b.w. (MORE100, p.o.) and 200 mg/kg b.w. (MORE200, p.o.) and that administered metformin at 250 mg/kg b.w. (Met, p.o.) as a reference group. Mice were administered drugs once daily for 4 weeks. The normal group was fed the normal diet, and the other groups were kept on HFD during drug administration.

**Measurement of Physiological Parameters.** At the end of the experiment, the body weight and food intake were measured. Also, the caloric intake was calculated by food intake multiplying 3.1 kcal/g in the standard diet and multiplying 5.24 kcal/g in the HFD mice.

**Measurement of Serological Parameters.** The animals were sacrificed using a 5% isoflurane in O2 (75%) and N2O (25%) euthanasia station after 24 h fasting. Whole blood was harvested from the abdominal aorta using a syringe. The blood samples were isolated by centrifugation twice at 3000 rpm for 10 min at room temperature and the serum samples were isolated for serological analysis. The following were measured including levels of glucose (GLU), aspartate transaminase (AST), alanine aminotransferase (ALT), total cholesterol (TCHO), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) in the serum samples using an automated clinical chemistry analyzer (FDC7000s, Fujifilm Co, Tokyo, Japan). The insulin levels were analyzed using an insulin enzyme-linked immunosorbent Assay (ELISA) kit (Cat. 90082, Crystal Chem Inc., IL) according to the manufacturer’s protocol. The concentration of insulin in the serum samples was determined using a standard curve. The equation calculated the level of homeostasis model assessment of insulin resistance (HOMA-IR) as follows: HOMA-IR = [fasting insulin (μU/mL) × fasting serum glucose (mM)]/22.5.

**Histopathological Observation.** The gastrocnemius tissues were obtained for histological observations and protein expression analysis. The gastrocnemius muscle tissues were immobilized with 4% paraformaldehyde for 24 h to make a specimen section after dehydration paraffin embedding and stained with hematoxylin and eosin (H&E). The change in muscle tissues was observed under a microscope (original magnification 200×). The size of fibers was measured in the image using the ImageJ software (https://imagej.nih.gov/ij/) and presented the mean cross-sectional area of the muscle fibers.
**Cell Culture and Treatment**. The C2C12 cells (ATCC, VA), a mouse myoblast line, were cultured in DMEM (Corning, NY) supplemented with 10% fetal bovine serum (Merck Millipore, MA) with penicillin/streptomycin (Corning) at 37 °C in a 5% CO₂ incubator. When more than 90% confluence was reached, the medium was changed to DMEM supplemented with 2% horse serum (Thermo Fisher Scientific, MA) for 4 days and then treated with MORE at different concentrations (0.5, 1, and 2 mg/mL) and metformin 2.5 mM/mL for 24 h. No toxicity was observed up to 2 mg/mL by an MTT assay (Figure S1).

**Immunocytochemistry Staining**. The C2C12 cells were seeded on Thermax plastic coverslips (Nunc, Thermo Fisher Scientific) and differentiated with 2% horse serum for 4 days. After treatment with MORE or metformin for 24 h, the C2C12 myotubes were washed three times with 1× PBS, fixed in 4% paraformaldehyde, placed on ice for 20 min, and permeabilized in 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO) for 5 min at room temperature. Subsequently, the cells were washed with 1× PBS, blocked in 0.5% bovine serum albumin (BSA) for 2 h, and incubated with the anti-MHC (Cat. Sc-376157, Santa Cruz Biotechnology Inc., TX) antibody at 4 °C overnight. The myotubes were washed three times with 1× PBS and incubated with a goat antimouse antibody conjugated with Alexa Fluor 488 (Thermo Fisher Scientific, Cat. A11001). The nuclei were counterstained with DAPI. The cells were visualized using a fluorescence microscope (Leica DM2500, Leica Microsystems, Wetzlar, Germany). The MHC-positive cells were observed by green fluorescence in blue multinucleated myotubes with DAPI.

**Western Blot Analysis**. To isolate the total protein, the skeletal muscle tissues and C2C12 myotubes were homogenized with a protein lysis buffer containing a protease inhibitor and phosphatase inhibitor, homogenized using a homogenizer (T10 basic, IKA, Staufen im Breis-gau, Germany), and centrifuged at 14000 rpm at 4 °C for 20 min. The supernatants were collected, and the total protein concentration in each sample, an equal amount of protein (30 μg), was used to capture the image, which was quantified by densitometry using ImageJ programming software (ImageJ, NIH, MD). The expression of each target protein was normalized to β-actin (Sigma-Aldrich) as an internal control.

**Statistical Analysis**. All data are presented as mean ± standard deviation (mean ± SD; n = 5 per each group in vivo and n = 3 in vitro). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by a Dunnett’s test between normal and control groups or control and drug groups. A p value <0.05 was considered significant. All statistical analyses were performed on the GraphPad Prism software Version 5.0 (GraphPad Software, CA).

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c03372. Cell viability assay: effects of MORE on the viability of C2C12 cells. The data used to support the study are available from the corresponding author upon request. (PDF)

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**Author Contributions**

H.W.J. and Y.-K.P. designed the study. P.W., Y.L., T.Z., C.Y., and S.Y.K. performed the experiments. P.W., S.Y.K., and S.J.K. conducted the statistical analyses. P.W. and H.W.J. wrote the manuscript. S.Y.K., H.W.J., Y.-K.P., and S.J.K. revised the manuscript, and all authors approved the final version.

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**Notes**

The authors declare no competing financial interest. All procedures were performed in accordance with the guidelines of the Dongguk University of Korea’s Medicine ethics committee.

**ABBREVIATIONS**

MORE Morinda officinalis root extract
T2DM type 2 diabetes mellitus
HFD high-fat diet
STZ streptozotocin
TCM Traditional Chinese medicine
LDL-C LDL-cholesterol
ALT alanine aminotransferase
AST aspartate transaminase
TKM Traditional Korean medicine
FBG fasting blood glucose

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