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The Korean Traditional Anti-obesity drug Gyeongshingangjeehwan Stimulates AMPKα Activation in Skeletal Muscle of OLETF Rats

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Our previous study demonstrated that the Korean traditional medicine Gyeongshingangjeehwan (GGEx) inhibits obesity and insulin resistance in obese type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. We investigated whether GGEx may affect AMP-activated protein kinase α (AMPKα) since AMPKα activation is known to stimulate fatty acid oxidation in skeletal muscle of obese rodents. After OLETF rats were treated with GGEx, we studied the effects of GGEx on AMPKα and acetyl-CoA carboxylase (ACC) phosphorylation, and the expression of AMPKα, PPARα, and PPARα target genes. The effects of GGEx on mRNA expression of the above genes were also measured in C2C12 skeletal muscle cells. Administration of GGEx to OLETF rats for 8 weeks increased phosphorylation of AMPKα and ACC in skeletal muscle. GGEx also elevated skeletal muscle mRNA levels of AMPKα1 and AMPKα2 as well as PPARα and its target genes. Consistent with the *in vivo* data, similar activation of genes was observed in GGEx-treated C2C12 cells. These results suggest that GGEx stimulates skeletal muscle AMPKα and PPARα activation, leading to alleviation of obesity and related disorders.

Key Words: AMPK; C2C12; GGEx; Korean traditional medicine; Skeletal muscle; PPARα

INTRODUCTION

AMP-activated protein kinase (AMPK) is a serine/threonine kinase that plays an important role in energy metabolism at both cellular and whole-body levels (Hardie et al., 1998; Kemp et al., 1999). AMPK functions as a heterotrimeric complex composed of α, β, and γ subunits. Activation of AMPK requires phosphorylation of Thr^{172} in the activation loop of the α subunit. The activated AMPKα phosphorylates acetyl-CoA carcoxylase (ACC) on Ser^{221}, resulting in the inhibition of its activity and reduced formation of malonyl-CoA. The latter effect in turn results in the activation of carnitine palmitoyltransferase I, a step required for the stimulation of fatty acid oxidation in mitochondria (Ruderman and Prentki, 2004). The activation of AMPKα also induces peroxisome proliferator-activated receptor α (PPARα) gene expression, which regulates the expression of genes critical for fatty acid oxidation (Lee et al., 2002, 2006; Suzuki et al., 2007). PPARα target genes include those involved in hydrolysis of plasma triglycerides, fatty acid uptake, binding, and fatty acid β-oxidation, thereby leading to lipid homeostasis (Yoon, 2009, 2010).

Gyeongshingangjeehwan (GGEx), which comprises *Liriope platyphylla* F.T. Wang & T. Tang (Liliaceae), *Platycodon grandiflorum* A. DC. (Campanulaceae), *Schisandra chinensis* K. Koch (Magnoliaceae), and *Ephedra sinica* Stapf (Ephedraceae), is thought to exhibit beneficial pharmacological effects on the endocrine, cardiovascular, and immune systems as practiced in Korean traditional medicine (Lee, 1993; Kim, 1997; Eun et al., 2006), although the mechanism of its action remains unknown. Four herbs of GGEx, *L. platyphylla*, *P. grandiflorum*, *S. chinensis*, and *E. sinica* are reported to have anti-diabetes, lipid-lowering, and anti-obesity effects (Park et al., 2007; Zheng et al., 2007; Yao

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et al., 2008), supporting that GGEx may regulate efficiently obesity, type 2 diabetes, and lipid disorders. Recently, our results showed that GGEx prevents obesity and insulin resistance in obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Jeong et al., 2008; Shin et al., 2010). Based on the role of GGEx in obesity and the actions of AMPK and PPARα on fatty acid oxidation in skeletal muscle, we hypothesized that GGEx can stimulate AMPKα and PPARα pathways in skeletal muscle.

We treated obese OLETF rats and C2C12 skeletal muscle cells with GGEx. We show that GGEx treatment increased the AMPKα phosphorylation and mRNA levels. Moreover, it increased mRNA levels of PPARα and its target genes responsible for fatty acid oxidation. These studies suggest that GGEx activates AMPKα and AMPKα-dependent PPARα, leading to reduced obesity and related disorders.

MATERIALS AND METHODS

Preparation of GGEx

GGEx was prepared from food-grade aqueous extracts of the four herbs (42.86% *L. platyphylla*, 28.57% *P. grandiflorum*, 14.29% *S. chinensis*, and 14.28% *E. sinica* - expressed as % dry weight) (Hwalim, Busan, Korea). The proportions used in this study are same as the proportions that are used to treat patients. Boucher specimens for *Liriope platyphylla* (FOS-05-01), *Platycodon grandiflorum* (FOS-05-02), *Schisandra chinensis* (FOS-05-03), and *Ephedra sinica* (FOS-05-04) were deposited at the Department of Formula Sciences, Dongeui University. Briefly, three dried herbs with their contents weighted were boiled together in distilled water for 22 h at 95℃. The aqueous extracts were then filtered and freeze-dried under vacuum for the production of GGEx.

Animal treatments

Eight male OLETF and four Long-Evans Tokushima Otsuka (LETO) rats were provided as a generous gift from the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan). Ten-week old animals were housed at the Dongeui University under pathogen-free conditions using a standard 12-h light/dark cycle. Prior to treatment, rats were fed standard rodent chow and water *ad libitum*. Rats were then divided into 3 groups (n = 4/group): a lean LETO group given water (Normal), an obese OLETF control group given water (Control), and an OLETF group given GGEx (GGEx, 121.7 mg/kg/day) orally by gavage once a day for 8 weeks. At the end of the 8-week period, the animals were sacrificed under diethyl ether anesthesia and their tissues were harvested, weighed, snap-frozen in liquid nitrogen and stored at -80℃ until use. All animal experiments were approved by the Institutional Animal Care and Use Committee of Dongeui University and followed National Research Council Guidelines.

Differentiation of C2C12 cells

Mouse myogenic C2C12 cells were routinely cultured in DMEM containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), penicillin G (100 U/ml), streptomycin sulfate (100 μg/ml), amphotericin B (0.25 μg/ml), and 2-mercaptoethanol (50 μM). To induce differentiation of skeletal muscle cell line C2C12, culture medium was changed to 2% horse serum instead of 10% fetal bovine serum when cells reached 70% confluence. The cells were differentiated into multinucleated myotubes for 5 days and then treated with chemicals for 24 h.

Immunoblotting

Skeletal muscle tissue was immediately lysed in ice-cold lysis buffer containing protease inhibitors. Lysates were centrifuged at 12,000 rpm for 20 min at 4℃ and the resulting supernatants were subjected to electrophoresis on a 8% polyacrylamide gel. The separated proteins were transferred to PVDF membrane (Millipore, Billerica, MA, USA). The membrane was incubated with rabbit antibodies to AMPKα (Thr^{172} phosphorylated) and ACC (Ser^{221} phosphorylated) as primary antibodies. After incubating with goat anti-rabbit secondary antibody, blots were visualized using a ECL immunoblotting analysis system (Amersham, Piscataway, NJ).

RT-PCR

Total cellular RNA from skeletal muscle tissues and differentiated C2C12 cells (C2C12 myotubes) was prepared

Table 1. Sequences of primers used for the RT-PCR assays

Genes	GeneBank	Primer sequences	Size (bp)
AMPKα1	NM00103367	Forward : 5'-agagggccgcaataaaagat-3'	177
		Reverse : 5'-tgttgtacaggcagctgagg-3'	
AMPKα2	NM178143	Forward : 5'-cgcctctagtcctccatcag-3'	219
		Reverse : 5'-atgtcacacgctttgctctg-3'	
PPARα	NM011144	Forward : 5'-gcagctcgtacaggtcatca-3'	202
		Reverse : 5'-ctcttcatccccaagcgtag-3'	
CPT-1	L07736	Forward : 5'-tatgtgaggatgctgcttcc-3'	586
		Reverse : 5'-ctcggagagctaagcttgtc-3'	
VLCAD	AF017176	Forward : 5'-cgtcagaggtgtactttgatgg-3'	268
		Reverse : 5'-catggactcagtcacatactgc-3'	
MCAD	NM007382	Forward : 5'-gacatttggaaagctgctagtg-3'	380
		Reverse : 5'-tcacgagctatgatcagcctctg-3'	
ACOX	J02752	Forward : 5'-actatatttggccaattttgtg-3'	196
		Reverse : 5'-tatggcagtggtttccaagcc-3'	
UCP3	NM009364	Forward : 5'-ggagccatggcagtgacctgt-3'	180
		Reverse : 5'-tgtgatgttgggccaagtccc-3'	
β-actin	J00691	Forward : 5'-tggaatcctgtggcatccatgaaa-3'	350
		Reverse : 5'-taaaacgcagctcagtaacagtcc-3'	

using the Trizol reagent (Invitrogen). After 2 μg total RNA was reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (MMLV-RT; Promega, Madison, WI, USA) and an antisense primer, cDNA was generated. The RNA was denatured for 5 min at 72℃ and immediately placed on ice for 5 min. Denatured RNA was mixed with MMLV-RT, MMLV-RT buffer, and a dNTP mixture, and incubated for 1 h at 42℃. Synthesized cDNA fragments were amplified by PCR in an MJ Research Thermocycler (Waltham, MA, USA). The PCR primers used for gene expression analysis are shown in Table 1. The cDNA was mixed with PCR primers, *Taq* DNA polymerase (Nanohelix, Daejon, Korea), and a dNTP mixture. The reaction consisted of 30 cycles of denaturation for 1 min at 94℃, annealing for 1 min at 58~60℃, and elongation for 1 min at 72℃. The PCR products were analyzed by electrophoresis on a 1% agarose gel. Relative expression levels are presented as a ratio of target gene cDNA vs. β-actin cDNA. PCR products were quantified from agarose gels using the GeneGenius (Syngene, Cambridge, UK).

Statistical analysis

Unless otherwise noted, all values are expressed as the mean ± standard deviation (SD). All data were analyzed by ANOVA and the unpaired student's *t*-test for statistically significant differences between groups.

RESULTS AND DISCUSSION

AMPK has been the subject of intense investigation and considerable pharmacological research because of the key roles that it has in regulating energy homeostasis. GGEx is widely used as an anti-obesity drug in Korean local clinics, although the cellular and molecular mechanisms underlying its effects remain unknown. Therefore, this study was undertaken to investigate whether skeletal muscle AMPK is involved in the regulation of obesity by GGEx.

Effects of GGEx on AMPK phosphorylation in skeletal muscle of OLETF rats

In multiple mammalian tissues, AMPK controls glucose and lipid metabolism (Carling, 2004; Long and Zierath,

2006; Hardie, 2008). The activation of AMPK results in the phosphorylation of several target molecules and subsequent fatty acid oxidation in skeletal muscle, liver, adipose tissue and heart, as well as glucose transport in muscle, cardiac glycolysis, and the inhibition of anabolic processes and ion channel activities (Hardie et al., 1998; Kemp et al., 1999). To determine the effects of GGEx on AMPK activation in skeletal muscle, we examined the AMPK and ACC phosphorylation in skeletal muscle of obese OLRTF rats. GGEx substantially induced the phosphorylation of the catalytic α subunit of AMPK on Thr^{172} compared with OLETF control rats (Fig. 1). The phosphorylation of ACC on Ser^{221} was also increased by GGEx compared with controls (Fig. 1). With obesity, reduced skeletal muscle AMPK activity and ACC phosphorylation accompanied by increased levels of malonyl-CoA may contribute to the accumulation of intramuscular lipids (Bandyopadhyay et al., 2006; Steinberg et al., 2006). It is suggested, therefore, that GGEx may act as an AMPK activator for the treatment of obesity and obesity-related disorders.

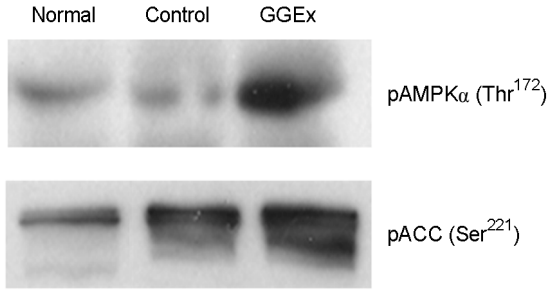

Fig. 1. GGEx stimulates the phosphorylation of AMPKα and ACC in skeletal muscle of obese OLETF rats. Adult male LETO and OLETF rats were treated with water or GGEx for 8 weeks. Immunoblotting analysis for AMPKα phosphorylated (pAMPKα) on Thr^{172} and ACC phosphorylated (pACC) on Ser^{221} was examined as described in Materials and Methods. Normal, lean LETO rats; Control, obese OLETF rats; GGEx, GGEx-treated obese OLETF rats.

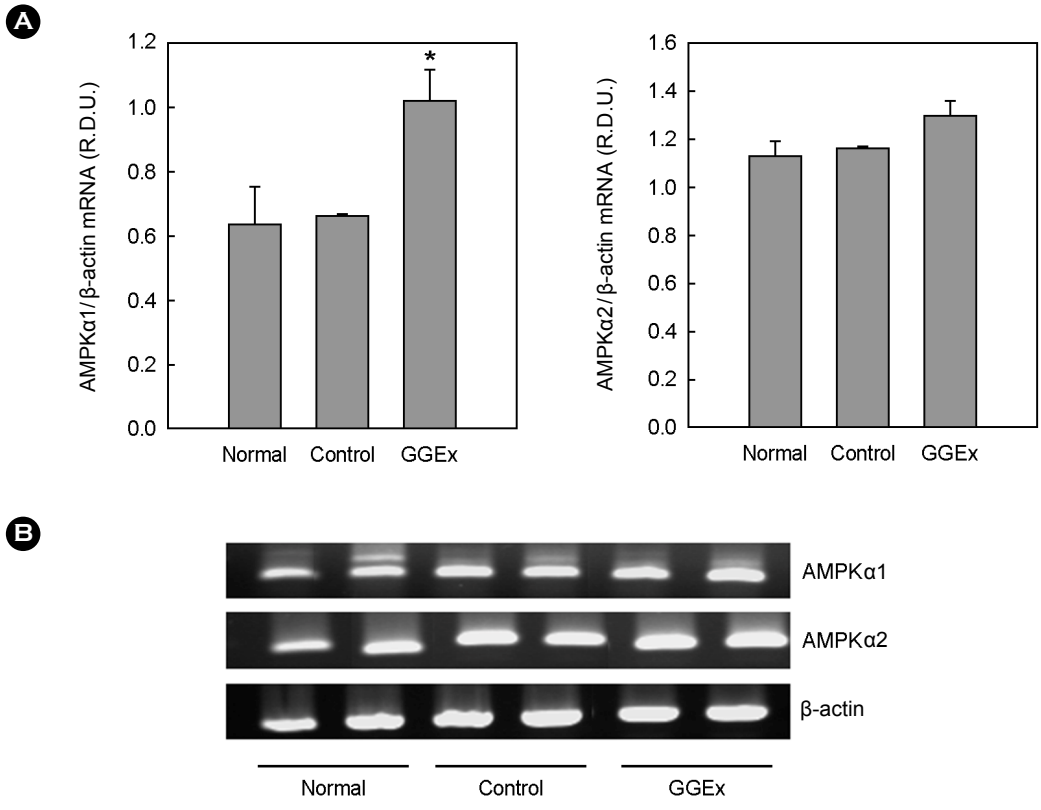

Fig. 2. GGEx increases the mRNA levels of AMPKα1 and AMPKα2 in skeletal muscle of obese OLETF rats. (A) Adult male LETO and OLETF rats were treated with water or GGEx for 8 weeks. Total cellular RNA was extracted from skeletal muscle tissue and mRNA levels were measured using RT-PCR. All values are expressed as the mean ± SD of relative density units (R.D.U.) using β-actin as a reference. #$P<0.05$ compared with normal group. *$P<0.05$ compared with control group. (B) Representative PCR bands from one of three independent experiments are shown. Normal, lean LETO rats; Control, obese OLETF rats; GGEx, GGEx-treated obese OLETF rats.

Effects of GGEx on mRNA levels of AMPKα, PPARα, and PPARα target genes in skeletal muscle of OLETF rats

The AMPK pathway in skeletal muscle has been reported to have profound effects on the regulation of lipid metabolism. The ability of AMPK to induce lipid oxidation can thus lower skeletal muscle lipid deposition (Collier et al., 2006; Long and Zierath, 2006), maybe contributing to alleviation of obesity. In particular, muscle AMPK activation induces PPARα gene expression, which regulates the expression of genes critical for fatty acid oxidation (Lee et al., 2002, 2006; Suzuki et al., 2007). To evaluate whether GGEx can upregulate AMPKα mRNA expression similar to its stimulatory effect on AMPKα phosphorylation, we measured mRNA expression of AMPKα1 and AMPKα2,

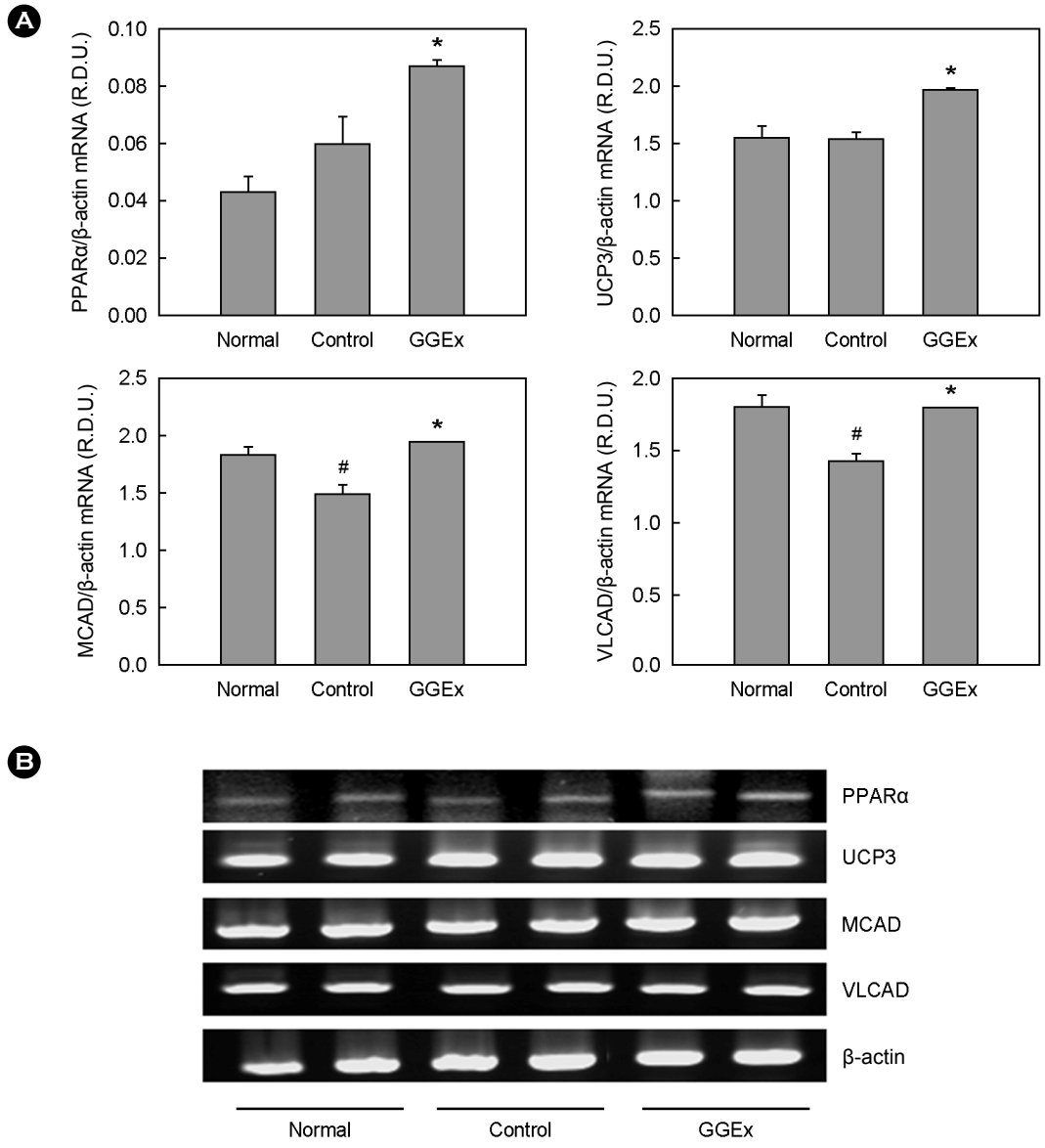

Fig. 3. GGEx18 increases the mRNA expression levels of PPARα and its target genes in skeletal muscle of obese OLETF rats. (A) Adult male LETO and OLETF rats were treated with water or GGEx for 8 weeks. Total cellular RNA was extracted from skeletal muscle tissue and mRNA levels were measured using RT-PCR. All values are expressed as the mean ± SD of relative density units (R.D.U.) using β-actin as a reference. #$P<0.05$ compared with normal group. *$P<0.05$ compared with control group. (B) Representative PCR bands from one of three independent experiments are shown. Normal, lean LETO rats; Control, obese OLETF rats; GGEx, GGEx-treated obese OLETF rats.

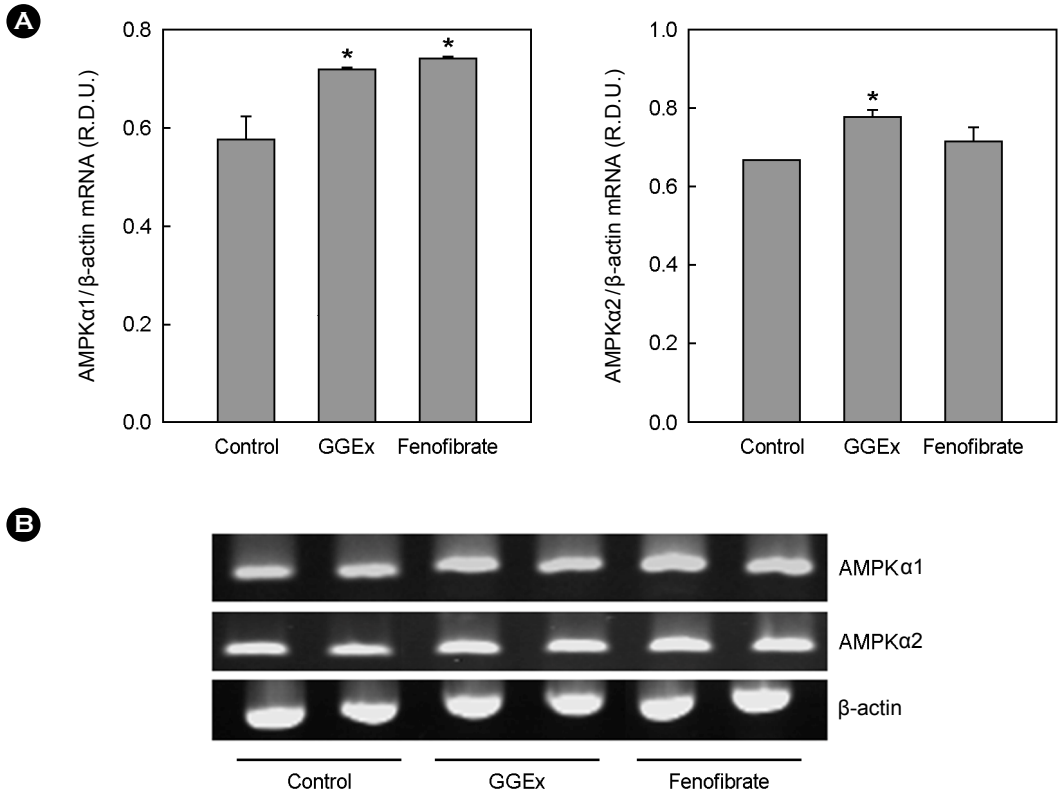

Fig. 4. GGEx increases the mRNA levels of AMPKα1 and AMPKα2 in C2C12 skeletal muscle cells. (A) Non-differentiated C2C12 myoblasts were differentiated into myotubes as described in the Materials and Methods. C2C12 cells were treated with DMSO (Control), 1 μg /ml GGEx, and 10 μM fenofibrate. RNA was extracted from C2C12 cells, and mRNA levels of AMPKα1, AMPKα2, and β-actin were measured as described in the Materials and Methods. All values are expressed as the mean ± SD of relative density units (R.D.U.) using β-actin as a reference. **P*<0.05 compared with control group. (B) Representative PCR bands from one of three independent experiments are shown.

which induces lipid oxidation and thus lowers triglyceride storage of skeletal muscle (Collier et al., 2006). In this study, GGEx significantly increased mRNA levels of AMPKα1 although AMPKα2 mRNA levels were not changed by GGEx (Fig. 2). We then measured mRNA levels of PPARα and its target genes responsible for fatty acid oxidation. Obese control rats had decreased mRNA levels of PPARα target genes, such as medium chain acyl-CoA dehydrogenase (MCAD) and very long chain acyl-CoA dehydrogenase (VLCAD) compared with normal LETO rats. However, mRNA expression of PPARα, MCAD, and VLCAD were markedly increased by GGEx treatment (Fig. 3). We also tested the effects of GGEx on mRNA levels of uncoupling protein 3 (UCP3), which has a PPAR response element in its promoter and is involved in the regulation of fatty acid oxidation (Acin et al., 1999; Brun et al., 1999). GGEx significantly increased UCP3 mRNA levels compared to those of control mice (Fig. 3).

Effects of GGEx on mRNA levels of AMPK, PPARα, and PPARα target genes in C2C12 skeletal muscle cells

We investigated the stimulatory effects of GGEx on AMPKα-dependent PPARα activation using *in vitro* C2C12 skeletal muscle cells. In this system, C2C12 skeletal myoblasts were differentiated into post-mitotic multinucleated myotubes that acquired a muscle-specific, contractile phenotype. In parallel with the *in vivo* data, treatment of C2C12 cells with GGEx increased mRNA expression of AMPKα1 and AMPKα2 (Fig. 4). mRNA levels of PPARα and the PPARα target genes MCAD, VLCAD, and UCP3 were also elevated by GGEx (Fig. 5). The PPARα activator fenofibrate also increased AMPKα1, PPARα, MCAD, and VLCAD mRNA levels. These results suggest that GGEx may activate skeletal muscle AMPKα and AMPKα-dependent PPARα

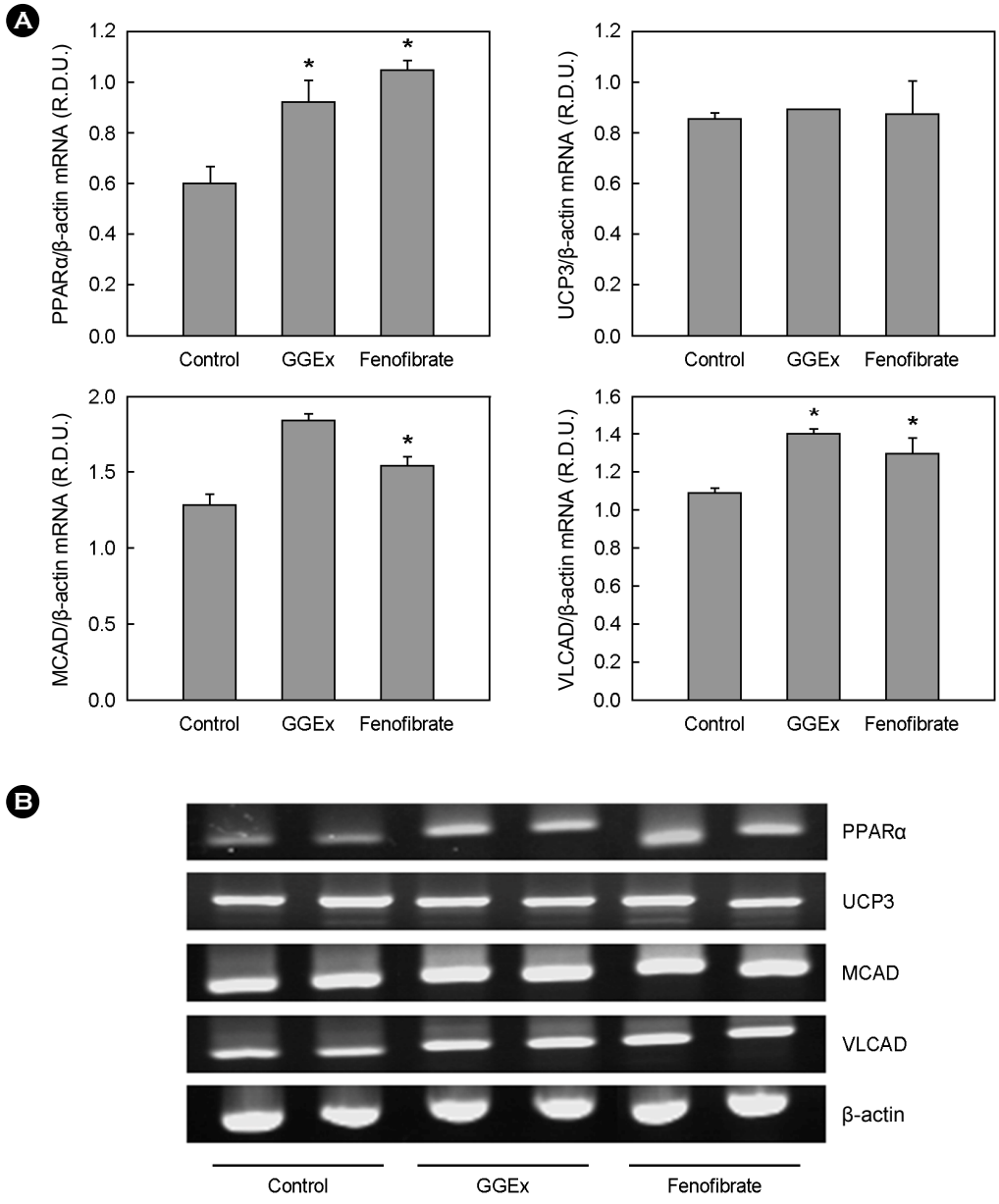

Fig. 5. GGEx increases the mRNA expression levels of PPARα and its target genes in C2C12 skeletal muscle cells. (A) Non-differentiated C2C12 myoblasts were differentiated into myotubes as described in the Materials and Methods. C2C12 cells were treated with DMSO (Control), 1 μg/ml GGEx, and 10 μM fenofibrate. RNA was extracted from C2C12 cells, and mRNA levels of PPARα, PPARα target genes, and β-actin were measured as described in the Materials and Methods. All values are expressed as the mean ± SD of relative density units (R.D.U.) using β-actin as a reference. *$P<0.05$ compared with control group. (B) Representative PCR bands from one of three independent experiments are shown.

similar to fenofibrate.

In conclusion, these studies demonstrate that GGEx treatment not only stimulates AMPKα and ACC phosphorylation, but also increases the expression of AMPK, PPARα, and PPARα target genes involved in fatty acid β-oxidation in skeletal muscle of obese mice and C2C12 skeletal muscle cells. These changes may lead to reduced fat deposition of skeletal muscle and improved obesity in obese animals. Further investigation will be necessary to identify bioactive compounds from GGEx to develop natural therapeutic agents or dietary supplements to treat obesity-related metabolic disease.

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REFERENCES

Acín A, Rodriguez M, Rique H, Canet E, Boutin JA, Galizzi JP. Cloning and characterization of the 5' flanking region of the human uncoupling protein 3 (UCP3) gene. Biochem Biophys Res Commun. 1999. 258: 278-283.

Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. Diabetes 2006. 55: 2277-2285.

Brun S, Carmona MC, Mampel T, Viñas O, Giralt M, Iglesias R, Villarroya F. Activators of peroxisome proliferator-activated receptor-alpha induce the expression of the uncoupling protein-3 gene in skeletal muscle: a potential mechanism for the lipid intake-dependent activation of uncoupling protein-3 gene expression at birth. Diabetes 1999. 48: 1217-1222.

Carling D. The AMP-activated protein kinase cascade--a unifying system for energy control. Trends Biochem Sci. 2004. 29: 18 -24.

Collier CA, Bruce CR, Smith AC, Lopaschuk G, Dyck DJ. Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacylglycerol storage in rodent skeletal muscle. Am J Physiol Regul Integr Comp Physiol. 2006. 291: E182-E189.

Eun JS, Jeon YK, Lee KA, Leem JY, Kwon J, Jang IS, Song JM. Effect of Bopaewon-tang on Immune Reaction. J Orient Physiol Pathol. 2006. 20: 125-130

Hardie DG. AMPK: a key regulator of energy balance in the single cell and the whole organism. Int J Obes (Lond). 2008. 32: S7 -S12.

Hardie DG, Carling D, Carlson M. The AMP-activated/SNF1 protein kinase subfamily: Metabolic sensors of the eukaryotic cell? Annu Rev Biochem. 1998. 67: 821-855.

Kemp BE, Mitchelhill KI, Stapleton D, Michell BJ, Chen ZP, Witters LA. Dealing with energy demand: The AMP-activated protein kinase. Trends Biochem Sci. 1999. 24: 22-25.

Jeong S, Chae K, Jung YS, Rho YH, Lee J, Ha J, Yoon KH, Kim GC, Oh KS, Shin SS, Yoon M. The Korean traditional medicine Gyeongshingangjeehwan inhibits obesity through the regulation of leptin and PPARalpha action in OLETF rats. J Ethnopharmacol. 2008. 119: 245-251.

Kim J. Clinical Prescriptions of Sasang Constitutional Medicine. Daeseong Publishing: Seoul. 1997.

Lee JM. Longevity and Life Preservation in Korean Oriental Medicine. Haengnim Publishing: Seoul. 1993.

Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim MS, Oh GT, Yoon M, Lee KU, Park JY. AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1. Biochem Biophys Res Commun. 2006. 340: 291-295.

Lee Y, Yu X, Gonzales F, Mangelsdorf DJ, Wang MY, Richardson C, Witters LA, Unger RH. PPAR alpha is necessary for the lipopenic action of hyperleptinemia on white adipose and liver tissue. Proc Natl Acad Sci U S A. 2002. 99: 11848-11853.

Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest. 2006. 116: 1776-1783.

Park YS, Yoon Y, Ahn HS. Platycodon grandiflorum extract represses up-regulated adipocyte fatty acid binding protein triggered by a high fat feeding in obese rats. World J Gastroenterol. 2007. 13: 3493-3499.

Ruderman N, Prentki M. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. Nat Rev Drug Discov. 2004. 3: 340-351.

Shin SS, Jung YS, Yoon KH, Choi S, Hong Y, Park D, Lee H, Seo BI, Lee HY, Yoon M. The Korean traditional medicine gyeongshingangjeehwan inhibits adipocyte hypertrophy and visceral adipose tissue accumulation by activating PPARalpha actions in rat white adipose tissues. J Ethnopharmacol. 2010. 127: 47-54.

Steinberg GR, Michell BJ, van Denderen BJ, Watt MJ, Carey AL, Fam BC, Andrikopoulos S, Proietto J, Görgün CZ, Carling D, Hotamisligil GS, Febbraio MA, Kay TW, Kemp BE. Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. Cell Metab. 2006. 4: 465-474.

Suzuki A, Okamoto S, Lee S, Saito K, Shiuchi T, Minokoshi Y. Leptin stimulates fatty acid oxidation and peroxisome proliferator-activated receptor alpha gene expression in mouse C2C12 myoblasts by changing the subcellular localization of the alpha2 form of AMP-activated protein kinase. Mol Cell Biol. 2007. 27: 4317-4327.

Yao HT, Chang YW, Chen CT, Chiang MT, Chang L, Yeh TK.

Shengmai San reduces hepatic lipids and lipid peroxidation in rats fed on a high-cholesterol diet. J Ethnopharmacol 2008. 116: 49-57.

Yoon M. The role of PPARα in lipid metabolism and obesity: Focusing on the effects of estrogen on PPARα actions. Pharmacol Res. 2009. 60: 151-159.

Yoon M. PPARα in Obesity: Sex Difference and Estrogen Involvement. PPAR Res. 2020. 2010: 584296.

Zheng CD, Duan YQ, Gao JM, Ruan ZG. Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. J Chin Med Assoc. 2010. 73: 319-324.