Identification of Functional Food Factors as β2-Adrenergic Receptor Agonists and Their Potential Roles in Skeletal Muscle

Miho CHIKAZAWA and Ryuichiro SATO

1 Food Biochemistry Laboratory, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113–8657, Japan
2 Nutri-Life Science Laboratory, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo 113–8657, Japan
3 AMED-CREST, Japan Agency for Medical Research and Development, Chiyoda-ku, Tokyo 100–0004, Japan

(Received July 20, 2017)

Summary Maintaining skeletal muscle functions by controlling muscle metabolism is of utmost importance. β2-Adrenergic receptor (β2-AR), which is expressed in skeletal muscle, is a member of the G-protein-coupled receptor family that plays a critical role in the maintenance of muscle mass. In the present study, using luciferase reporter assays in β2-AR-expressing HEK293 cells, we discovered several food factors that exhibited agonistic activity at mouse or human β2-AR. Osthole, gramine, and hordenine were identified as both mouse and human β2-AR agonists, whereas berberine was identified as a mouse β2-AR agonist only. Additionally, intramuscular injection of gramine or hordenine in mice facilitated gene expression of several cAMP response element binding protein targets, which is thought to result in increased skeletal muscle protein synthesis. This study provides evidence that several food factors might exert potent health effects on skeletal muscle by enhancing cAMP signaling through the activation of β2-AR.

Key Words skeletal muscle, beta 2-adrenergic receptor, luciferase assay, agonist, cAMP

Skeletal muscle mass is controlled by balancing protein synthesis and degradation. Decreased protein synthesis and/or increased protein degradation for a prolonged period of time, which may be caused by inactivity, inadequate diet, reduced hormone levels or disease, contribute to a decrease in skeletal muscle mass and the onset of atrophy.

Skeletal muscle hypertrophy, characterized by increased protein synthesis rates and myofiber size in adults, and atrophy are regulated through multiple signaling pathways. G-protein coupled receptor (GPCR) is one of the key regulators of skeletal muscle hypertrophy (1). GPCR belongs to a large family of membrane proteins that binds various endogenous ligands and transmits intracellular signaling (2). Although the function of GPCR in skeletal muscle is not adequately understood, it has been reported that some GPCRs play an important role in the regulation of skeletal muscle mass (3). β2-Adrenergic receptor (β2-AR) is one of the well-studied members of the GPCR family and is the major adrenergic receptor isoform expressed in skeletal muscle. Activation of β2-AR by catecholamines, such as norepinephrine and epinephrine, induces the dissociation of guanine nucleotide binding proteins (G protein) into the G protein alpha subunit (Gαα) and the heterodimer of beta and gamma subunits (Gβγ) from the intact receptor. Released Gαα is then activated, and it stimulates adenylyl cyclase (AC) activation and adenosine 3’,5’-cyclic monophosphate (cAMP) production (4, 5). The increase in cAMP concentration promotes muscle fiber type alterations and myoblast fusion. Phosphorylation of cAMP response element binding protein (CREB) by cAMP elicits CREB-mediated gene transcription. Some CREB-responsive genes regulate skeletal muscle hypertrophy. For example, salt-inducible kinase 1 is induced by CREB phosphorylation and, in turn, increases the expression of MEF2, which regulates skeletal muscle differentiation and repair (6). Many previous studies have demonstrated that chronic treatment with β2-AR agonists induced hypertrophy and skeletal muscle repair in vivo (3). Similarly, β2-AR selective agonist clenbuterol administration ameliorated skeletal muscle atrophy induced by hindlimb unweighting (7), denervation (8), and dexamethasone treatment (9) through the activation of protein synthesis and inhibition of protein degradation by controlling lysosomal and proteasomal activity in animal models. These results indicate that β2-AR activation by its agonists may be effective for increasing muscle mass and preventing atrophy.

Currently, the beneficial effect of food factors on skeletal muscle is poorly understood. Osthole, a natural coumarin, is a constituent of the mature fruit of Fructus Cnidii (Cnidium monnieri) and other medical plants. Osthole has been reported to exert various effects, such as neuroprotective, osteogenic, and anti-inflammatory (10). Gramine is a natural indole alkaloid that exists in higher plants, such as giant reed (Arundo donax Linn.)

*To whom correspondence should be addressed.
E-mail: aroysato@mail.ecc.u-tokyo.ac.jp
It has been demonstrated that gramine derivatives exhibited anti-viral activity against enterovirus 71 (12) and neuroprotective activity by inhibiting the entry of Cu^{2+} into neuroblastoma cells (13). Hordenine is found in bitter orange (Citrus aurantium) (14) and germinated barley (Hordeum vulgare L.). Although various bioactivities of these food factors has been previously reported, their effect for muscle hypertrophy has not been evaluated to date.

Previously it has been reported that ursolic acid and tomatidine, food factors derived from apples and tomato plants, reduce skeletal muscle atrophy by preventing the functioning of activating transcription factor-4 (ATF-4) (15). In addition, it has recently been reported that citrus limonoid obacunone acted as an agonist of TGR5, a member of the GPCR family that is expressed in skeletal muscle (16), and diet supplementation by obacunone increased skeletal muscle mass in mice (17). Considering these reports, identification of novel food-derived GPCR agonists seems to be promising for designing regulatory tools for muscle mass. We, therefore, aimed to identify novel food factors that exhibit β2-AR agonist activity and to reveal the physiological effects of such factors on the stimulation of β2-AR signaling.

MATERIALS AND METHODS

Reagents. Osthole (purity >98.0%), gramine (purity >98.0%), hordenine (purity >98.0%), and berberine (purity >98.0%) were purchased from TCI Chemicals. Clenbuterol hydrochloride (purity ≥98.0%) was purchased from LKT Laboratories. ICI 118551 hydrochloride (purity ≥98.0%), hordenine (purity ≥98.0%), and berberine (purity ≥98.0%) were purchased from Abcam.

Plasmid constructs. pCRE-luc reporter plasmid containing four copies of cAMP response element (CRE) consensus sites was obtained from Agilent Technologies. p3×FLAG-β2-AR, an expression plasmid for β2-AR signaling. We, therefore, aimed to identify novel food factors that exhibit β2-AR agonist activity and to reveal the physiological effects of such factors on the stimulation of β2-AR signaling.

**MATERIALS AND METHODS**

**Reagents.** Osthole (purity >98.0%), gramine (purity >98.0%), hordenine (purity >98.0%), and berberine (purity >98.0%) were purchased from TCI Chemicals. Clenbuterol hydrochloride (purity ≥98.0%) was purchased from LKT Laboratories. ICI 118551 hydrochloride (purity >99%) was purchased from Abcam.

**Plasmid constructs.** pCRE-luc reporter plasmid containing four copies of cAMP response element (CRE) consensus sites was obtained from Agilent Technologies. p3×FLAG-β2-AR, an expression plasmid for β2-AR signaling.

**Cell culture.** HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 10% fetal bovine serum (FBS). The cells were incubated at 37°C under 5% CO2 atmosphere.

**Luciferase assays.** HEK293 cells were plated in 12-well plates at a density of 1.0×10⁴ cells/well, cultured for 20 h, and transfected by the calcium phosphate method with 100 ng of pCRE-luc, 10 ng of empty vector (p3×FLAG-CMV-7.1) or p3×FLAG-β2-AR, and 100 ng pCMV-β-Gal (an expression plasmid for β-galactosidase) for the determination of β2-AR agonist activity. Four hours after transfection the medium was replaced with DMEM supplemented with 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 5% dextran-charcoal-stripped FBS. After 24 h the indicated test compounds or control vehicle, dimethyl sulfoxide (DMSO), was added to the medium. All test compounds were dissolved in DMSO. The final DMSO concentration of the cultured medium was 0.1%. After incubation, luciferase and β-galactosidase activities were measured as described previously (18). Normalized luciferase values were determined by dividing luciferase activity by β-galactosidase activity.

**Mice.** All experiments were performed according to the guidelines of the Animal Usage Committee of the Faculty of Agriculture, University of Tokyo (Tokyo, Japan) and were approved by the committee (Permission No. P11-590). Male 7-wk-old C57BL/6j mice obtained from CLEA Japan, Inc. were housed in the animal care facility under controlled temperature and humidity conditions with a 12 h light/dark cycle. For intramuscular injections mice were anesthetized with isoflurane. Quadriicepses in both legs were injected directly with 2 mg/kg of body weight of clenbuterol or 20 mg/kg of body weight of osthole, gramine, and hordenine diluted in 10% DMSO in sterile water (100 μL/leg). Control mice were intramuscularly injected with 10% DMSO in sterile water (100 μL/leg). Six hours after the administration of food factors, mice were killed under isoflurane anesthesia, and quadriiceps muscles were rapidly excised.

**Real-time PCR.** Total RNA was extracted from mouse skeletal muscle using an RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. Four micrograms of total RNA was used to synthesize and amplify cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR analysis (stained with SYBR green) was performed on an Applied Biosystems 7000 sequence detection system. Expression was normalized to the 18S control. Forward and reverse primer sequences are listed in Table 1.

**Statistical analysis.** All data are presented as mean ± SD. Statistical significance was evaluated by using the unpaired two-tailed Student’s t-test or, when appropriate, Dunnett’s or Tukey’s HSD test.

**RESULTS**

**Food factors as mouse β2-AR agonists**

To evaluate β2-AR agonistic activity of food factors we executed reporter gene assay using HEK293 cells transfected with a mouse β2-AR expression plasmid together
with a CRE-luc reporter plasmid. This assay system enabled us to evaluate the agonistic activity of each factor, which was indicated by the increased luciferase activity. Cells were treated with one of our 357 purified in-house food factors at 100 μM for 24 h (Supplemental Online Materials, Table S1). We selected several food factors which fulfill the following conditions: (1) those elevating luciferase activity by more than 4-fold compared with DMSO-treated cells in 24 h treatment; (2) those significantly increasing luciferase activity in β2-AR-expressing cells compared with empty vector-transfected cells; (3) those whose agonist activity against β2-AR has not been reported previously. Repeated luciferase assays revealed that osthole, gramine, hordenine, and berberine were potential β2-AR agonists (Fig. 1A).

Luciferase activities increased after 3 h of incubation in the presence of one of these factors or clenbuterol, only when β2-AR was expressed in HEK293 cells (Fig. 1B). These food factors showed much weaker agonistic activities compared with that of a synthesized agonist, clenbuterol. These results suggest that these agonists triggered an increase in intracellular cAMP levels, thereby activating endogenous CREB and inducing luciferase gene expression. The short culture period of 3 h allowed us to neglect cellular toxicity or side effects caused by these factors. All of the four food factors increased luciferase activities in a concentration-dependent manner in β2-AR-expressing HEK293 cells (Fig. 1C). However, berberine increased luciferase activity even in the absence of the receptor. To further verify β2-AR agonist activity of the food factors we next examined the inhibitory effect of a selective β2-AR antagonist, ICI-118551, on food factor-induced CRE-luc activity. ICI-118551 is known to act as a highly selective inverse β2-AR agonist (19). HEK293 cells expressing β2-AR were preincubated with ICI-118551 for 30 min prior to the addition of food factors. Increased luciferase activity by each factor was almost completely abolished by this pre-incubation.

Fig. 1. Mouse β2-AR activation by its agonists. (A) Structures of clenbuterol, osthole, gramine, hordenine and berberine. (B) Food factors and clenbuterol induce β2-AR activity. Twenty-four hours after transfection, cells were treated with 100 μM of indicated compounds for another 3 h. Relative luciferase activity in HEK293 cells was measured by the luciferase assay. The data are shown as the mean±SD of triplicate cultures. Different letters above bars indicate a significant difference (p<0.01) according to Tukey’s HSD test. Data are representative of three individual experiments.

Fig. 2. Activation of human β2-AR by food-derived agonists. Twenty-four hours after transfection, cells were preincubated for 30 min with the antagonist ICI-118551 (1 μM) and subsequently treated with 100 μM of indicated compounds for another 3 h. Relative luciferase activity in HEK293 cells was measured by the luciferase assay. The data are shown as the mean±SD of triplicate cultures. Different letters above bars indicate a significant difference (p<0.01) according to Tukey’s HSD test. Data are representative of three individual experiments.

Statistically significant differences compared to (B) control vector or (D) DMSO treated group are indicated by asterisks (*p<0.05; **p<0.01; ***p<0.001). In (C) different letters above bars indicate a significant difference (p<0.01) according to Tukey’s HSD test. Data are representative of three individual experiments.
Taken together, these results confirm these food factors as the mouse $\beta_2$-AR agonists. Osthole, gramine, and hordenine act as human $\beta_2$-AR agonists.

$\beta_2$-AR expression in skeletal muscle has previously been confirmed in several species. In fact, mouse and human $\beta_2$-ARs share 88% sequence homology (20). To make sure that these four food factors also act as human $\beta_2$-AR agonists, luciferase assays were performed using a human $\beta_2$-AR expression plasmid. Similar to mice, luciferase activities were increased by osthole, gramine, and hordenine in HEK293 cells expressing human $\beta_2$-AR (Fig. 2). In addition, increased luciferase activity was cancelled by ICI-118551 pre-treatment in the same manner as with mouse $\beta_2$-AR. In contrast to mice, no increase in luciferase activity was observed when cells were treated with berberine.

**DISCUSSION**

Using luciferase reporter assays we identified osthole, gramine, and hordenine as mouse/human $\beta_2$-AR agonists and berberine as a mouse agonist. Berberine increased CRE-driven luciferase expression level not only in cells expressing mouse $\beta_2$-AR, but also in cells expressing empty vector at a slightly lower level. Other mechanisms may exist for the activation of CREB by berberine aside from the activation of $\beta_2$-AR signaling. Moreover, intramuscular injection experiments revealed that gramine and hordenine increased CREB target gene expression by acting as $\beta_2$-AR agonists. We also performed oral administration of several food factors in order to determine their $\beta_2$-AR agonistic activities in skeletal muscle. However, no significant increases in CREB target gene expression were observed, except for very slight increasing tendencies (data not shown). If these food factors are to be utilized as dietary supplements, a mixture containing several of them might have a greater potential for maintenance of skeletal muscle.
Effects of Food-Derived β2-AR Agonists in Skeletal Muscle

Previously it has been demonstrated that osthole had an inhibitory effect on cAMP phosphodiesterase (PDE), thereby increasing intracellular cAMP levels (10). Given that osthole enhanced CRE-driven CREB-induced luciferase expression, this food factor may increase the intracellular cAMP levels through β2-AR signaling and PDE inhibition pathways. It is suggested that the stimulation of β2-AR induces phosphatidylinositol 3-kinase (PI3K) activation via Gαs protein, which causes skeletal muscle hypertrophy (25, 26). A previous study has demonstrated that osthole activated the PI3K/Akt pathway in L6 myotubes, although this effect was not determined in C2C12 myotubes (27). Considering these reports, there is a possibility that osthole activates the PI3K/Akt signaling pathway by functioning as a β2-AR agonist in skeletal muscle.

In a previous study, administration of a high concentration of a gramine analog, 2,5,6-tribromo-1-methylgramine from a bryozoon Zoototryon pellucidum, was reported to increase the cAMP levels in the rat aorta (28). Our study indicates that gramine analogs may increase the cAMP level through their β2-AR agonistic activities.

Hordenine has been shown to inhibit cAMP production in human epidermal melanocytes (29). Although it remains unknown whether hordenine activates β2-AR in melanocytes, this result conflicts with our current findings in skeletal muscle. It is possible that hordenine regulates cAMP production not only through β2-AR but also through other pathways.

Our study demonstrated that gramine and hordenine facilitated the expression of the CREB target genes, Nrf4a1 and PGC-1α4, when intramuscularly administered to mice. It has been reported that Nrf4a1 and PGC-1α4 expression contributes to the improvement of skeletal muscle function. Transgenic expression of Nrf4a1 in mice skeletal muscle exhibits an increase in myofiber size, and deletion of global or muscle Nrf4a1 decreases the increase in skeletal muscle mass and myofiber size (30). PGC-1α4 is induced by resistance training and clenbuterol treatment, and is also essential for clenbuterol-induced hypertrophy (31). It is then possible that administration of gramine and hordenine, which increases the expression of these genes, will provide positive effect for muscle mass maintenance and contribute to the improvement of skeletal muscle function. On the other hand, the expression of Nrf4a3 was not increased by food factors, contrary to the results for Nrf4a1. Although the reason for this is not clearly understood, it is possible that the effect of β2-AR is cancelled out by the function of food factors against other molecules. In addition, the effect of food factors for Nrf4a3 expression may be detected with multiple and longer treatment for mice since luciferase activity of food factors is sustained for 24 h (data not shown).

Sarcopenia, an aging-related skeletal muscle atrophy, is defined as a loss in skeletal muscle mass and strength by older persons (32, 33). Progression of sarcopenia reduces the functional capacity of skeletal muscle and increases the risk of chronic metabolic disorders (34). It has been reported that administration of clenbuterol facilitated slow-to-fast alteration of myosin heavy chain, increased skeletal muscle mass, and prevented its functional loss caused by skeletal muscle atrophy (35, 36). Our study demonstrates that intramuscular administration of gramine and hordenine increased the expression of hypertrophic genes in mice quadriceps, which is suggested to have been mediated, at least in part, by the activation of β2-AR. These results suggest the potential of the food factors to be utilized as functional dietary supplements for the maintenance of healthy skeletal muscle.

Acknowledgments
This study was supported by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 15H05781 to R.S., No. 17K15267 to M.C.), the Cross-ministerial Strategic Innovation Promotion Program (No. 14533567 to R.S.), and the Japanese Agency for Medical Research and Development (AMED-CREST, No. 16gm0910008h0001 to R.S.). The funders had no role in study design, data collection or analysis, decision to publish, or preparation of the manuscript. The authors would like to thank Enago (www.enago.jp) for the English language review.

Supporting Information
Supplemental Online Material is available on J-STAGE.

REFERENCES
1) Egerman MA, Glass DJ. 2014. Signaling pathways controlling skeletal muscle mass. Crit Rev Biochem Mol Biol 49: 59–68.
2) Kristiansen K. 2004. Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. Pharmacol Ther 103: 21–80.
3) Jean-Baptiste G, Yang Z, Khoury C, Gaudio S, Greenwood MT. 2005. Peptide and non-peptide G-protein coupled receptors (GPCRs) in skeletal muscle. Peptides 26: 1528–1536.
4) Kitaura T. 2013. How β2-adrenergic agonists induce skeletal muscle hypertrophy? J Phys Fitness Sports Med 2: 423–428.
5) Berdeaux R, Stewart R. 2012. cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. Am J Physiol Endocrinol Metab 303: E1–E17.
6) Berdeaux R, Goebel N, Banaszynski L, Takemori H, Wandless T, Shelton GD, Montminy M. 2007. SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. Nat Med 13: 597–603.
7) Yilmaz H, Todd SL, Borst SE, Park S. 2005. Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin-proteasome pathway. J Appl Physiol 99: 71–80.
8) Goncalves DAP, Silveira WA, Lira EC, Graça FA, Paula-Gomes S, Zanon NM. Kettelhut IC, Navegantes LCC. 2012. Clenbuterol suppresses proteasomal and lysosomal proteolysis and atrophy-related genes in degenerated rat soleus muscles independently of Akt. Am J Physiol Endocrinol Metab 302: E123–E133.
9) Pellegrino MA, D’Antona G, Bortolotto S, Boschi F, Pastoris O, Bottinelli R, Polla B, Reggiani C. 2004. Clenbuterol antagonizes glucocorticoid-induced atrophy and fibre type transformation in mice. Exp Physiol 89: 89–100.

10) Zhang Z-R, Leung WN, Cheung HY, Chan CW. 2015. Exosome: A review on its bioactivities, pharmacological properties, and potential as alternative medicine. Evid Based Complement Alternat Med 2015: 919616.

11) Hong Y, Hu H-Y, Xie X, Sakoda A, Sagehashi M, Li F-M. 2009. Glutamate-induced growth inhibition, oxidative damage and antioxidant responses in freshwater cyanobacterium Microcystis aeruginosa. Aquatic Toxicol 91: 262–269.

12) Wei Y, Shi L, Wang K, Liu M, Yang Q, Yang Z. Ke S. 2014. Discovery of glutamate derivatives that inhibit the early stage of EV71 replication in vitro. Molecules 19: 8949–8964.

13) Lajarín-Cuesta R, Nanclores C, Arranz-Tagarro J-A, González-Lafuente L, Arribas RL, Dyle MC, Bullard SA, Dierdorff JM, Nelson BC, Putzbach K, Sharpless KE, Sander LC. 2007. Expression of CAST channels and Ser/Thr phosphatases: A new dual strategy for the treatment of neurodegenerative diseases. J Med Chem 59: 6256–6280.

14) Nelson BC, Putzbach K, Sharpless KE, Sander LC. 2007. Mass spectrometric determination of the predominant adrenergic protoalkaloids in bitter orange (Citrus aurantium). J Agric Food Chem 55: 9769–9775.

15) Ebert SM, Dyle MC, Bullard SA, Dierdorff JM, Murry DJ, Fox DK, Bongers KS, Adams CM. 2015. Identification and small molecule inhibition of an activating transcription factor 4 (ATF4)-dependent pathway to age-related skeletal muscle weakness and atrophy. J Biol Chem 290: 25497–25511.

16) Ono E, Inoue J, Hashidume T, Shimizu M, Sato R. 2011. Anti-Obesity and anti-hyperglycemic effects of the dietary citrus limonoid nomilin in mice fed a high-fat diet. Biochem Biophys Res Commun 410: 677–681.

17) Horiba T, Katsukawa M, Mita M, Sato R. 2015. Dietary obacunone supplementation stimulates muscle hypertrophy, and suppresses hyperglycemia and obesity through the TGR5 and PPARγ pathway. Biochem Biophys Res Commun 463: 846–852.

18) Sato R, Miyamoto W, Inoue J, Terada T, Imanaka T, Maeda M. 1999. Sterol regulatory element-binding protein negatively regulates microsomal triglyceride transfer protein gene transcription. J Biol Chem 274: 24714–24720.

19) Hopkinson HE, Latif ML, Hill SJ. 2000. Non-competitive antagonism of β2-agonist-mediated cyclic AMP accumulation by ICI 118551 in BC3H1 cells endogenously expressing constitutively active β2-adrenoceptors. Br J Pharmacol 131: 124–130.

20) Čikšo Š, Czikková S, Chrenek P, Makarevich AV, Burkuš J, Janištová Ž, Fabian D, Koppel J. 2014. Expression of adrenergic receptors in bovine and rabbit oocytes and preimplantation embryos. Reprod Domest Anim 49: 92–100.

21) Bruno NE, Kelly KA, Hawkins R, Bramah-Lawani M, Amelio AL, Nwachukwu JC, Nwachukwu J, Conkright MD. 2014. Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. EMBO J 33: 1027–1034.

22) Kawasaki K, Kawanaka K. 2011. The effects of β-adrenergic stimulation and exercise on NR4A3 protein expression in rat skeletal muscle. J Physiol Sci 61: 1–11.

23) Pearl MA, Ryall JG, Maxwell MA, Ohkura N, Lynch GS, Muscat GEO. 2006. The orphan nuclear receptor, NOR-1, is a target of β-adrenergic signaling in skeletal muscle. Endocrinology 147: 5217–5227.

24) Martinez-Redondo V, Jannig PR, Correia JC, Ferrreira DMS, Cervinka I, Lindvall JM, Simha I, Izadi M, Pettersson-Klein AT, Agudelo L, Gimenez-Cassina A, Brum PC, Dahlman-Wright K, Rus JL. 2016. Peroxisome proliferator-activated receptor gamma coactivator-1alpha isoforms selectively regulate multiple splicing events on target genes. J Biol Chem 291: 15169–15184.

25) Kline WO, Panaro FJ, Yang H, Bodine SC. 2007. Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. J Appl Physiol 102: 740–747.

26) Zhang W, Nono N, Deng M, Mao Q, Shaw SK, Tseng Y-T. 2011. β-Adrenergic Receptor-PI3K signaling crosstalk in mouse heart: Elucidation of immediate downstream signaling cascades. PLoS One 6: e26581.

27) Lee W-H, Lin R-J, Lin S-Y, Chen Y-C, Lin H-M, Liang Y-C. 2011. Osthole enhances glucose uptake through activation of AMP-activated protein kinase in skeletal muscle cells. J Agric Food Chem 59: 12874–12881.

28) Iwata S, Saito S, Kon-ya K, Shizuri Y, Ohiwata Y. 2001. Novel marine-derived halogen-containing ganine analogues induce vasorelaxation in isolated rat aorta. Eur J Pharmacol 432: 63–70.

29) Kim S-C, Lee JH, Kim MH, Lee JA, Kim YB, Jung E, Kim Y-S, Lee J, Park D. 2013. Hordenine, a single compound produced during barley germination, inhibits melanogenesis in human melanocytes. Food Chem 141: 174–181.

30) Tontonoz P, Cortez-Toledo O, Wroblewski K, Hong C, Lin L, Carranza R, Conneely O, Metzger D, Chao LC. 2007. The orphan nuclear receptor Nur77 is a determinant of myotubule size and muscle mass in mice. Mol Cell Biol 27: 1125–1138.

31) Ruiz JL, White JP, Ruo RR, Kleiner S, Brannan KT, Harrison BC, Greene NP, Wu J, Estall JL, Irving BA, Diao LC. 2015. The orphan nuclear receptor Nur77 is a determinant of myotubule size and muscle mass in mice. Mol Cell Biol 35: 1125–1138.

32) Cruz-Jentoft AJ, Barbat F, Briskin M, Zhou Y, Fischer FF, Bratt F, Alves M, Oses E. 2010. Sarcopenia: European consensus on definition, epidemiology, and pathophysiology. J Bone Metab 17: 1–11.

33) Thomas DR. 2007. Loss of skeletal muscle mass in aging: Examining the relationship of starvation, sarcopenia and cachexia. Clin Nutr 26: 389–399.

34) Kim TN, Choi KM. 2013. Sarcopenia: Definition, epidemiology, and pathophysiology. J Bone Metab 20: 1–10.

35) Umeki D, Ohnuki Y, Mototani Y, Shiozawa K, Suita K, Fujita T, Okumura S. 2015. Protective effects of clenbuterol against dexamethasone-induced masseter muscle atrophy and myosin heavy chain transition. PLoS One 10: e0128263.

36) Lynch GS, Ryall JG. 2008. Role of β-adrenergic receptor signaling in skeletal muscle: Implications for muscle wasting and disease. Physiol Rev 88: 729–767.