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## Guided Publication Analysis

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## Master 2

### Parcours

## Nutrition, Santé, Innovation – Nutrition et Santé

Année 2020 - 2021

UE Nutrition, muscle, mobilité

### Guided Publication Analysis

A votre disposition , 3 articles.

2 d'entre eux sont des revues de synthèse sur le rôle de PPAR beta/delta et de la voie des TGF-beta quant au contrôle de la masse musculaire :

- Manickam et Wahli, Biochimie 2017
- Sartori et al. Trends Endocrinol Metab 2014

Vous prendrez le soin de les lire préalablement à l'analyse de l'article proposé.

Résumez en 15-20 lignes chacune de ces 2 revues en focalisant sur le rôle de PPAR delta et de la voie des TGFb dans le contrôle de la masse musculaire

Ce travail vous apportera une aide à l'analyse de l'article proposé comme TD.

Dans le 3ème article ci-dessous, vous répondrez aux questions suivantes :

- 1) Donner le contexte de l'étude et le (ou les) objectif(s).
- 2) Décrire et interprétez les figures présentées (Figures 1 à 5) ; donner un titre pour chacune d'elles.
- 3) Donner une conclusion générale à l'article.
- 4) Donner un titre à l'article.

Vous pouvez m'envoyer vos résumés et analyse à [lydie.combaret@inrae.fr](mailto:lydie.combaret@inrae.fr) avant le 29-11-2020.

## 1. Introduction

Skeletal muscle mass and quality are essential factors in maintaining quality of life and can decline due to disease, aging, and inactivity.[1,2] Skeletal muscle loss with aging is associated with chronic disease states, falls, and mortality.[3] Increasing skeletal muscle mass is of interest to both athletes and individuals wishing to maintain a healthy physique. Physical exercise and protein supplementation can prevent skeletal muscle loss and promote skeletal muscle growth, but pharmacological treatments that aid this process have not yet been developed.[4] Recently, several food extracts have been described as helping to improve the quality of skeletal muscle and increase muscle mass. Apple pomace extract enhances the strength and weight of skeletal muscle,[5] while green tea extract has been shown to attenuate skeletal muscle loss and improve skeletal muscle function.[6] L-citrulline improves exercise performance and increases skeletal muscle weight by up-regulating PGC-1 $\alpha$  expression.[7] These results suggest that the consumption of food extracts exerts beneficial effects on skeletal muscle function and morphology. Therefore, this study attempts to identify the food extracts that can improve skeletal muscle function and mass, and investigate these molecular mechanisms.

Mitochondria play a critical role in maintaining skeletal muscle mass.[8] Aging is associated with an increase in reactive oxygen species and mitochondrial stress, which induces mitochondrial dysfunction.[9] When mitochondrial content is released into the cell cytosol due to mitochondrial stress, nucleus damage occurs and eventually results in the death of the entire muscle cell.[9] Exercise enhances mitochondrial biogenesis and may protect against the age-associated apoptosis of muscle cells and muscle wasting.[10,11] Therefore, the restoration of mitochondrial function and biogenesis represents a potential strategy for maintaining skeletal muscle mass. Our previous study showed that apigenin attenuates obesity-induced skeletal muscle atrophy by regulating mitochondrial dysfunction in mice.[12]

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a secreted cytokine that increases skeletal muscle atrophy and inhibits myogenic differentiation.[13,14] Recently, excessive TGF- $\beta$  expression has been associated with skeletal muscle weakness.[15] Myostatin, a member of the TGF- $\beta$  superfamily, has been reported to regulate skeletal muscle mass.[16] The deletion of myostatin induces skeletal muscle outgrowth, whereas the overexpression of myostatin increases skeletal muscle atrophy.[16] Growth differentiation factor 11 (GDF11), a TGF- $\beta$  superfamily member which increases in quantity with age, inhibits skeletal muscle regeneration and induces the loss of skeletal muscle mass and function.[17,18] TGF- $\beta$ , myostatin, and GDF11 regulate identical downstream signaling pathways including Smad2/3 phosphorylation.[15,17] Therefore, the regulation of the TGF- $\beta$  family in skeletal muscle is essential for the prevention of skeletal muscle atrophy and for the maintenance of skeletal muscle function.

*Hydrangea serrata* is a plant belonging to the Hydrangeaceae family and is also referred to as mountain hydrangea or “tea of heaven.” It is primarily distributed in the mountainous areas of Korea and Japan and its leaves contain phylloidalin, a natural sweetener which produces sweetness of approximately 1000-fold higher than sugar.[19] *H. serrata* is usually used as a tea (called Gamro-cha) and can be used in a variety of foods as a sugar substitute for diabetic patients. *H. serrata* is a different species to the *Hydrangea macrophylla* flowering plant from Japan.

## 2. Experimental Section

### 2.1. Materials

Total MHC (myosin heavy chain), MHC1, MHC2A, and MHC2B antibodies were purchased from the Developmental Studies Hybridoma Bank, University of Iowa (Iowa City, IA, USA). Antibodies against PPAR $\delta$ , NOX4, phospho-Smad3, and OXPPOS Rodent WB antibody cocktail were obtained from Abcam (Cambridge, MA, USA). pAkt, Akt, pS6K1, S6K1, p-p38, p38, and voltage-dependent anion channel (VDAC) antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). Anti-MyoD, GAPDH, and  $\beta$ -actin were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

### 2.2. Extraction of *H. serrata*

Dried *H. serrata* leaves were purchased from Hannongwon (Daegu, Republic of Korea). The leaves of *H. serrata* were dipped in 20 times their weight of 70% ethanol and extracted overnight in a shaking incubator at room temperature. The reaction mixture was filtered and the supernatant was lyophilized.

### 2.3. Dosage Information

*H. serrata* extract (0.25%, 0.5%) was added into an American Institute of Nutrition (AIN)-93M diet (Dyets, Bethlehem, PA, USA). 0.25% and 0.5% of *H. serrata* can be converted to 250 mg kg<sup>-1</sup> BW and 500 mg kg<sup>-1</sup> BW which are equivalent to 1.2 g and 2.4 g for a 60 kg human, respectively.<sup>[20]</sup> These concentrations are achievable in humans through supplements. Eleven-week old male C57BL/6 mice were provided ad libitum access to a AIN-93M diet with 0.25% and 0.5% *H. serrata* for 8 weeks. 1 and 2  $\mu$ g mL<sup>-1</sup> of *H. serrata* did not show cytotoxicity in C2C12 myoblasts.

### 2.4. Cell Culture and Differentiation

C2C12 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U mL<sup>-1</sup> penicillin and 100  $\mu$ g mL<sup>-1</sup> streptomycin (Invitrogen, Carlsbad, CA, USA) in a 5% CO<sub>2</sub> incubator at 37 °C. When the cells reached confluence, the medium was changed with differentiation medium (DMEM with 2% horse serum) every day for 2 or 4 days. For the TGF- $\beta$  experiment, C2C12 cells were differentiated for 3 days and stimulated with TGF- $\beta$  (R&D systems, Minneapolis, MN, USA) for 24 h.

### 2.5. Luciferase Assay in C2C12 Cells

C2C12 cells were seeded at  $4 \times 10^4$  cells per well in 24-well plates and transfected with MyoD-luc or pcDNA vector using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. These vectors were kindly provided by Prof. Gyu Un Bae (Sookmyung Women's University) After 1 day of transfection, the cells were treated with *H. serrata* for 24 h. Luciferase activity was measured using a Dual-Light Luciferase Reporter Gene Assay System (Invitrogen).

### 2.6. Animal Experiments

All animal experiments were performed according to procedures approved by the Institutional Animal Care and Use Committee of the Korea Food Research Institute (KFRI-IACUC, KFRI-M-10011). Eleven-week old male C57BL/6 mice were purchased from Orient Bio Inc. (Seongnam, Korea). After 1 week of acclimation, the mice were divided into three groups: control (CTL), *H. serrata* 0.25% (HSL), and *H. serrata* 0.5% (HSH). The mice were housed at a constant temperature (21–25 °C) with a 12 h light and 12 h dark cycle with free access to food and water. The animals were fed on an AIN-93M diet with 0.25% *H. serrata* and 0.5% *H. serrata* for 8 weeks.

### 2.7. Exercise Endurance of Mice on a Treadmill

Exercise endurance was measured on a treadmill as previously described.<sup>[21]</sup> After 2 days of acclimation, the total running distance and maximum speed of the mice on a motorized treadmill were measured (Daejong Instrument Industry, Republic of Korea). The treadmill incline was set at 15%. The number of mice used in exercise test is 12–15 per group. Mice were euthanized and dissected 3 days after the treadmill test.

### 2.8. Hematoxylin and Eosin (H&E) Stain

The mouse tibialis anterior was fixed in 10% formalin, embedded in paraffin, and 5  $\mu$ m sections were prepared, as previously described.<sup>[21]</sup> Tibialis anterior sections were stained with H&E.

Olympus BX51 was used to capture images, and cross-sectional area (CSA) was quantified with IMT iSolution DT 9.2 software.

### **2.9. Western Blot Analysis in C2C12 Cell and Mouse Skeletal Muscle Tissue**

Cell lysates were prepared with RIPA buffer (Thermo Fisher Scientific, Rockford, IL, USA) and sonicated for 15 s at 40 W. Equal amount of protein were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidenedifluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Protein from mouse quadricep muscles (12–15 mice per group) was extracted in RIPA buffer with beads. Equal amounts of protein from each mouse quadricep was combined, separated with SDS-PAGE, and transferred to PVDF membranes. The membranes were blocked with 5% skim milk and incubated with primary antibodies overnight at 4 °C followed by incubation with secondary antibodies conjugated to horseradish peroxidase (Novus Biologicals, Littleton, CO, USA).

### **2.10. Transmission Electronic Microscopy in Mouse Skeletal Muscle Tissues**

Mouse gastrocnemius muscle sections were fixed in 2.5% glutaraldehyde solution. Mitochondrial morphology was observed under a LEO 912AB EF-TEM microscope (Carl Zeiss, Oberkochen, Germany) at the Korean Basic Science Institute in Chuncheon.

### **2.11. Citrate Synthase Activity and Complex I and II Activity in Mouse Skeletal Muscle Tissues**

The mitochondria were prepared using a mitochondria isolation kit (Thermo Fisher Scientific, Rockford, IL, USA) in mouse quadricep muscles (12–15 mice per group). Equal amounts of isolated mitochondria from each mouse quadriceps were divided into three groups and combined. Citrate synthase activity was measured using commercial kits (Sigma-Aldrich, MO, USA). Mitochondrial complex I and II activity were measured with a Complex I and II Enzyme Activity Microplate Assay Kit (Abcam).

### **2.12. Quantitative Real-Time PCR Analysis in Mouse Skeletal Muscle Tissues**

Total RNA was extracted using an RNeasy fibrous tissue mini kit (Qiagen, Germantown, MD, USA), and cDNA was synthesized using ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan). Quantitative real-time PCR was conducted with SYBR Green Master Mix (Toyobo, Osaka, Japan) on a ViiA7 System (Applied Biosystems, Foster City, CA). The primer sequences were as follows: TGF- $\beta$  forward 5' TGA CGT CAC TGG AGT TGT AC 3'; TGF- $\beta$  reverse 5' GGT TCA TGT CAT GGA TGG TG 3'; Myostatin forward 5' ACG CTA CCA CGG AAA CAA TC 3'; Myostatin reverse 5' GGA GTC TTG ACG GGT CTG AG 3'; GDF11 forward

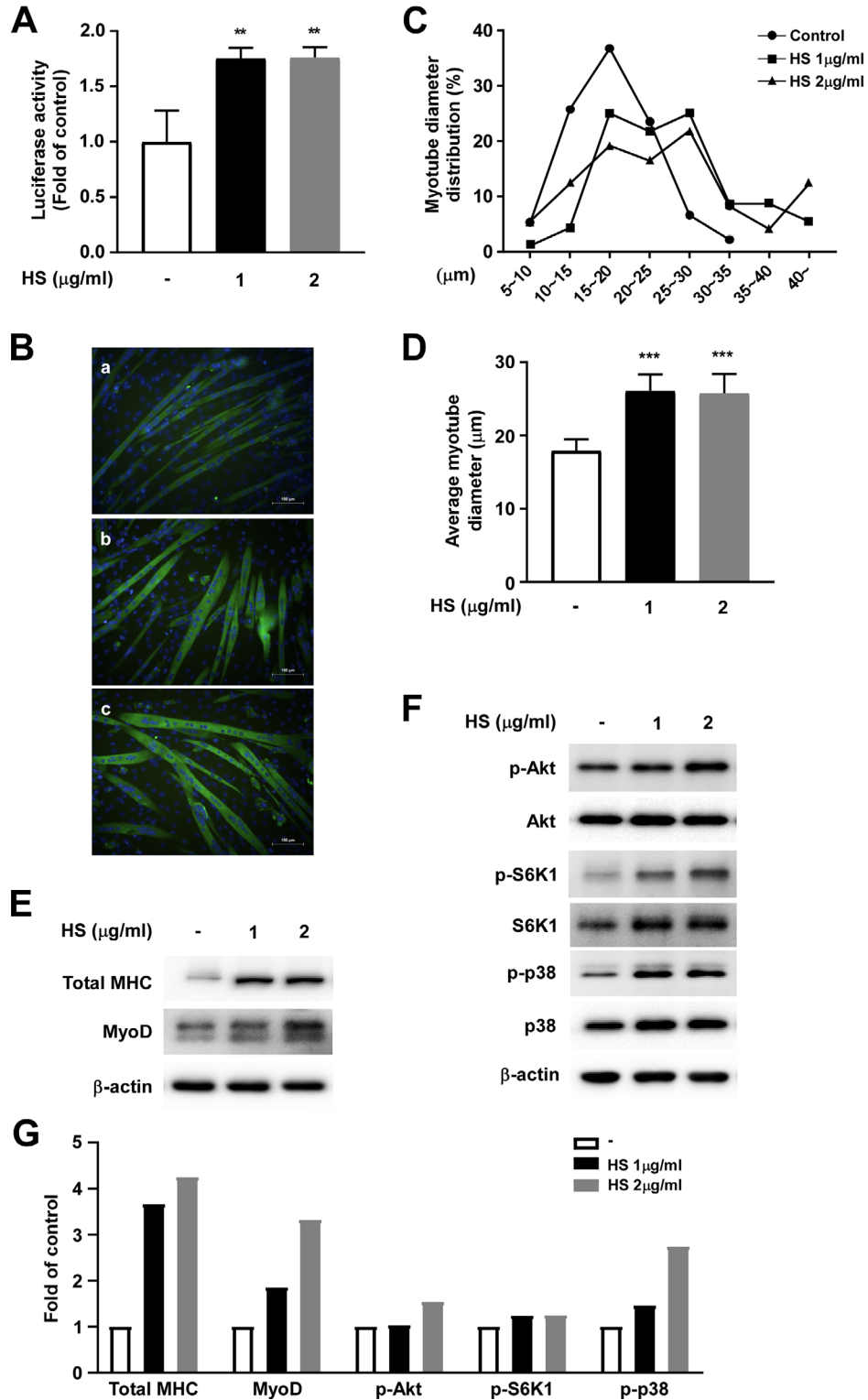
5' AGC ATC AAG TCG CAG ATC CT 3'; GDF11 reverse 5' CTT ATG ACC GTC TCG GTG GT-3'.

### **2.13. Statistical Analysis**

Data were analyzed with GraphPad Prism version 7.0 (GraphPad Software, Inc., La Jolla, CA) and expressed as means  $\pm$  SD (in vitro) or SEM (in vivo). One-way ANOVA was used for statistical analyses followed by Dunnett's multiple comparison test. A probability value of  $p < 0.05$  was used as the criterion for statistical significance.

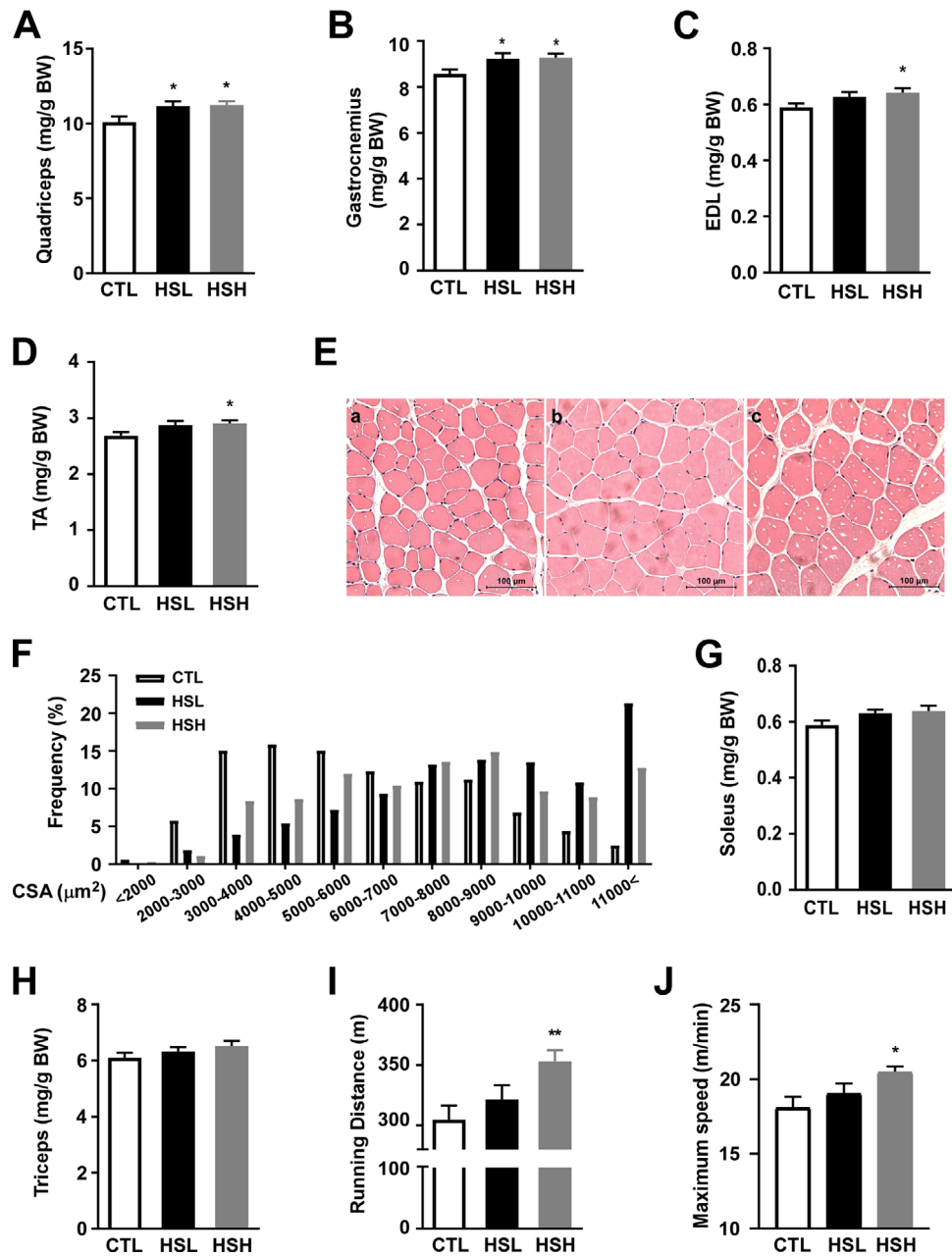
**Figure 1.** Effect of *Hydrangea serrata* (HS) on myogenic differentiation in C2C12 cells.

A) MyoD luciferase activity. C2C12 cells were transfected with MyoD-Luc or pcDNA vector for 48 h. n = 3. B) C2C12 cells were differentiated in the presence or absence of HS for 2 days. Cells were stained using anti-total MHC antibody and nuclei were stained with DAPI. a, control; b, HS 1  $\mu\text{g}/\text{mL}$ -1; c, HS 2  $\mu\text{g}/\text{mL}$ -1. Scalebar= 100  $\mu\text{m}$ . C) Distribution curve of myotube diameter. D) Average myotube diameter. n = 4. E) Protein expression of total MHC and MyoD in C2C12 cells. F) Phosphorylation of Akt, S6K1, and p38. C2C12 cells were differentiated in the presence or absence of HS for 2 days. G) Quantification of protein expression. Protein expression was analyzed by Western blot. Data are means  $\pm$  SD. \*\*p < 0.01, \*\*\*p < 0.001 versus control.



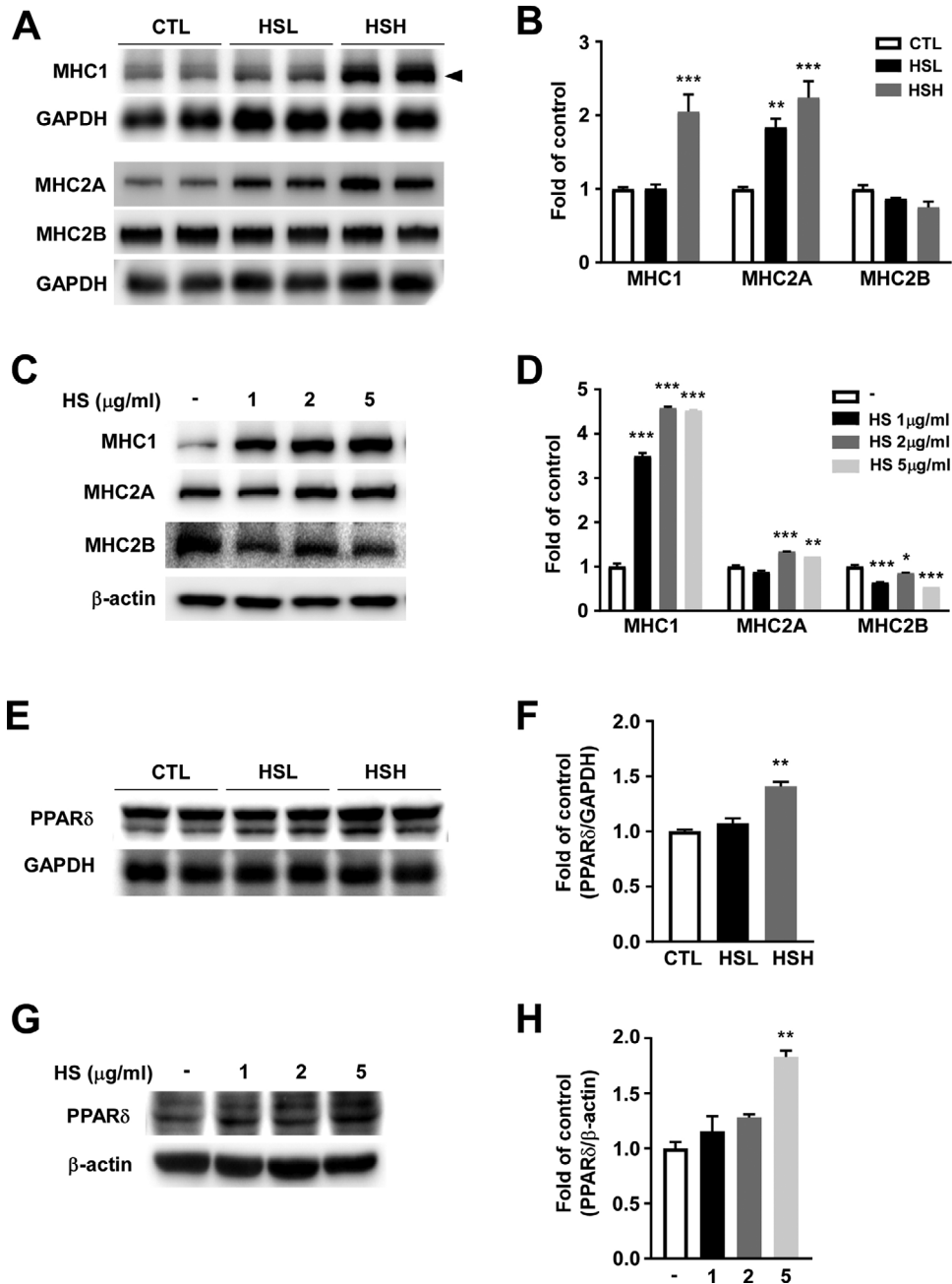
**Figure 2.** Effect of *Hydrangea serrata* (HS) on skeletal muscle hypertrophy and running endurance.

Male C57BL/6 mice were provided ad libitum access to a standard diet (CTL, control) or a standard diet supplemented with 0.25% *H. serrata* (HSL) or 0.5% *H. serrata* (HSH) for 8 weeks. n = 12 to 15. Skeletal muscle weight of A) quadriceps, B) gastrocnemius, C) extensor digitorum longus and D) tibialis anterior. E) Representative images of H&E staining of section of tibialis anterior (a, CTL; b, HSL; c, HSH). F) Frequency distribution of muscle fiber cross-sectional area (CSA). Skeletal muscle weights of G) soleus and H) triceps. I) Running distance and J) maximum speed of mice on an accelerating treadmill. Data are means  $\pm$  SEM. \*p < 0.05, \*\*p < 0.01 versus CTL.



**Figure 3.** Effect of *Hydrangea serrata* (HS) on muscle fiber type and PPAR $\delta$  protein expression.

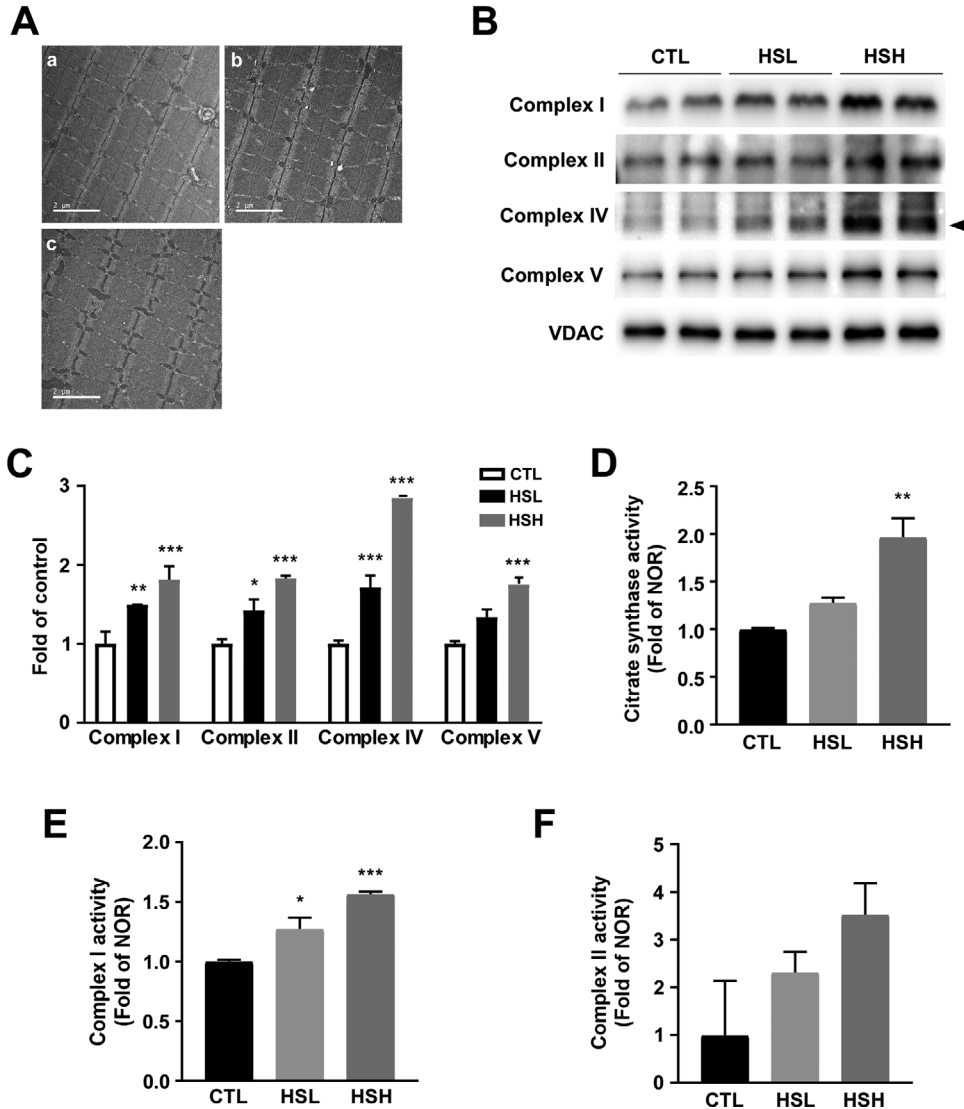
A) MHC expression in mouse quadriceps. Male C57BL/6 mice were provided ad libitum access to a standard diet (CTL, control) or a standard diet supplemented with 0.25% *H. serrata* (HSL) or 0.5% *H. serrata* (HSH) for 8 weeks. Proteins from 12 to 15 mouse quadriceps were combined. B) Quantification of protein expression of MHC in mouse quadriceps. C) MHC expression in C2C12 cells. C2C12 cells were differentiated in the presence or absence of HS for 2 days. D) Quantification of protein expression of MHC in C2C12 cells. E) Protein expression of PPAR $\delta$  in mouse quadriceps. Proteins from 12 to 15 mice quadriceps were combined. F) Quantification of protein expression of PPAR $\delta$  in mice quadriceps. G) PPAR $\delta$  expression in C2C12 cells. C2C12 cells were differentiated in the presence or absence of HS for 2 days. H) Quantification of protein expression of PPAR $\delta$  in C2C12 cells. Protein expression was analyzed by Western blot. Data are means  $\pm$  SD. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus CTL.





**Figure 4.** Effect of *Hydrangea serrata* (HS) on mitochondrial complex expression and activities in mouse skeletal muscle.

A) Transmission electronic microscope (TEM) images of mouse gastrocnemius muscle (original magnification  $\times 10000$ ). Data are representative of three independent experiments. B) Protein expression of the mitochondrial OXPHOS respiratory complex in mouse quadriceps. Isolated mitochondria from 12 to 15 mouse quadriceps were combined. C) Quantification of protein expression of the mitochondrial complex. D,E) Citrate synthase activity and complex I activity in mouse quadriceps. Equal amounts of isolated mitochondria from each mouse quadriceps (12–15 mice) were divided into three groups. Data are means  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus CTL



**Figure 5.** Effect of *Hydrangea serrata* (HS) on the TGF- $\beta$  signaling pathway.

A) TGF- $\beta$ , B) MSTN, and C) GDF11 mRNA expression in mouse quadriceps. n = 12 to 15, data are means  $\pm$  SEM. \*\*\*p < 0.001 versus CTL. mRNA levels were determined by qRT-PCR. D) Protein expressions of phospho-smad3 and NOX4 in mouse quadriceps. Proteins from 12 to 15 mice quadriceps were combined. E) Quantification of protein expression of p-Smad3 and NOX4. F) Representative images of Western blots for MHC, phospho-Smad 3 and Smad 3 in C2C12myotubes and G) Quantification of protein expression of total MHC and p-Smad3. C2C12 cells were differentiated for 3 days and stimulated with TGF- $\beta$  in the presence or absence of HS for 24 h. Protein expression was analyzed by Western blot. Data are means  $\pm$ SD. \*p < 0.05, \*\*p < 0.01 versus CTL, #p < 0.05 versus TGF- $\beta$  treated group

