Kim and Ahn *Applied Biological Chemistry* (2023) 66:24
https://doi.org/10.1186/s13765-023-00783-5

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Filbertone, (2E)-5-methyl-2-hepten-4-one, regulates thermogenesis and lipid metabolism in skeletal muscle of a high-fat diet fed mice

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Abstract

Filbertone, the principal flavor compound of hazelnuts, is known to have preventive effects against hypothalamic inflammation, obesity and adipocity in vitro and in vivo. However, the effect of filbertone in skeletal muscle remains unknown. In the present study, we determined the effect of filbertone in skeletal muscle of mice fed a high-fat diet (HFD). To identify the underlying molecular and cellular processes of filbertone, we performed whole transcriptome sequencing in skeletal muscle. The muscle transcriptome analysis revealed that the upregulated differentially expressed genes (DEGs) in filbertone-fed mice were substantially associated with several pathways including thermogenesis, fatty acid degradation, oxidative phosphorylation, and branched chain amino acids (BCAAs) degradation. Furthermore, the expression level of thermogenic genes such as uncoupling protein 1 (Ucp1; $p < 0.05$), cell death-inducing DNA fragmentation factor alpha-like effector A (Cidea; $p < 0.05$), peroxisome proliferator-activated receptor alpha (Ppara; $p < 0.05$) and lipid droplet-associated protein genes such as Plin3 ($p < 0.05$), Plin4 ($p < 0.05$), and Plin5 ($p < 0.05$) were significantly upregulated in muscle tissue of HFD with filbertone fed mice compared to HFD only fed mice. Filbertone also elevated the protein level of UCP1 ($p < 0.05$) and PPARα ($p < 0.05$). In addition, filbertone reduced the accumulation of intracellular lipids in C_2C_{12} myotubes ($p < 0.05$). On the basis of these results, we suggest that filbertone has a crucial effect in the regulation of muscle lipid metabolism and energy balance.

Keywords Filbertone, Thermogenesis, Skeletal muscle, Lipid metabolism

Introduction

Insulin resistance is strongly associated with the development of impaired glucose homeostasis, dyslipidemia, diabetes mellitus and cardiovascular diseases [1, 2]. Skeletal muscle, composing approximately 40–50% of body mass in mammals, plays a vital role in whole-body energy homeostasis [3–5]. Skeletal muscle is the primary organ responsible for insulin-stimulated glucose elimination [6, 7], and the loss of skeletal muscle mass is intimately associated with the development of several pathologies including obesity, type 2 diabetes mellitus and age-related muscle wasting (sarcopenia) [8, 9]. The appropriate uptake and elimination of glucose and lipid in muscle can aid to impede obesity, diabetes, and metabolic disorders [10]. Physical exercise can improve mitochondrial function, muscle mass, muscle fiber type remodeling, whole-body insulin sensitivity, and whole-body metabolic rate [11, 12]. As an alternative, taking supplements can be beneficial, but these days, natural product-derived supplements have fewer negative effects and are gaining a great deal of interest among athletes [13].

The uncoupling protein (Ucp) family, transmembrane proteins located in the mitochondrial inner-membrane, reduces the proton gradients which is generated by the

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electron transport chain. Among the family, uncoupling protein 1 (Ucp1), found in brown adipose tissue, is associated with heat generation by non-shivering thermogenesis [14–17]. Two different studies have demonstrated that overexpression of Ucp1 in skeletal muscle prevents HFD-induced obesity and fat mass by enhancing energy expenditure [18, 19]. In addition, increased mitochondrial Ucp1 in muscle may have a protective effect to regulate reactive oxygen species production [20]. In contrast, a genetic ablation of Ucp1 is associated with the increase of obesity [21]. Therefore, the activation of Ucp1 expression could be a potential therapeutic approach for strategies aimed at reducing obesity, diabetes and metabolic syndromes.

Filbertone, (2E)-5-methylhept-2-en-4-one, is a major aroma component in fruits of hazel trees (Corylus maxima and Corylus avellana). Hazelnuts are known to comprise various phytochemicals, related to anti-inflammatory, antioxidant, and lipid-lowering properties, such as phytosterols and polyphenols [22]. Filbertone was firstly determined in hazelnut oil [23]. The Flavor and Extract Manufacturers Association (FEMA) considers filbertone to be a safe chemical [24] and it is used in fragrance as a substance that imparts the natural flavor of exotic fruits and citrus [25, 26]. Recently, it has been reported that the beneficial effects of filbertone include anti-inflammation, anti-hyperglycemia and anti-obesity activities in vitro and in vivo [27, 28]. However, the mechanisms associated with the activities of filbertone have not been exhaustively investigated and comprehended.

We have pursued an unbiased approach, in which a total RNA-sequencing (RNA-seq) based-transcriptome analysis was conducted to identify the beneficial effect of filbertone on downstream pathways involved in the regulation of lipid metabolism, amino acid metabolism and energy balance. In this study, we probed the potential effect of filbertone in the regulation of myocyte lipid metabolism and thermogenesis in muscle under conditions of HFD.

Materials and methods

Reagents

Filbertone ((E)-5-methyl-hept-2-en-4-one, 98%), dimethyl sulfoxide (DMSO), phenyl methyl sulfonyl fluoride (PMSF), sodium pyrophosphate, ethylenediaminetetraacetic acid (EDTA), sodium floride, sodium orthovanadate, chloroform, methanol and dithiothreitol (DTT), Oleic Acid-Albumin were purchased from Sigma Aldrich (St. Louis, MO, USA). The cOmplete protease inhibitor cocktail was purchased from Roche (Indianapolis, IN, USA). Micro BCA protein assay kit and SuperSignal West Pico PLUS Chemiluminescent Substrate were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Nitrocellulose blotting membrane (0.2 μm NC) was supplied by GE Healthcare (Piscataway, NJ, USA).

Animal studies

Five-week-old male C57BL/6 wild-type (WT) mice were purchased from Orient Bio Inc. (Busan, Korea). The animals were controlled in a temperature (22 ± 2℃), humidity (55%) and lighting (12 h light/12 h dark cycle) with access to free food and water. After one week of adaptation, the animals were randomly assigned into three groups without statistical significance among the groups. Animals were maintained on a standard chow diet (Orient Bio Inc., 5L79, Korea), a HFD (60 kcal% fat; Research Diets Inc, D12492, USA), or a HFD supplemented with 0.2% filbertone (HFD + Fil) for 10 weeks. Animals were anesthetized with CO_2 asphyxiation after a 5 h fasting at the end of the experiment. The muscle tissues were frozen in liquid nitrogen and stored at -80℃ until further analysis. Each group had 8–10 mice, and 3 independent experiments were performed. All animal care and experiments were performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Ulsan (Approval No. IACUC-LNY-16-010).

RNA isolation and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) and RNase-Free DNase Set (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNAs were generated using M-MLV reverse transcriptase (Promega, Madison, WI, USA) with 1.0 μg of total RNA. PCR reactions were conducted using TB Green Premix Ex Taq II (TaKaRa Bio Inc., Foster, CA, USA) on a Thermal Cycler Dice Real-Time PCR System (TaKaRa Bio Inc., Otsu, Shiga, Japan) with specific primers for each gene. All qRT-PCR data were analyzed by TP800 software (TaKaRa Bio Inc.). Primer sets are listed in Additional file 1: Table S1. The expression of target mRNAs was normalized by that of *Rplp0* (36B4) as the standard.

RNA-seq experiment

DNase-treated total RNA (1 μg) was used to construct multiplexed libraries using Illumina's Truseq Stranded Total RNA Kit (San Diego, CA, USA). Then, the total RNA was subjected to Ribo-Zero Removal Kit (Illumina) for ribosomal RNA removal. The remaining RNA was purified, fragmented, reverse-transcribed, adapter ligated, and finally PCR amplified to enrich the DNA fragments. RNA-Seq libraries were sequenced at paired-end 150-bp read length on an Illumina NovaSeq 6000 instrument. Illumina's BaseSpace was used to convert bcl files to FASTQ files and demultiplex the samples.

Identification of differentially expressed genes and functional enrichment analysis

After filtering out low quality sequencing reads (Trimmomatic ver. 0.39), HISAT2 (ver. 2.2.1) was used to map quality-filtered sequencing data to a reference genome (Mus_musculus.GRCm39). Unique readings were selected using SAMtools, then being quantified with featureCount (Subread v2.0.3). After removing genes with low expression levels, the counts were normalized with the DESeq2 package (R v4.2.2) [29]. DEGs were identified using the limma package (v3.54.1) with the criteria of p-value < 0.05 and |log2 (Fold Change)| >0.3. To identify key genes among DEGs, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using the DAVID (v6.8) (https://david.ncifcrf.gov). R software (R v4.2.2) was used to create the volcano map, bar graph, and heatmap of significant DEGs for data visualization.

Protein sample preparation and western blotting

Total Protein was lysed with RIPA buffer 1% NP-40, 5 mM $Na_4P_2O_7$, 1 mM EDTA, 20 mM NaF, 2 mM Na_3VO_4, 1× cOmplete protease inhibitor cocktail and 1 mM PMSF. After centrifugation (13,500 ×g for 10 min at 4 ℃) using Eppendorf 5415R (Hamburg, Germany), the supernatant was transferred to new e-tube. Protein concentration was determined by using Micro BCA Protein Assay Kit (Thermo Fisher Scientific). 20–30 μg of protein was subjected to 8–10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and transferred to a nitrocellulose membrane. The NC blots were blocked with 5% (w/v) skim milk solution for 1 h and hybridized with primary antibodies for overnight at 4 ℃, respectively. The following antibodies were used: anti-PPARα (ProteinTech Group, Rosemont, IL, USA), anti-UCP-1 (Abcam, Cambridge, UK), anti-ACSL1 (Cell Signaling Technology, Danvers, MA, USA) and anti-α-Tubulin (Sigma-Aldrich). Protein bands were determined by SuperSignal West Pico PLUS Chemiluminescent Substrate. Protein detection and quantification was conducted using Fusion Solo S (Vilber, France). The intensity of bands was normalized to α-Tubulin.

Muscle triglyceride measurement

Tissue triglyceride (TAG) levels were measured as previously described [30]. Frozen tissue (50 mg) was homogenized with 1 mL chloroform/methanol (2:1) on ice with vortex. After adding 300 μl distilled water, the mixture was mixed and then centrifuged at 1000 x g for 20 min. The lower(chloroform) phase was collected and evaporated under nitrogen stream. The dried samples were suspended in 0.5 mL chloroform. TAG levels were determined by colorimetric assay using Infinity Triglycerides Reagents (Thermo Fisher Scientific), following the manufacturer's instructions for colorimetric assay.

Myocyte triglyceride measurement

Mouse myoblast C_2C_{12} cells (ATCC, Manassas, VA, USA) were maintained in DMEM containing 10% FBS (Gibco, Grand Island, NY, USA) at 37 °C in a 5% CO2 incubator. At approximately 80–90% confluence, C_2C_{12} myoblasts were differentiated into myotubes by culturing them in DMEM supplemented with 2% horse serum (Gibco) for 4–5 days. C_2C_{12} myotube cells were treated with oleic acid (100 μM) complexed to fatty acid-free BSA (Sigma) and the test compound for 24 h. After that, cells were collected with a lysis buffer containing 0.1% IGEPAL CA-630 (Sigma-Aldrich) in PBS. The harvested cells were sonicated for 5 s and then centrifuged at 10,000 x g for 10 min at 4 °C. The supernatant was removed and stored on ice. TAG levels were quantified using Infinity™ Triglycerides Liquid Stable Reagent (Thermo Scientific), following the manufacturer's instructions for colorimetric assay. TAG accumulation was determined by Synergy HTX (BioTek Instruments, VT, USA).

Statistical analysis

Statistical comparisons were assessed by Mann-Whitney U test or one-way ANOVA with Tukey's post hoc test using GraphPad Prism 7.0 (San Diego, CA, USA). P values less than 0.05 were considered statistically significant.

Results

RNA-seq transcriptome analysis of filbertone in skeletal muscle

To obtain insight into the molecular mechanism of filbertone in skeletal muscle, we conducted RNA-seq analysis of filbertone with HFD fed mice compared to HFD alone fed mice or chow diet (CD) fed mice. Log_2-transformed normalized counts were used to determine the differential gene expression between HFD + Fil, HFD and CD. The RNA-seq analysis identified a total of 996 DEGs (p value < 0.05 and log_2 < − 0.3 or > 0.3), 455 up-regulated genes and 541 down-regulated genes, when comparing HFD + Fil to HFD, as shown in the volcano plot (Fig. 1A). Filbertone markedly increased Ucp1 and Cidea expression (Fig. 1A; Table 1). The analysis of KEGG pathway was performed to identify potential pathways for 455 up-regulated DEGs and 541 down-regulated DEGs. KEGG pathway analysis revealed that the up-regulated DEGs were strongly associated with several pathways involved in metabolic pathways, thermogenesis, fatty acid degradation, oxidative phosphorylation and branched chain amino acids (BCAAs) (valine, leucine and isoleucine)

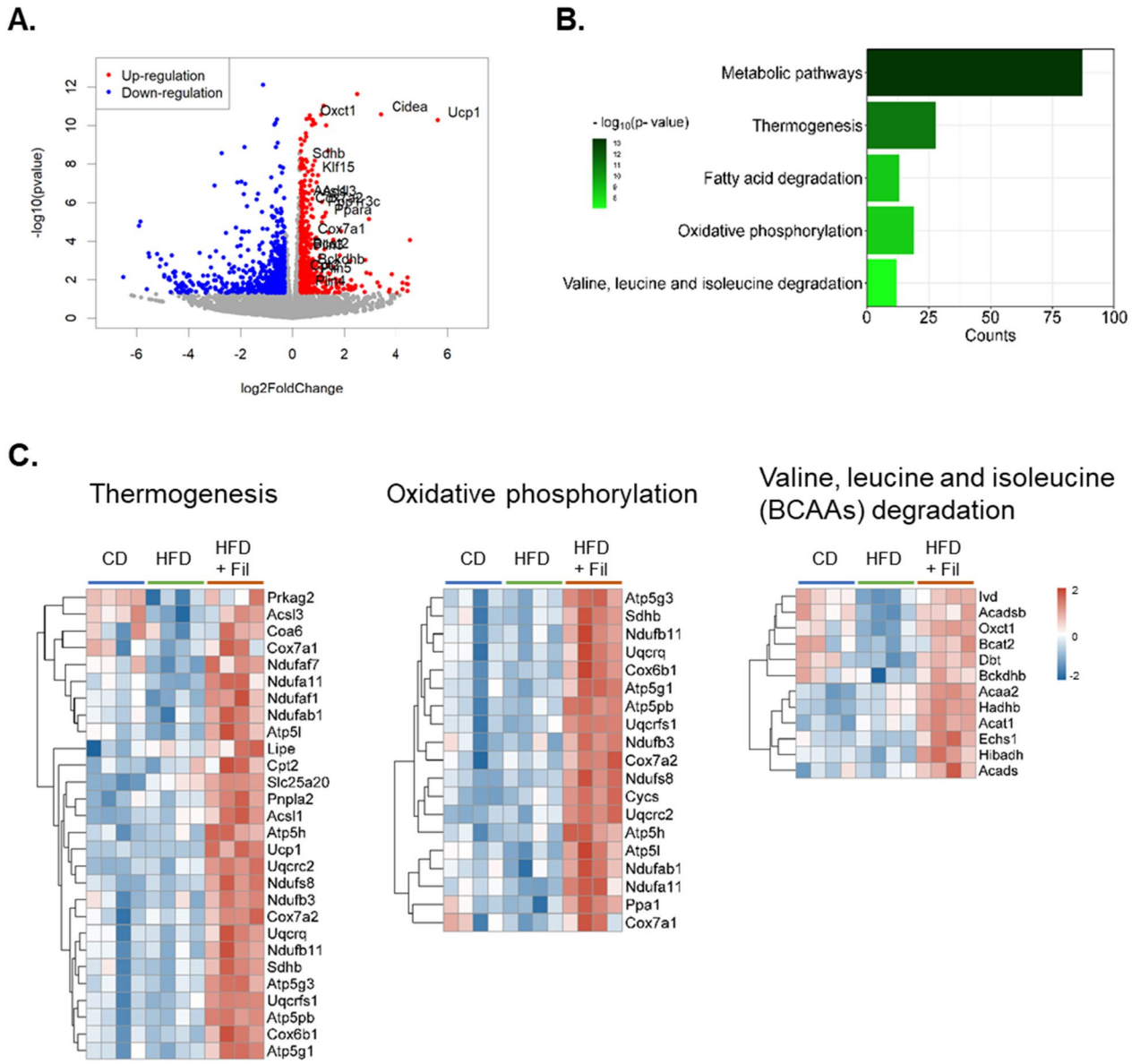

Fig. 1 Delineation of filbertone regulated genes and pathways in skeletal muscle. **A** Volcano plot of differentially expressed genes (DEGs) between high-fat diet (HFD) and HFD supplemented with Filbertone (HFD + Fil). DEGs involved the thermogenesis pathway were labeled. Genes having significant differences ($p < 0.05$ and |log2 (Fold Change)| >0.3) were indicated by colored plots. Red plots represented genes that were up-regulated by HFD + Fil compared to HDF whereas blue plots represented genes that were down-regulated. **B** Bar plot depicting the most enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for DEGs. The x-axis depicts the number of genes enriched in a specific pathway, with the color indicating the change in p-value. **C** Heatmap of certain enriched pathways. Following KEGG pathway analysis of upregulated genes by filbertone, the gene sets involved in thermogenesis, oxidative phosphorylation and branched chain amino acids (BCAAs) degradation pathways were visualized (n = 4 per group)

degradation (Fig. 1B; Table 2 A). The down-regulated DEGs were not involved in metabolic process but related to cancer pathways, platelet activation, breast cancer, focal adhesion and prostate cancer (Table 2B). Filbertone-regulated genes were involved in thermogenesis (Ucp1, Cidea and Ppara), fatty acid thioesterification (Acsl1 and Acsl3), oxidative phosphorylation (Sdhb, Cox7a1 and Cox7a2) and BCAAs degradation (Oxct1, Bcat2 and Bckdhb) (Fig. 1C).

Identification of filbertone regulated genes in skeletal muscle

Consistent with the RNA-seq results, qRT-PCR results revealed that filbertone increased the expression of

Table 1 Top 20 up-regulated genes with HFD supplemented with Filbertone (HFD-Fil) compared to high-fat diet (HFD)

Gene ID	Gene description	log2FC
Ucp1	Uncoupling protein 1 (mitochondrial, proton carrier)	5.631
Cidea	Cell death-inducing DNA fragmentation factor, alpha -like effector A	3.410
Hspa1a	Heat shock protein 1 A	2.487
Dkk3	Dickkopf WNT signaling pathway inhibitor 3	1.869
Tbx1	T-box 1	1.818
Banp	BTG3 associated nuclear protein	1.569
Lrtm2	Leucine-rich repeats and transmembrane domains 2	1.569
Pck1	Phosphoenolpyruvate carboxykinase 1, cytosolic	1.399
Prkcz	Protein kinase C, zeta	1.380
Ankrd2	Ankyrin repeat domain 2 (stretch responsive muscle)	1.368
Cbx8	Chromobox 8	1.361
Cfd	Complement factor D (adipsin)	1.290
Pitx3	Paired-like homeodomain transcription factor 3	1.283
Entpd4b	Ectonucleoside triphosphate diphosphohydrolase 4B	1.230
Katnal2	Katanin p60 subunit A-like 2	1.225
Acot1	Acyl-CoA thioesterase 1	1.222
Actc1	Actin, alpha, cardiac muscle 1	1.202
Gm5420	Predicted gene 5420	1.173
Ppara	Peroxisome proliferator activated receptor alpha	1.161
Snph	Syntaphilin	1.137

genes involved in thermogenesis (Ucp1; $p < 0.05$, Cidea; $p < 0.05$, Ppara; $p < 0.05$, and Cpt2; $p < 0.05$), fatty acid thioesterification (Acsl1; $p < 0.05$ and Acsl3; $p < 0.05$), oxidative phosphorylation (Sdhb; $p < 0.05$, Cox7a1; $p < 0.05$, and Cox7a2; $p < 0.05$) and BCAAs degradation (Oxct1; $p < 0.05$, Bcat2; $p < 0.05$, and Bckdhb; $p < 0.05$) (Fig. 2A and D). Filbertone also upregulated the gene expression of Protein phosphatase 1 regulatory subunit 3c (Ppp1r3c), also known as Protein Targeting To Glycogen (PTG), which encodes a master modulator of glycogen synthesis [31] ($p < 0.05$; Fig. 2E). In addition, filbertone increased the gene expression of Kruppel-like factor 15 (Klf15), highly expressed in skeletal muscle and cardiac muscle, which encodes a positive regulator of glucose, lipid, and BCAAs metabolism [32] ($p < 0.05$; Fig. 2F). Moreover, filbertone increased the expression of genes involved in the lipid droplet-associated proteins including perilipin 3 (Plin3; $p < 0.05$), perilipin 4 (Plin4; $p < 0.05$), and perilipin 5 (Plin5; $p < 0.05$) (Fig. 2G). Plin5, mainly expressed in oxidative tissues such as skeletal muscle, heart, and liver, which plays a vital role in lipid homeostasis [33]. The regulation of up or down-regulated genes in skeletal muscle determined by RNA-seq analysis was confirmed by qRT-PCR.

Effect of filbertone in muscle lipid accumulation

We assessed whether filbertone inhibits the accumulation of intracellular triglycerides in the skeletal muscle of HFD + Fil fed mice compared with that of HFD fed mice. HFD-induced TAG accumulation is remarkably 35% decreased in the skeletal muscle of HFD + Fil fed mice compared to HFD fed mice ($p < 0.05$; Fig. 3A). Consistent with the effect of filbertone in vivo, neutral lipid accumulation was significantly decreased in C_2C_{12} myotube with filbertone treatment in dose dependent manner (25Gene regulation of thermogenesis, fatty acid thioesterification, oxidative phosphorylation and BCAAs metabolism with filbertone in skeletal muscleμM; 15%, $p < 0.05$ and 50μM; 33%, $p < 0.05$; Fig. 3B).

Effect of filbertone on thermogenesis in skeletal muscle

We also examined the effect of filbertone on thermogenic factors in the skeletal muscle. The protein levels of UCP1, PPARα and ACSL1 in the skeletal muscle were measured by western blotting analysis (Fig. 4A). Consistent with the results of the RNA-seq and qRT-PCR in previous results (Figs. 1 and 2), filbertone significantly increased the protein levels of UCP1 (2.9 fold, $p < 0.05$), PPARα (1.7 fold, $p < 0.05$), and ACSL1 (2.3 fold, $p < 0.05$) in the skeletal muscle of HFD + Fil fed mice compared with that of HFD fed mice (Fig. 4B, D).

Discussion

Skeletal muscle is not only one of the metabolically active organs along with liver, adipose tissue and heart, but also the principal location for energy balance management. Filbertone, the key flavoring component in hazel tree fruits (Corylus maxima and Corylus avellana), has recently been proposed to have a beneficial effect on obesity and inflammation. Filbertone improved HFD-induced obesity, hyperglycemia and hyperinsulinemia by activating AMPK signaling [27]. Filbertone also prevented HFD-induced hypothalamic inflammation by inhibiting MAPK and NF-κB signaling [28]. In this study, we investigated the possible influence of filbertone on the regulation of myocyte lipid metabolism and thermogenesis in muscle under conditions of HFD by using RNA-seq based-transcroptome analysis. Our results provided an important effect of filbertone in muscle lipid accumulation. Filbertone significantly decreased muscle TAG accumulation in context of chronic nutrient excess. Moreover, filbertone treatment resulted in an inhibition of lipid accumulation with oleic acid loading in C_2C_{12} myotubes. Filbertone also induced genes involved in Lipid droplet associated proteins (C) including Plin3, Plin4 and Plin5 in skeletal muscle. Plin5, known as Lipid storage droplet protein 5 (also named MLDP and

Table 2 Top 10 Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways with up and down regulated genes by filbertone treatment

A. KEGG pathways with up-regulated genes			
Ranking	**Term**	**Gene count**	**P-value**
1	Metabolic pathways	87	4.10E-14
2	Thermogenesis	28	7.80E-12
3	Fatty acid degradation	13	2.60E-09
4	Oxidative phosphorylation	19	3.50E-09
5	Valine, leucine and isoleucine degradation	12	9.00E-08
6	Diabetic cardiomyopathy	21	1.70E-07
7	Non-alcoholic fatty liver disease	17	1.10E-06
8	PPAR signaling pathway	13	1.40E-06
9	Chemical carcinogenesis—reactive oxygen species	20	1.60E-06
10	Fatty acid metabolism	11	2.00E-06
B. KEGG pathways with down-regulated genes			
Ranking	**Term**	**Gene count**	**P-value**
1	Pathways in cancer	35	3.50E-07
2	Platelet activation	14	1.10E-05
3	Breast cancer	14	6.20E-05
4	Focal adhesion	16	1.20E-04
5	Prostate cancer	11	1.50E-04
6	Human papillomavirus infection	22	2.10E-04
7	Small cell lung cancer	10	4.40E-04
8	Endocrine resistance	10	4.40E-04
9	EGFR tyrosine kinase inhibitor resistance	9	6.70E-04
10	MicroRNAs in cancer	18	1.20E-03

OXPAT), is specifically expressed in highly oxidative tissues, such as skeletal muscle and heart [34]. Previous studies show that Plin5 knock-out mice induces insulin resistance in skeletal muscle through regulation of ceramide accumulation [35], enhances oxidative stress in the heart [36], and also induces myocardial ischaemia/reperfusion (I/R) injury via elevation of oxidative stress [37]. Intriguingly, it has been reported that muscle-specific overexpression of Plin5 protects against diet-induced weight gain on both HFD and CD, and also increases muscle FGF21 gene expression (not liver FGF2 and circulating FGF21) [38]. Our findings support that filbertone has a beneficial influence in muscle lipid metabolism.

A crucial function of filbertone unveiled here is activation of thermogenesis in skeletal muscle via upregulation of Ucp1 and Cidea in filbertone supplement with HFD fed mice compared to HFD alone fed mice. Previous evidence demonstrates that muscle specific overexpression of Ucp1 ameliorates chronic diet-induced obesity and insulin resistance [18, 19]. Conversely, deletion of Ucp1 is involved in the development of obesity and the suppression of thermogenesis [21]. Excitingly, a recent report shows that knock-out of Cidea reduces the gene expression of Ucp1 in human adipocytes and the transcriptional activity of Ucp1 is regulated by Cidea via inhibition of liver X receptor α [39]. Hence, the activation of thermogenesis by filbertone through the up-regulation of Ucp1 and Cidea in muscle is considered as advantageous for combating obesity and insulin resistance.

In addition, our findings indicate that filbertone may be effective on insulin resistance via the modulation of several genes in muscle. We found that protein phosphatase 1 regulatory subunit 3c, which is a glycogen-targeting subunit of protein phosphatase 1 (PP1) complex (known as PTG), and Krüppel-like factor 15 (Klf15) are up-regulated by filbertone. Ablation of PTG has been associated with development of insulin resistance and diabetes [31]. Recent result suggest that Knock-out of Klf15 in skeletal muscle is related to the development of insulin resistance [40] and abnormality of muscle endurance [41]. Adipose tissue-specific Klf15 overexpression can protect against insulin resistance and HFD induced obesity [42]. Interestingly, Deletion

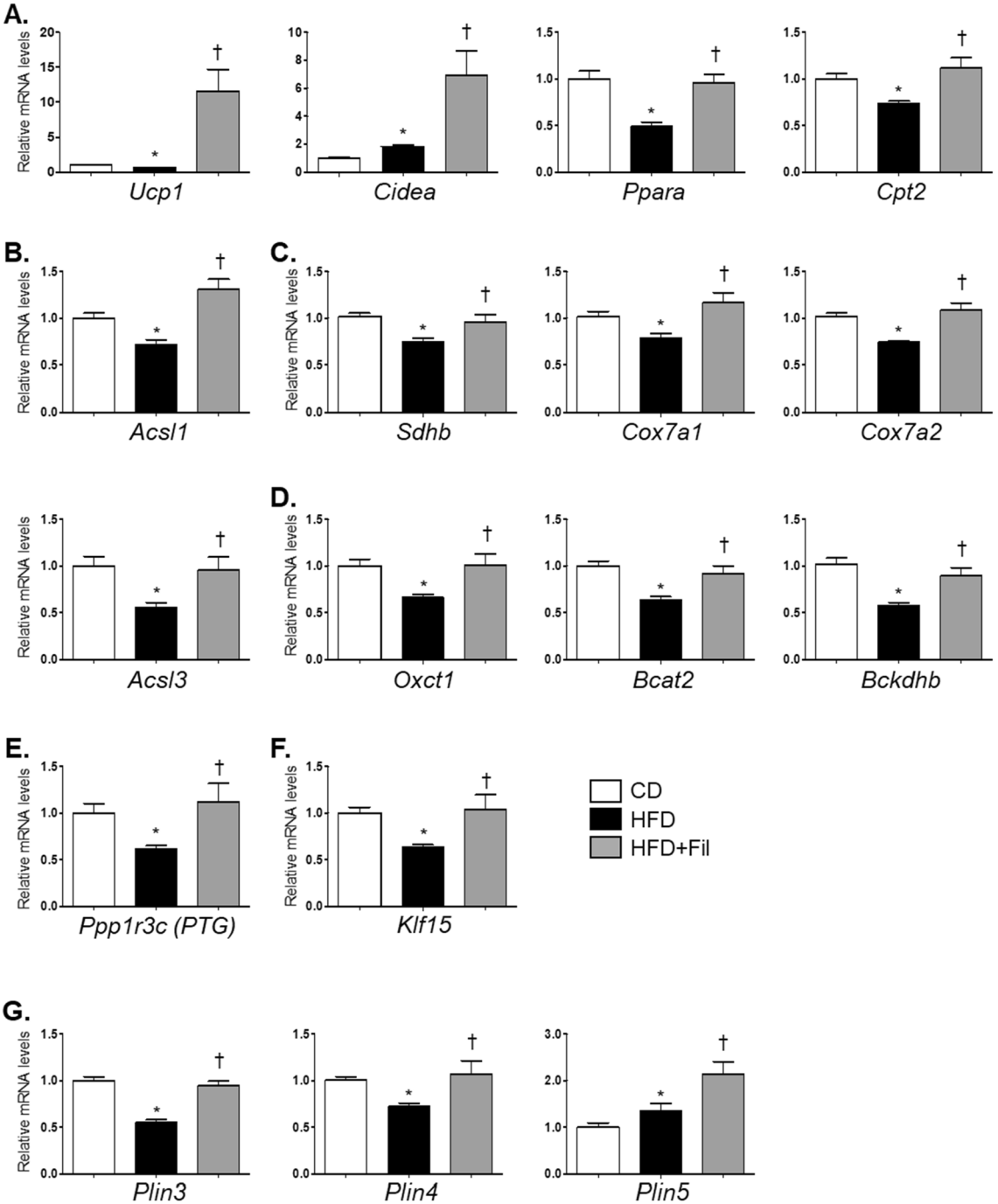

Fig. 2 Gene regulation of thermogenesis, fatty acid thioesterification, oxidative phosphorylation and BCAAs metabolism with filbertone in skeletal muscle. The expression of genes involved in **A** thermogenesis, **B** fatty acid thioesterification, **C** oxidative phosphorylation, **D** BCAAs metabolism, **E** glycogen synthesis, **F** nutrient metabolism, **G** lipid metabolism was measured by quantitative RT-PCR in skeletal muscle (n = 8–10 mice per group). The data represent mean ± SEM. * $p < 0.05$ vs. CD, and † $p < 0.05$ vs. HFD by 1-way ANOVA with Tukey's multiple-comparisons post hoc test

of Klf15 in cardiac muscle is involved in pressure-overload cardiac hypertrophy, whereas Klf15 overexpression in cardiomyocytes inhibits hallmark characteristics of cardiac remodeling [43]. In addition, our results reveal that filbertone induces genes involved in catabolism of BCAAs (leucine, isoleucine, and valine). Previous reports showed that defect of the catabolism of BCAAs is associated with the development of pressure

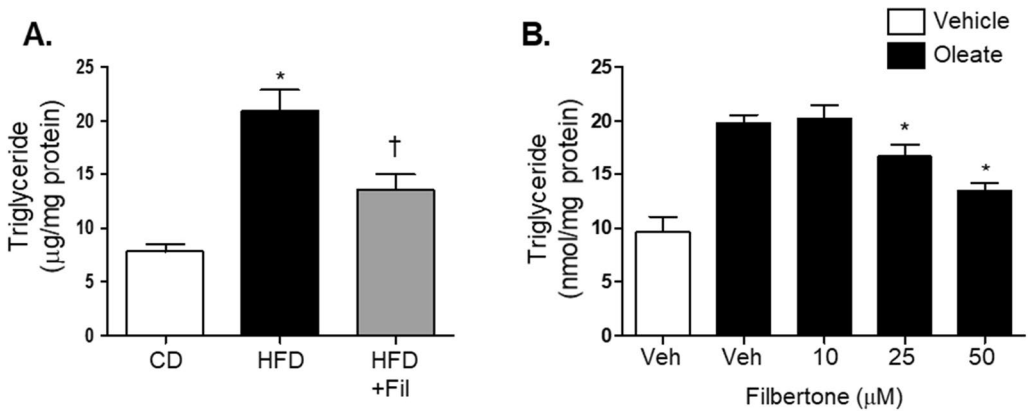

Fig. 3 Filbertone inhibits triglycerides accumulation in skeletal muscle. Total triglyceride content was measured in **A** gastrocnemius muscle (n = 8–10 mice per group). Data bars represent mean ± SEM. * $p < 0.05$ vs. CD; † $p < 0.05$ vs. HFD by one-way ANOVA with Tukey multiple comparison post-hoc test. Total triglyceride content was measured in (**B**) C_2C_{12} myotubes (n = 4 per group). Data bars represent mean ± SD. * $p < 0.05$ vs. Oleate with vehicle by one-way ANOVA with Tukey multiple comparison post-hoc test

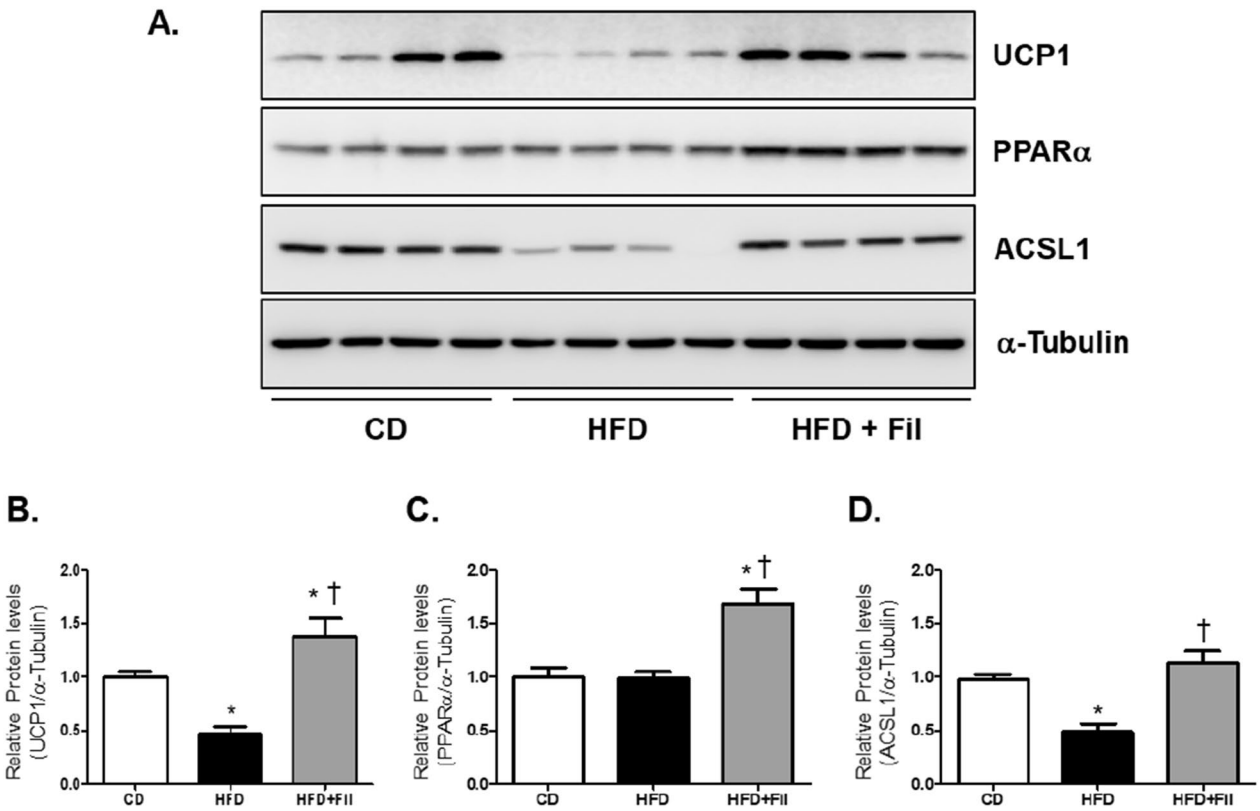

Fig. 4 Filbertone increases thermogenic factors in skeletal muscle. The western blot analysis of **B** UCP1, **C** PPARα and **D** ACSL1 was determined in gastrocnemius muscle (n = 8–10 mice per group). **A** Representative Western blots are shown in the upper panel. Data bars represent mean ± SEM. * $p < 0.05$ vs. CD; † $p < 0.05$ vs. HFD by one-way ANOVA with Tukey multiple comparison post-hoc test

overload–induced heart failure [44] and ischemia-reperfusion injury [45]. Therefore, up-regulation of these genes by filbertone is considered to be beneficial for insulin resistance, diabetes, and cardiovascular disease.

Our findings demonstrate that filbertone treatment elevates genes involved in the pathway of thermogenesis, lipid homeostasis and BCAAs metabolism in skeletal muscle. Therefore, further studies will be essential

to determine whether filbertone can control energy homeostasis and insulin signaling in other tissue such as liver, heart and pancreas. In conclusion, our results suggest that filbertone should be considered an ingredient for a development strategy of functional food to improve obesity-induced metabolic diseases.

Abbreviations

ACSL	Acyl-CoA synthetase long chain family member
BCAA	Branched chain amino acid
CD	Chow diet
DEG	Dfferentially expressed gene
HFD	High-fat diet
KEGG	Kyoto encyclopedia of genes and genomes
KLF	Krüppel-like factor
PLIN	Perilipin
PPAR	Proxisome proliferator-activated receptor
PTG	Protein targeting to glycogen
qRT-PCR	Real-time quantitative reverse transcription PCR
RNA-seq	RNA-sequencing
TAG	Triglyceride
UCP	Uncoupling protein

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00783-5.

Additional file 1: Table S1. Mouse primer sequence for qRT-PCR.

Acknowledgements
Not applicable.

Author contributions
BA contributed to the study conception and design. Experiments, data collection and analysis were performed by HK and BA. The manuscript was written by HK and BA. BA acquired funding. Both authors read and approved the final manuscript.

Funding
This work was supported by the National Research Foundation of Korea funded by the Ministry of Science and ICT (NRF-2021R1F1A1049873) and a Grant (B.Y.A., 2022 F-4) from the Korean Diabetes Association.

Availability of data and materials
The datasets used and analyzed in this study are included in this published article. Additional information will be available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

Received: 5 March 2023 Accepted: 10 April 2023
Published online: 02 May 2023

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