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Antiobesity effect and metabolite analysis of catechin functional kimchi

Geun-Hye Hong[1,2], So-Young Lee[1,2] and Kun-Young Park[1,2*]

Abstract

The antiobesity effects of catechin functional kimchi (CFK) were studied in C57BL/6 mice with high-fat diet (HFD)-induced obesity. We prepared four types of kimchi: commercial kimchi (CK), standard kimchi (SK), green tea functional kimchi (GFK), and CFK. CFK decreased the adipo-/lipogenesis-related genes of CCAAT/enhancer binding protein α (C/EBPα), peroxisome proliferator-activated receptor-γ (PPARγ), and sterol regulatory element-binding protein-1 (SREBP-1) in the liver and epididymal tissues ($p < 0.05$). On the other hand, CFK showed the highest lipolysis-related gene expression of hormone-sensitive lipase (HSL) and β-oxidation related gene expression of carnitine palmitoyltransferase 1 (CPT-1). CFK produced the lowest inflammation-related mRNA expression of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) among all groups in the epididymal tissues ($p < 0.05$). In addition, UPLC–Q-TOF-MS showed that CFK is composed mainly of 39 active compounds, e.g., epigallocatechin gallate (EGCG), catechins, apigenin, myricetin, kaempferitin, rutin, quercetin, and other substances with anti-inflammatory, blood cholesterol reduction, blood sugar reduction, body fat reduction, antioxidant, and anticancer functions. Thus, CFK exhibited an antiobesity effect through its modulation of lipid metabolism and active compounds.

Keywords Kimchi, Catechin, Lactic acid bacteria (LAB), Antiobesity, Active compounds

Introduction

Fermentation has been one of the ways to store food for a long time, but interest in fermented foods is increasing around the world as research reports that it improves the taste and functionality of foods [1]. Among these, kimchi is a traditional Korean fermented food containing probiotics, prebiotics, and postbiotics [2]. It is made using Baechu cabbage and radish as the main ingredients and adding sub-ingredients such as garlic, ginger, and red pepper powder [3]. The origins of kimchi are estimated to be at least 2000 years ago and are known to have originated from salting vegetables in the winter when fresh vegetables were not available [4, 5]. Kimchi, which is simply salted vegetables, was manufactured until the end of the Three Kingdoms Period (approximately 57 BC-667 AD) and is estimated to have been consumed as a side dish [5]. First, kimchi was made using radish as the main ingredient, and later it was made using cabbage and red pepper powder. As different ingredients began to be mixed, the current form of kimchi began to appear in the late seventeenth century [6].

Kimchi uses a variety of vegetables, so it has a high content of vitamins, minerals, and dietary fiber [7], and contains various functional ingredients such as capsaicin, allyl compounds, and chlorophyll [6]. Kimchi provides important nutrients in the daily lives of Koreans and is related to maintaining health [8]. Research has been reported on various health functionalities of kimchi, including intestinal health [8, 9], antioxidant and anti-aging [10, 11], plasma lipid suppression and antiobesity [12–15], and immune-enhancing [8] effects. Isothiocyanate, indole-3-carbinol, and allyl sulfur compounds (allicin, diallyl sulfide, etc.), beta-sitosterol,

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ascorbic acid, carotenoids, flavonoids, tocopherol, selenium, dietary fiber, polyunsaturated fatty acids, etc., found in kimchi has been shown to appear due to functional substances [16, 17].

The ingredients of kimchi, especially the types of subingredients, fermentation temperature, and various conditions, affect the taste, characteristics, fermentation, and functionality of kimchi [3, 5]. It has been reported that kimchi using Amtak baechu cabbage [18] and organically cultivated baechu cabbage [19] has increased anticancer effects. It was confirmed that kimchi using mistletoe extract, a functional substance, had an enhanced anticancer effect compared to regular kimchi [20]. In addition, the antiobesity effect increased in kimchi containing green tea as subingredient [21], and the antiobesity effect was also confirmed to be significantly enhanced in kimchi containing catechin and lactic acid bacteria, the active ingredients of green tea [22]. Kimchi using mixed strains as a starter had excellent taste and showed high health functionalities such as antioxidant and anticancer properties [23]. Kimchi using lactic acid bacteria isolated from kimchi as a starter also showed excellent anti-obesity effects [8]. Therefore, subingredients play an important role in determining the functionality of kimchi.

In this study, we investigated the effects of CFK on lipid accumulation and antiobesity in high-fat diet (HFD)-fed obese mice. We investigated the lipid accumulation and antiobesity effects of commercial kimchi (CK), standard kimchi (SK), green tea functional kimchi (GFK), and catechin functional kimchi (CFK) in high-fat diet (HFD)-fed obese mice. It was confirmed through real-time quantitative polymerase chain reaction (RT-qPCR), and the active compounds of kimchi were also studied.

Materials and methods

Sample preparation

Kimchi was prepared using Baechu cabbages and subingredients purchased from Pungmi Kimchi Co. (Suwon, Korea). Kimchi was manufactured by implementing a slight adaptation of a previously documented recipe of naturally fermented kimchi [8, 14, 24, 25]. At the optimal fermentation period (pH 4.0–4.3), the mixture was prepared by drying it using a freeze dryer (FD 5512, Ilshin BioBase CO., Korea) and then adding methanol. The admixture of kimchi and methanol underwent filtration to isolate the methanol extract, which was then subjected to concentration by using a vacuum rotary evaporator (EYELA, Tokyo Rikakikai Co., Tokyo, Japan) [26].

Animal study

The mice study was obtained from the Institutional Animal Care and Use Committee of CHA University (IACUC-200050) [25]. The mice used in the experiment were purchased from Orient Bio (Seong-nam, Korea) as a 6-week-old C57BL/6 strain with body weight of 20 ± 2 g and maintained at 23 ± 2 °C and 55 ± 5% relative humidity for a 12-h light–dark cycle. The experimental mice accommodated within a specific pathogen-free (SPF) room situated at the CHA Bio complex (Seongnam, Korea) and provided unrestricted access to a diet (DooYeol Biotech, Seoul, Korea) and water. The mice were classified into seven groups considering their body weights ($n = 10$ per group): AIN-93G diet group (Normal, Nor); 45% high-fat diet group (High-fat diet, HFD); 45% high-fat diet and 1.5% NaCl with same salinity as kimchi (Salt); 45% high-fat diet and 1.5 mg/kg/day CK (CK); 45% high-fat diet and 1.5 mg/kg/day SK (SK); 45% high-fat diet and 1.5 mg/kg/day GFK (GFK); and 45% high-fat diet and 1.5 mg/kg/day CFK (CFK). After adaptation period, the groups administered the kimchi sample (CK, SK, GFK, and CFK) were orally administered 0.1 mL of kimchi extract for 16 weeks, and a 45% HFD was supplied at the same time [22, 25].

Measurement of mRNA expression levels

The total RNA of liver and epididymal tissues was isolated using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). cDNA was obtained using oligo dT18 (Invitrogen), reverse transcriptase buffer (Invitrogen), dNTPs (Invitrogen), reverse transcriptase (Invitrogen), and RNase inhibitor (Invitrogen). cDNA synthesis of isolated total RNA was performed using a 2720 thermal cycler (Applied Biosystems, Foster, CA, USA). The synthesized cDNA was amplified using a thermal cycler Bio-Rad CFX-96 real-time system (Bio-Rad, Hercules, CA, USA). The primers for 18s rRNA, CCAAT/enhancer binding protein α (C/EBPα), peroxisome proliferator-activated receptor-γ (PPARγ), sterol regulatory element-binding protein-1 (SREBP-1), lipoprotein lipase (LPL), diacylglycerol O-acyltransferase 1 (DGAT), carnitine palmitoyltransferase I (CPT-1), hormone-sensitive lipase (HSL), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (Bioneer Corp., Daejeon, Korea) Table 1 lists the primer sequences of each gene [27]. Relative mRNA transcription levels were calculated using the $2^{-\Delta\Delta CT}$ formula [28].

Analysis of kimchi metabolites using UPLC–Q-TOF MS

Freeze-dried kimchi sample powder from the 3rd week was homogenized with 80% methanol using a bullet blender (Next Advance, Troy, NY, USA). After

Table 1 Primer sequences of obesity-related genes used for RT–qPCR in obesity through HFD-induced mice

Gene	Forward sequence	Reverse sequence
C/EBPα	5′-TGC TGG AGT TGA CCA TGT AC-3′	5′-AAA CCA TCC TCT GGG TCT CC-3′
PPARγ	5′ -TTT TCA AGG GTG CCA GTT TC-3′	5′-AAT CCT TGG CCC TCT GAG AT-3′
SREBP-1	5′-CGG AGA CAG GGA GTT CTC AG-3′	5′-TGG GGG ATA TGC TCT ACC AG-3′
LPL	5′-CAG CTG GGC CTA ACT TTG AG-3′	5′-CCT CTC TGC AAT CAC ACG AA-3′
DGAT1	5′-GCA GAC CGC GAG TTC TAC AG-3′	5′-CTC ATG GAA GAA GGC TGA GG-3′
HSL	5′-AGA CAC CAG CCA ACG GAT AC-3′	5′-CAT CAC CCT CGA AGA AGA GC-3′
CPT-1	5′-TAT CGC CAC CTG CTG AAC C-3′	5′-TTG AAG GTG ACG AAG GTG GT-3′
TNF-α	5′-CAGGCGGTGCCTATGTCTC-3′	5′-CGATCACCCCGAAGTTCAGTAG-3′
IL-6	5′-ATG AAG TTC CTC TCT GCA A-3′	5′-AGT GGT ATC CTC TGT GAA G-3′
18S rRNA	5′-TCG AGG CCC TGT AAT TGG AA-3′	5′-CCC TCC AAT GGA TCC TCG TT-3′

centrifuging, the supernatants were dried completely using a CentriVap concentrator SpeedVac (Labconco Co., Kansas City, MO, USA) and analyzed by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC–Q-TOF MS). The kimchi samples were analyzed using a UPLC–Q-TOF MS system (Xevo G2-S; Waters, Milford, MA, USA) [29]. The samples were injected into an Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 m; Waters Corp., Milford, MA, USA), equilibrated with water containing 0.1% formic acid (FA), and eluted with a gradient of acetonitrile containing 0.1% formic acid. A flow rate of 0.35 mL/min and a column temperature of 40℃ were employed. The eluted metabolites were analyzed by Q-TOF MS in positive electrospray ionization (ESI) mode. The desolvation flow rate and temperature were 800 L/h and 400 °C, respectively, and the source temperature was 100℃. A TOF MS scan range of 50–1500 m/z, scan time of 0.2 s, and capillary and sampling cone voltages of 3 kV and 30 V, respectively, were used. Leucine-enkephalin ([M + H] = 556.2771), which was used as a lock mass, was infused at a flow rate of 0.35 mL/min and frequency of 10 s to ensure mass measurement accuracy of the metabolites analyzed by the instrument. A quality-control (QC) sample prepared by mixing all samples was analyzed in triplicate before the start and after every five samples. The MS/MS spectra were obtained in the range of m/z 50 – 1500 using a collision energy ramp from 10 to 30 eV.

The metabolites were identified based on online databases (ChemSpider database in UNIFI, METLIN database (www.metlin.scripps.edu), and human metabolome databases (www.hmdb.ca) [30].

Statistical analysis

The RT-qPCR experimental results are presented as the means ± standard errors (SE). Duncan's multiple range tests and one-way analysis of variance (ANOVA) were used to determine the significant intergroup differences. The analysis was performed using IBM SPSS version 23 (SPSS Inc.). The significance of the experimental results was tested at the $p < 0.05$ level.

Results and discussion

Effects of CFK on mRNA expression of the adipogenesis-related genes

The antiobesity effect was confirmed by orally administering four types of kimchi (CK, SK, GFK, and CFK) to C57BL/6 mice along with a 45% high-fat diet for 16 weeks. Obesity is influenced by lipid metabolism through the regulation of adipo-/lipogenesis and lipolysis [31]. C/EBPα is a key contributor to the initial phases of adipogenic differentiation [32]. PPARγ plays an important role in generating adipose tissue by differentiating preadipocytes into adipocytes. Furthermore, it is well-established that there is mutual regulation between PPARγ and C/EBPα, which contributes to the promotion and maintenance of the state of adipocyte differentiation [33, 34]. The mRNA expression levels of adipogenesis-related genes (C/EBPα, PPARγ) in the liver tissues were measured and showed a significant decrease in all the groups that were administered kimchi compared to the HFD group (Fig. 1A). The Salt group (4.86 ± 0.81) appears to have increased fat synthesis by increasing the expression of the C/EBPα gene compared to the HFD group (3.21 ± 0.47). It can be seen that salt intake through kimchi is different from simply administering salt, and this is thought to be due to the various minerals contained in the salt (solar salt, bamboo salt) used to make kimchi [11, 26]. The C/EBPα mRNA expression was 2.90-fold lower in the CFK group (1.11 ± 0.47) than in the HFD group ($p < 0.05$). PPARγ gene expression was also significantly lower in the CFK group than in the HFD group. As a result of H&E staining analysis of liver tissues in the

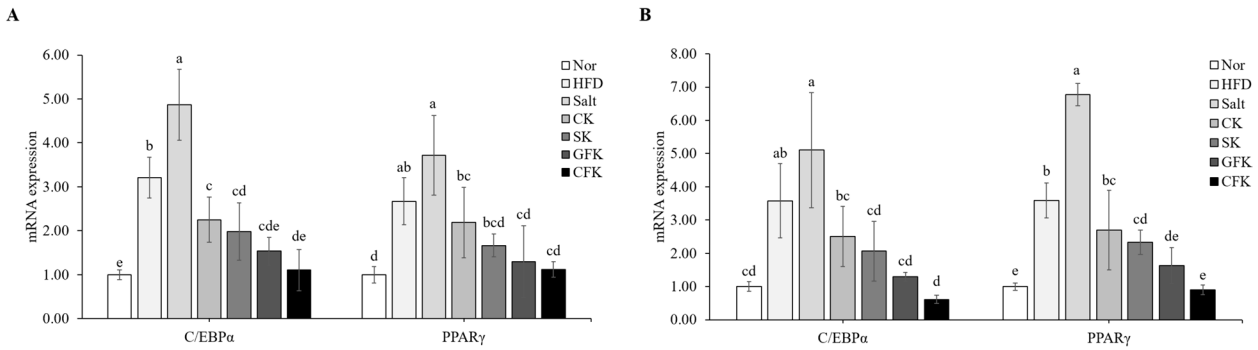

Fig. 1 Effects of catechin functional kimchi (CFK) on the mRNA expression levels of adipogenesis-related genes in **A** liver tissues, and **B** epididymal tissues in high-fat diet (HFD)-induced obese mice. Nor, AIN-93G diet group; HFD, 45% high-fat diet group; Salt †: 45% high-fat diet and 1.5% NaCl; CK, 45% high-fat diet and 1.5 mg kg^{-1} day^{-1} commercial kimchi; SK, 45% high-fat diet and 1.5 mg kg^{-1} day^{-1} standard kimchi; GFK, 45% high-fat diet and 1.5 mg kg^{-1} day^{-1} green tea functional kimchi; CFK, 45% high-fat diet and 1.5 mg kg^{-1} day^{-1} catechin functional kimchi; C/EBPα, CCAAT/enhancer binding protein α; PPARγ, peroxisome proliferator-activated receptor gamma. Data represent means ± SEs. †Same salinity as kimchi (1.5%). [a–e]Mean values with different letters on the bars are significantly different ($p < 0.05$) according to Duncan's multiple-range test

previously reported paper [22, 25], it was confirmed that the number and size of fat globules in the liver tissues of the CFK group with low expression of C/EBPα and PPARγ were reduced by 36.67% compared to the HFD group. Therefore, when the mRNA expression of C/EBPα and PPARγ is decreased, lipid synthesis is reduced.

The mRNA expression analysis of adipogenesis-related genes in epididymal tissues showed that C/EBPα mRNA expression was 5.85-fold lower in the CFK group (0.61 ± 0.13) than in the HFD group ($p < 0.05$) (Fig. 1B). Additionally, PPARγ gene expression was also significantly lower in the CFK group than in the HFD group (3.58 ± 1.12), and significantly lower in the order of CFK (0.90 ± 0.14), GFK (1.63 ± 0.37), SK (2.33 ± 0.37), and CK (2.70 ± 1.20) groups. This result was similar to the research result of Lee et al. [35], in which administered kimchi helped reduce the expression of adipogenesis-related genes. In addition, epigallocatechin gallate (EGCG) in green tea is known to regulate the differentiation of preadipocytes into adipocytes [36], and it is thought to control obesity by inhibiting the expression of these genes (C/EBPα, PPARγ).

Effects of CFK on mRNA expression of the lipogenesis-related genes

The mRNA expression levels of lipogenesis-related genes (SREBP-1, LPL, DGAT1) in liver tissues were measured and were significantly lower in the CFK group than in the HFD group (Fig. 2A). SREBPs are known to promote the role of PPAR-γ as a key element in lipogenesis, and are reported to cause lipogenesis in the liver and adipose tissue by regulating the expression of fatty acid synthase (FAS) and lipoprotein lipase (LPL) [37, 38]. SREBP-1 mRNA expression was 4.31-fold lower in the CFK group (0.57 ± 0.10) than in the HFD group (2.46 ± 0.21), and significantly lower than that in the CK groups (1.50 ± 0.05). This result was similar to the research result of Cui et al. [14], who showed that kimchi reduced the expression of lipogenesis-related genes (SREPB-1c, FAS) in liver tissues. LPL is known to produce mature adipocytes at the adipogenesis stage and is a target factor that contributes to the activity of PPRAγ [39]. When the expression of LPL is reduced, lipogenesis can be suppressed. The mRNA expression of LPL in the CFK group (1.07 ± 0.22) was significantly lower than that in the HFD group (4.60 ± 0.25), and 6.77-fold lower in the CFK group than in the Salt group (7.22 ± 0.06) (Fig. 2A). In addition, the mRNA expression of LPL was also significantly lower in the CFK group than in the SK group (2.03 ± 0.23), which suggests that green tea catechins, which have a strong polyphenol effect, may have suppressed fat accumulation in mice.

In epididymal tissues, the mRNA expression levels of lipogenesis-related genes were significantly lower in the CFK group than in the HFD group ($p < 0.05$) (Fig. 2B). The Salt group showed significantly higher mRNA expression. Thus, simple saltwater intake is thought to induce more health problems. Lee et al. [35] reported that MSFK (kimchi with green tea) reduces the expression of SREPB-1c and FAS, which are related to lipogenesis. In this study, SREBP-1 mRNA expression was 6.86-fold lower in the CFK group (0.83 ± 0.48) than in the HFD group ($p < 0.05$) (Fig. 2B). In addition, it was significantly 5.64 and 4.04-fold lower than the CK (4.66 ± 1.81) and SK (3.34 ± 0.51) groups, respectively.

Diacylglycerol transferase (DGAT) is needed for the formation of adipose tissue and participates in the last stage of triacylglycerol synthesis [40, 41]. The CFK group

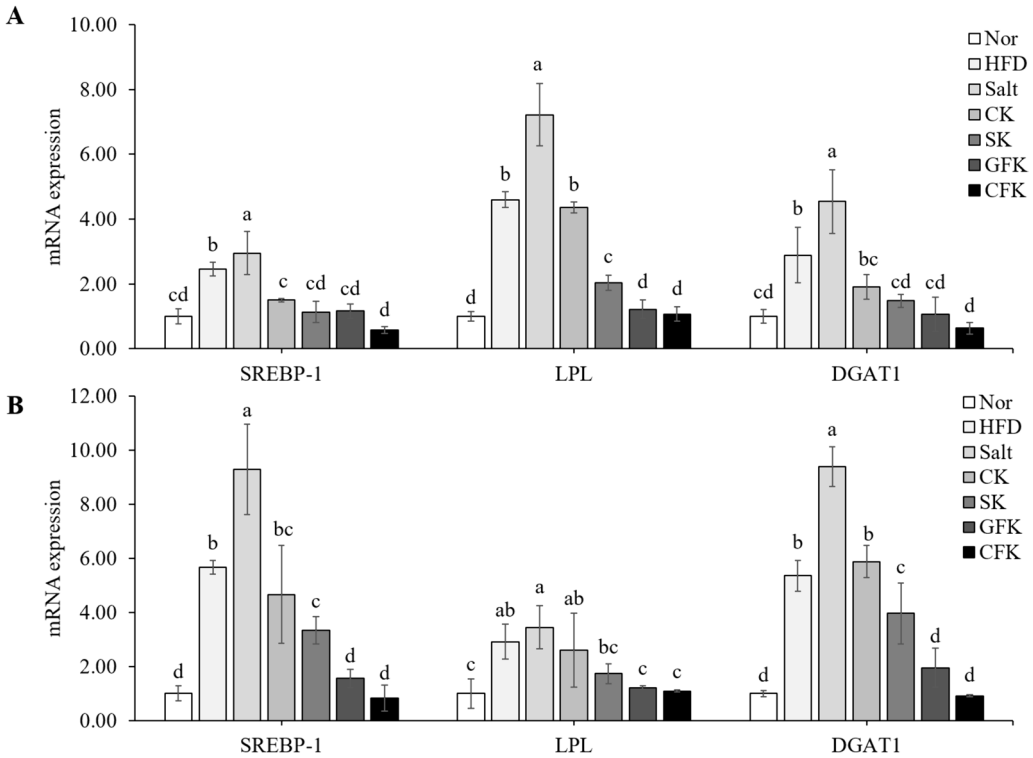

Fig. 2 Effects of CFK on the mRNA expression levels of lipogenesis-related genes in **A** liver tissues, and **B** epididymal tissues in high-fat diet (HFD)-induced obese mice. Group definitions are detailed in the legend of Fig. 1; SREBP-1, sterol regulatory element-binding protein-1; LPL, lipoprotein lipase; DGAT1, diacylglycerol O-acyltransferase 1. Data represent means ± SEs. [a–d]Mean values with different letters on the bars are significantly different ($p < 0.05$) according to Duncan's multiple-range test

(1.09 ± 0.04) exhibited significantly reduced expression levels of the DGAT1 genes compared to the Salt group. In addition, it was higher than in the SK (1.74 ± 0.37) and GFK (1.22 ± 0.07) groups ($p < 0.05$). These results were similar to the results of kimchi adding catechins, which reduces lipid accumulation in the liver and adipose tissues by overall reducing the levels of factors related to lipid synthesis [22]. According to previous study [22, 25], the body weight of CFK group (41.17 ± 3.00 g) decreased by 15.95% compared to the HFD group (48.98 ± 2.18 g) ($p < 0.05$), which appears to be due to a decrease in mRNA expression of lipogenesis-related genes.

Effects of CFK on mRNA expression of the lipolysis and inflammation-related genes

The gene associated with lipolysis, HSL was highest in the CFK group and significantly higher than the groups administered the other kimchi samples (Fig. 3A). Lipolysis is regulated by adipose triglyceride lipase (ATGL) and HSL [42], which acts on diglycerides in adipocytes to release fatty acid and glycerol molecules [43]. The HSL mRNA expression in the CFK group (2.98 ± 0.16) was significantly higher (814.50%) than that of the HFD group (0.33 ± 0.15) ($p < 0.05$) (Fig. 3A). In addition, it was significantly higher (2.45-fold) than in the CK (0.86 ± 0.10) and SK (0.99 ± 0.08) groups. Therefore, upregulation of HSL can be seen as promoting lipolysis. The gene associated with β-oxidation (CPT-1) was significantly higher in the CFK group (0.80 ± 0.15) than in the HFD group (0.58 ± 0.07) (Fig. 3A). CPT-1 mRNA expression was 4.25-fold higher in the CFK group than in the Salt group (0.19 ± 0.05) ($p < 0.05$). CPT-1 is an enzyme that plays an essential role in fatty acid oxidation and is regulated by acetyl-CoA carboxylase (ACC), a marker for final lipogenesis [44]. Therefore, this result was similar to the results of the study by Hong et al. [22], in which catechin and starter-added kimchi promoted HSL and CPT-1 expression.

CFK was effective in controlling the inflammation associated with HFD-mediated expression of inflammation-related genes in the epididymal fat tissues of mice ($p < 0.05$). Obesity is known to be associated with chronic inflammatory diseases [45]. The mRNA expression of the TNF-α gene was significantly lower in the CFK group (1.11 ± 0.29) than in the HFD group (2.72 ± 0.47) (Fig. 3B). The TNF-α mRNA expression was 2.88-fold lower in the CFK group than in the Salt group (3.18 ± 1.33) ($p < 0.05$). This was consistent with

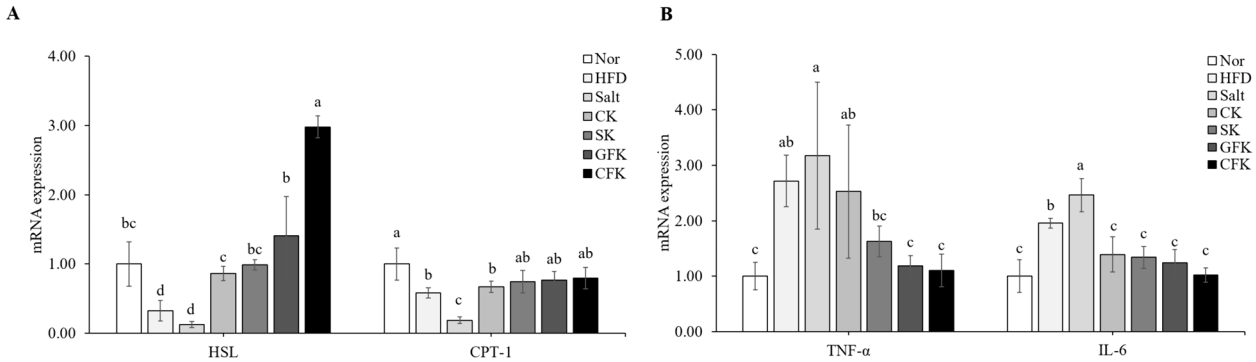

Fig. 3 Effects of CFK on the mRNA expression levels of genes related to **A** lipolysis, **B** inflammation, in epididymal tissues in high-fat diet (HFD)-induced obese mice. Group definitions are detailed in the legend of Fig. 1; HSL, hormone-sensitive lipase; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; CPT1, carnitine palmitoyltransferase I. Data represent means ± SEs. [a–d]Mean values with different letters on the bars are significantly different ($p < 0.05$) according to Duncan's multiple-range test

the results of Ziccardi et al. [46], who showed higher levels of TNF-α in adipose tissue and plasma of obese mice. The IL-6 mRNA expression was 52.15% lower in the CFK group (1.02 ± 0.13) than in the HFD group (1.96 ± 0.09) ($p < 0.05$). Cytokines are secreted by adipocytes and contribute to insulin resistance [47]. In this study, this result was similar to the results of the study by Woo et al. [48], who found that kimchi helps reduce the expression of inflammation-related factors, so it is thought to control obesity by suppressing the expression of these cytokines. These results imply that CFK inhibits adipogenesis and lipogenesis, reducing lipid accumulation in liver and adipose tissues.

Analysis of kimchi metabolites

Metabolite analysis was conducted on kimchi sample (3rd week) used in in vivo to confirm the differences. The data were statistically analyzed by PLS-DA (Fig. 4A), with good quality parameters ($R_2X = 0.658$, $R_2Y = 0.965$ and $Q_2 = 0.768$, $p = 8.86 \times 10^{-5}$) and acceptable cross-validation (when $R2 < 0.4$ and $Q2 < -0.1$) (Fig. 4B). The 0th week of SK, 3rd week of SK, 0th week of CFK, and 3rd week of CFK were separated significantly from each other, and changes in these metabolite profiles helped separate the four samples on the PLS-DA score map.

The heatmap (Fig. 5A) and box plot (Fig. 5B) were used to visualize differences in metabolite levels of the 0th week of SK, 3rd week of SK, 0th week of CFK, and 3rd week of CFK. The heatmap color represents the

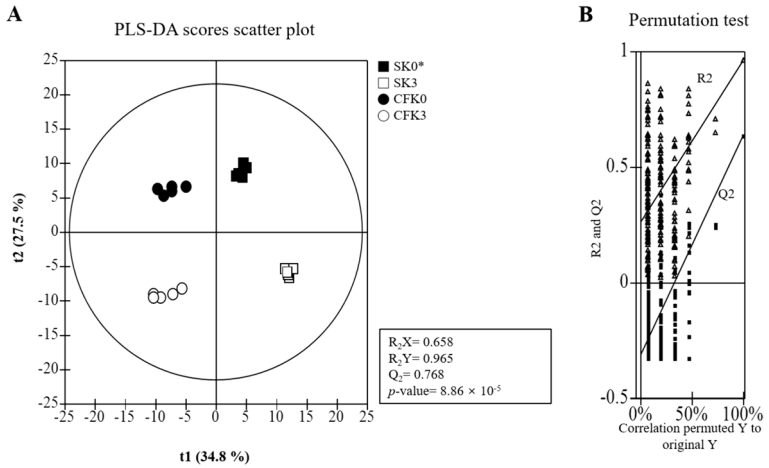

Fig. 4 Partial least squares discriminant analysis (PLS-DA) score plot of kimchi metabolites (**A**) and its quality parameters (**B**). Metabolites were analyzed using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC–Q-TOF MS). The statistical acceptability of the PLS-DA model was evaluated by R_2X, R_2Y, Q_2, and p value and validated by cross validation with a permutation test. SK0, 0th week of SK; SK3, 3rd week of SK; CFK0, 0th week of CFK; CFK3, 3rd week of CFK

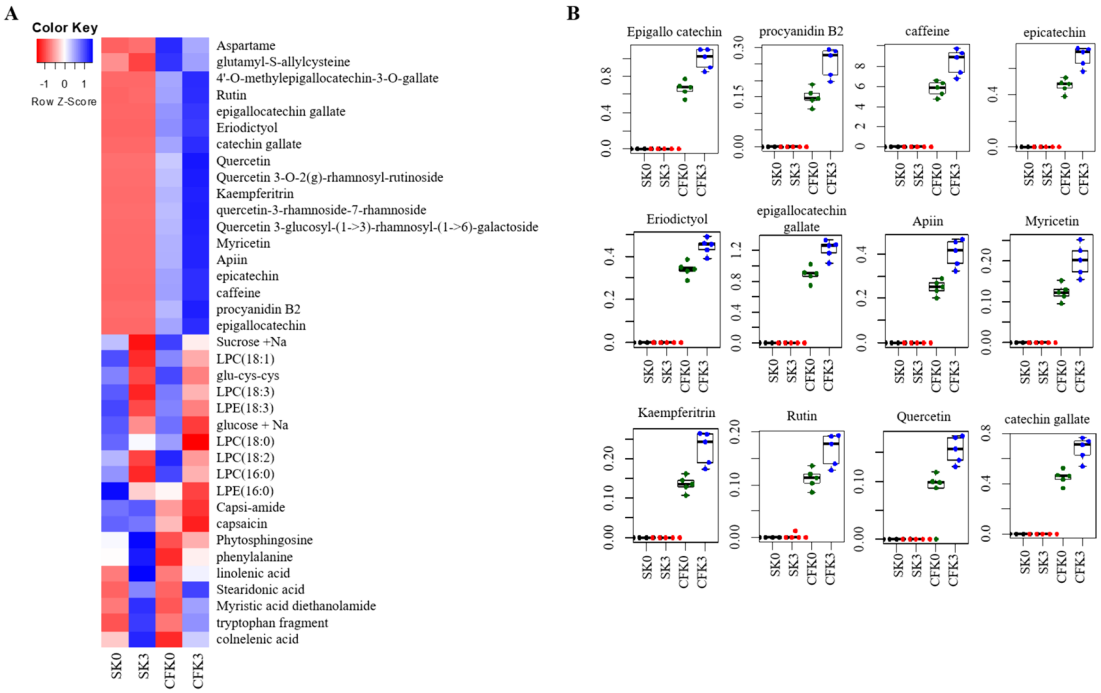

Fig. 5 Heatmaps (**A**) and box plot (**B**) of kimchi metabolites analyzed UPLC–Q-TOF MS. SK0, 0th week of SK; SK3, 3rd week of SK; CFK0, 0th week of CFK; CFK3, 3rd week of CFK. Y-axis is the relative abundance of compounds

z-score-transformed raw data of metabolites with a significant difference between both kimchi sample groups and is plotted on a blue-red color scale. The red and blue colors indicate an decrease and increase in metabolite levels, respectively (Fig. 5A).

From the box plot results, the active compounds with anti-inflammatory, cholesterol reduction, blood sugar reduction, body fat reduction, antioxidant, and anticancer functions such as epigallocatechin, procyanidin B2, caffeine, epicatechin, eriodictyol, epigallocatechin gallate, apiin, myricetin, kaempferitrin, rutin, quercetin, and catechin gallate were confirmed, and they were found to be significantly higher in CFK than in SK. In addition, more catechins were detected in the 3rd week of CFK than in 0th the week of CFK, which was similar to the results of study showing that an increase in the amount of catechins extracted increased when green tea was fermented using solar salt [49, 50]. Therefore, it is thought that as fermentation progresses, the active ingredients increase due to the combined action of kimchi subingredients (salt, catechin) and lactic acid bacteria.

Catechins have several pharmacological activities, such as anticancer, antitumor, antimutagenic, and anti-inflammatory activities [51]. Catechin gallate can inhibit the growth of adipocytes by inhibiting glucose absorption [52]. Epigallocatechin gallate (EGCG) is a major polyphenol and a major bioactive compound in green tea, and is a functional ingredient in foods and natural health products, often used in diet products related to obesity. Therefore, it has been reported that supplements containing EGCG may be a natural treatment option for obesity [53]. EGCG ester derivatives with anti-inflammatory functions can be used to prevent and treat inflammation-mediated diseases [54]. Eriodictyol, one of the flavonoids, exhibits anti-inflammatory and antioxidant activities and has protective effects on neurons, kidneys, and lungs [55]. Due to this role of eriodictyol, it is widely used to treat and prevent obesity and related complications [56]. Rutin may help strengthen and increase the flexibility of blood vessels such as arteries and capillaries [57]. Strengthening blood vessels can improve overall health by alleviating related conditions, including bruising, spider veins, and varicose veins. Quercetin is known to be a flavonoid that reduces blood pressure, blood sugar levels and fat accumulation [58, 59]. Myricetin is a natural flavonoid and has antioxidant and anticancer effects. It is also known to be effective against cardiovascular disease and diabetes [60]. The active ingredients of CFK are mainly substances related to reducing blood cholesterol and blood sugar, reducing body fat, and anti-inflammation. It is thought that these substances exert the effect of CFK regulating lipid metabolism. In a previously reported study [22, 25], it was confirmed that CFK increases *Bacteroidetes* and decreases *Firmicutes*. However, further research is

needed to determine how the active ingredients in CFK affect the gut microbiome.

In conclusion, this study confirmed that CFK is effective in suppressing lipid accumulation in HFD-induced mice by downregulating the expression of adipo-/lipogenesis-related genes and upregulating the expression of lipolysis-related genes in liver and epididymal tissues. Therefore, CFK has the potential to regulate lipid metabolism and prevent obesity and related metabolic diseases, which is thought to be due to the increased content of polyphenols such as caffeine. We plan to verify the specific mechanism and antiobesity effect of the active ingredient through future research.

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Author contributions
Geun-Hye Hong: Conceptualization, methodology, validation, formal analysis, data curation, writing original draft. So-Young Lee: Validation, data curation, visualization. Kun-Young Park: Conceptualization, writing—review and editing, resources, supervision, project administration.

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Availability of data and materials
The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate
This study was approved by the Institutional Animal Care and Use Committee of CHA University (Approval No. IACUC-200050).

Consent for publication
Not applicable.

Competing interests
There are no conflicts of interest in this article.

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References

1. Wilburn JR, Ryan EP. Fermented foods in health promotion and disease prevention: an overview. In: Frias J, Martinez-Villaluenga C, Penas E, editors. Fermented foods in health and disease prevention. London: Academic Press; 2017. p. 3–19.
2. Hong GH, Lee SY, Park ES, Park KY. Changes in microbial community by salt content in kimchi during fermentation. J Korean Soc Food Sci Nutr. 2021;50(6):648–53. https://doi.org/10.3746/jkfn.2021.50.6.648.
3. Park KY, Jeong JK, Lee YE, Daily JW III. Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. J Med Food. 2014;17(1):6–20. https://doi.org/10.1089/jmf.2013.3083.
4. Cheigh HS. History of kimchi. In: Cheigh HS, editor. kimchi culture and scientific technology Series 1. Seoul: Hyoil Publishing Co; 2002. p. 147–270.
5. Park KY, Ju JH. Kimchi and its health benefits. In: Park KY, Kwon DY, Lee KW, Park SM, editors. Korean functional foods: composition, processing and health benefits. Florida: USA: CRC Press; 2018. p. 43–73.
6. Park KY, Kim HY, Jeong JK. Kimchi and its health benefits. In: Frias J, Martinez-Villaluenga C, Penas E, editors. Fermented foods in health and disease prevention. London: Elsevier; 2017. p. 477–502.
7. Cheigh HS, Park KY. Biochemical, microbiological and nutritional aspects of kimchi. Crit Rev Food Sci Nutr. 1994;34(2):175–83. https://doi.org/10.1080/10408399409527656.
8. Park KY. The nutritional evaluation, and antimutagenic and anticancer effects of kimchi. J Korean Soc Food Sci Nutr. 1995;24(1):169–82.
9. Bong YJ, Jeong JK, Park KY. Fermentation properties and increased health functionality of kimchi by kimchi lactic acid bacteria starters. J Korean Soc Food Sci Nutr. 2013;42(11):1717–26.
10. Kim B, Park KY, Kim HY, Soon CA, Cho EJ. Anti-aging effects and mechanisms of kimchi during fermentation under stress-induced premature senescence cellular system. Food Sci Biotechnol. 2011;20:643–9. https://doi.org/10.1007/s10068-011-0091-9.
11. Park ES, Lee SM, Park KY. In vitro anti-obesity effects of kimchi prepared with solar salts without bittern on 3T3-L1 adipocytes. J Korean Soc Food Sci Nutr. 2018;47(2):9–234.
12. Choi SH, Kim HJ, Kwon MJ, Baek YH, Song YO. The effects of kimchi pill supplementation on plasma lipid concentration in healthy people. J Korean Soc Food Sci Nutr. 2001;30(5):913–20.
13. Kim JY, Lee YS. The effects of kimchi intake on lipid contents of body and mitogen response of spleen lymphocytes in rats. J Korean Soc Food Sci Nutr. 1997;26(6):1200–7.
14. Cui M, Kim HY, Lee KH, et al. Antiobesity effects of kimchi in diet-induced obese mice. J Ethnic Foods. 2015;2(3):137–44. https://doi.org/10.1016/j.jef.2015.08.001.
15. Kim HY, Park KY. Clinical trials of kimchi intakes on the regulation of metabolic parameters and colon health in healthy Korean young adults. J Funct Foods. 2018;47:325–33. https://doi.org/10.1016/j.jff.2018.05.052.
16. Park KY, Rhee SH. Functional foods from fermented vegetable products: kimchi (Korean fermented vegetables) and functionality. In: Shi J, Ho CT, Shahidi F, editors. Asian functional foods. USA: CRC Press; 2005. p. 341–80.
17. Park KY, Kim BK. Lactic acid bacteria in vegetable fermentation. In: Lahtnen S, Ouwehand AC, Salminen S, von Wright A, editors. Lactic acid bacteria. USA: CRC Press; 2012. p. 187–211.
18. Song GH, Park ES, Lee SM, Park DB, Park KY. Beneficial outcomes of kimchi prepared with Amtak Baechu cabbage and salting in brine solution: anticancer effects in pancreatic and hepatic cancer cells. J Environ Pathol Toxicol Oncol. 2018;37(2):151–61. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2018026204.
19. Yu T, Park ES, Song GH, Zhao X, Yi RK, Park KY. Kimchi markedly induces apoptosis in HT-29 human colon carcinoma cells. J Food Biochem. 2021;45(1):e13532. https://doi.org/10.1111/jfbc.13532.
20. Kim HY, Song JL, Chang HK, Kang SA, Park KY. Kimchi protects against azoxymethane/dextran sulfate sodium-induced colorectal carcinogenesis in mice. J Med Food. 2014;17(8):833–41. https://doi.org/10.1089/jmf.2013.2986.
21. Kim DW, Yang DH, Kim SY, Kim KS, Chung MG, Kang SM. Hypocholesterolemic effect of lyophilized, heat-killed *Lactobacillus rhamnosus* and *Lactobacillus plantarum*. Kor J Microbiol Biotechnol. 2009;37(1):69–74.
22. Hong GH, Lee SY, Yoo JI, Chung JH, Park KY. Catechin with lactic acid bacteria starters enhances the antiobesity effect of kimchi. J Med Food. 2023;26(8):560–9. https://doi.org/10.1089/jmf.2023.K.0067.
23. Lee JS, Lee MK, Ha TY, Bok SH, Park HM, Jeong KS, et al. Supplementation of whole persimmon leaf improves lipid profiles and suppresses body weight gain in rats fed high-fat diet. Food Chem Toxicol. 2006;44(11):1875–83. https://doi.org/10.1016/j.fct.2006.06.014.
24. Choi WY. Development of green tea added kimchi and its chemopreventive and antiobesity effects. PhD Dissertation, Pusan National University, Busan, Korea; 2001.
25. Hong GH. Anti-obesity effect of kimchi with the addition of lactic acid bacteria (LAB) and catechin on C57BL/6 mice. MS Thesis, CHA University, Gyeonggi, Korea; 2021.
26. Yu T, Park ES, Zhao X, Yi RK, Park KY. Lower Mg and S contents in solar salt used in kimchi enhances the taste and anticancer effects on HT-29 colon carcinoma cells. RSC Adv. 2020;10(9):5351–60. https://doi.org/10.1039/c9ra09032k.

27. Song GH, Park ES, Lee SM, Kim TY, Park KY. An atopic preventive drink (APD) reduces Th2 cytokines in LPS-treated RAW 264.7 cells. Tang. 2017;7:15.1-15.6. https://doi.org/10.5667/tang.2017.0014.
28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods. 2001;25(4):402–8. https://doi.org/10.1006/meth.2001.1262.
29. Gu EJ, Kim DW, Jang GJ, Song SH, Lee JI, Lee SB, et al. Mass-based metabolomic analysis of soybean sprouts during germination. Food Chem. 2017;217:311–9. https://doi.org/10.1016/j.foodchem.2016.08.113.
30. Long X, Kim YG, Pyo YK, et al. Inhibitory effect of Jangkanghwan (Korean traditional food) on experimental ulcerative colitis in mice. J Food Biochem. 2020;44(12):e13488. https://doi.org/10.1111/jfbc.13488.
31. Yu T. Kimchi prepared with organically cultivated baechu cabbage and washed-dehydrated solar salt enhance the anticancer and antiobesity effects. PhD Dissertation. CHA University, Gyeonggi, Korea; 2021.
32. Mota de Sá P, Richard AJ, Hang H, Stephens JM. Transcriptional regulation of adipogenesis. Compr Physiol. 2017;7(2):635–74. https://doi.org/10.1002/cphy.c160022.
33. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, et al. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev. 2002;16(1):22–6. https://doi.org/10.1101/gad.948702.
34. Tontonoz P, Hu E, Spiegelman BM. Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr Opin Genet Dev. 1995;5(5):571–6. https://doi.org/10.1016/0959-437x(95)80025-5.
35. Lee KH. Lactic acid bacteria starter fermented kimchi inhibits obesity and colitis-associated colon cancer in C57BL/6J mice. PhD Dissertation, Pusan National University, Busan, Korea; 2016.
36. Kao YH, Hiipakka RA, Liao S. Modulation of obesity by a green tea catechin. Am J Clin Nutr. 2000;72(5):1232–4. https://doi.org/10.1093/ajcn/72.5.1232.
37. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab. 2006;4(4):263–73. https://doi.org/10.1016/j.cmet.2006.07.001.
38. Shimano H. SREBPs: physiology and pathophysiology of the SREBP family. FEBS J. 2009;276(3):616–21. https://doi.org/10.1111/j.1742-4658.2008.06806.x.
39. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, et al. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J. 1996;15(19):5336–48.
40. Harris CA, Haas JT, Streeper RS, Stone SJ, Kumari M, Yang K, et al. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. J Lipid Res. 2011;52(4):657–67. https://doi.org/10.1194/jlr.M013003.
41. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, et al. Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat Genet. 2000;25(1):87–90. https://doi.org/10.1038/75651.
42. Gaidhu MP, Anthony NM, Patel P, Hawke TJ, Ceddia RB. Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. Am J Physiol Cell Physiol. 2010;298(4):C961–71. https://doi.org/10.1152/ajpcell.00547.2009.
43. Lafontan M, Langin D. Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res. 2009;48(5):275–97. https://doi.org/10.1016/j.plipres.2009.05.001.
44. Zammit VA. Carnitine palmitoyltransferase 1: central to cell function. IUBMB Life. 2008;60(5):347–54. https://doi.org/10.1002/iub.78.
45. Rocha VZ, Folco EJ. Inflammatory concepts of obesity. Int J Inflamm. 2011. https://doi.org/10.4061/2011/529061.
46. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over 1 year. Circulation. 2002;105(7):804–9. https://doi.org/10.1161/hc0702.104279.
47. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259(5091):87–91. https://doi.org/10.1126/science.7678183.
48. Woo M, Kim M, Noh JS, Park CH, Song YO. Preventative activity of kimchi on high cholesterol diet-induced hepatic damage through regulation of lipid metabolism in LDL receptor knockout mice. Food Sci Biotechnol. 2018;27(1):211–8. https://doi.org/10.1007/s10068-017-0202-3.
49. Yun H, Oh HJ, Choi SW. Difference of catechins extracted level when fermented sun-dried salt and green tea. J Korea Contents Assoc. 2012;12(11):278–85. https://doi.org/10.5392/JKCA.2012.12.11.278.
50. Yoon H, Choi CW. The influence of procyanidins, gallic acid and theaflavins extracted level when fermented sun-dried salt and green tea. J Korea Acad-Ind Coop Soc. 2014;15(6):3774–80. https://doi.org/10.5762/KAIS.2014.15.6.3774.
51. Zanwar AA, Badole SL, Shende PS, Hegde MV, Bodhankar SL. Antioxidant role of catechin in health and disease. In: Watson RR, Preedy VR, Zibadi S, editors. Polyphenols in human health and disease. London: Academic Press; 2014. p. 267–71.
52. Strobel P, Allard C, Perez-Acle T, Calderon R, Aldunate R, Leighton F. Myricetin, quercetin and catechin-gallate inhibit glucose uptake in isolated rat adipocytes. Biochem J. 2005;386(3):471–8. https://doi.org/10.1042/BJ20040703.
53. Wolfram S, Raederstorff D, Wang Y, Teixeira SR, Elste V, Weber P. TEAVIGOTM (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. Ann Nutr Metab. 2005;49(1):54–63. https://doi.org/10.1159/000084178.
54. Zhong Y, Chiou YS, Pan MH, Shahidi F. Anti-inflammatory activity of lipophilic epigallocatechin gallate (EGCG) derivatives in LPS-stimulated murine macrophages. Food Chem. 2012;134(2):742–8. https://doi.org/10.1016/j.foodchem.2012.02.172.
55. Xie Y, Ji R, Han M. Eriodictyol protects H9c2 cardiomyocytes against the injury induced by hypoxia/reoxygenation by improving the dysfunction of mitochondria. Exp Ther Med. 2019;17(1):551–7. https://doi.org/10.3892/etm.2018.6918.
56. Islam A, Islam MS, Rahman MK, Uddin MN, Akanda MR. The pharmacological and biological roles of eriodictyol. Arch pharm Res. 2020;43(6):582–92. https://doi.org/10.1007/s12272-020-01243-0.
57. Fusi F, Saponara S, Pessina F, Gorelli B, Sgaragli G. Effects of quercetin and rutin on vascular preparations: a comparison between mechanical and electrophysiological phenomena. Eur J Nutr. 2003;42(1):10–7. https://doi.org/10.1007/s00394-003-0395-5.
58. Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Galvez J, et al. In vivo quercetin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. Eur J Immunol. 2005;35(2):584–92. https://doi.org/10.1002/eji.200425778.
59. Egert S, Bosy-Westphal A, Seiberl J, Kürbitz C, Settler U, Plachta-Danielzik S, et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr. 2009;102(7):1065–74. https://doi.org/10.1017/S0007114509359127.
60. Ong KC, Khoo HE. Biological effects of myricetin. Gen Pharmacol. 1997;29(2):121–6. https://doi.org/10.1016/s0306-3623(96)00421-1.

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