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Original article

A Study on the Anti-obesity Effects of Original and Mutated Lentil Extracts and Arginine in a High-Fat Diet-Induced Obese Mouse Model

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ABSTRACT Lentils are a type of pulse crop recognized as a superfood, containing essential nutrients such as carbohydrate and proteins, as well as functional components like polyphenol, soyasaponins, and lipid-soluble compounds. A mutant population of the original lentil cultivar L8 was generated by breeding gamma-irradiated lentils. Through the feature-based molecular networking analysis, a mutant line L8-70-74(2) with improved dipeptidyl peptide-IV inhibitory activity was selected. This line was significantly enriched in arginine compared to the L8. Therefore, in a study involving an obese mouse model induced by a high fat diet (HFD), the anti-obesity effects of the 70 % acetone extracts of L8 and L8-70-74(2), along with two forms of arginine (L- or D-forms) were evaluated and compared. The administration of L8-70-74(2) and L-arginine led to a significant decrease in weight gain, the mass of gonadal adipose tissue, and the size of adipocytes. Moreover the administration of L8-70-74(2) and L-arginine were notably lowered calculated-low-density lipoprotein cholesterol and the serum level of adipokines (leptin and monocyte chemoattractant protein 1) in mice with obesity induced by HFD, while simultaneously increasing adiponectin levels. In addition, the administration of L8-70-74(2) and L-arginine resulted in the reduction of adipocyte area and the presence of crown-like structures within adipose tissue. These findings indicated that the selected mutant line and L-arginine may serve as potential sources for the prevention and treatment of obesity and metabolic disease.

Key words: Lentils, *Lens culinaris*, radiation-mutation, L-arginine, high-fat diet, anti-obesity

1. Introduction

Mutation breeding in lentils provided the benefit of improving genetic attributes while maintaining the superior characteristics of the original cultivar by inducing mutations artificially [1]. Gamma-irradiation has been widely used as a mutagenic method due to its effectiveness in crop improvement, particularly in enhancing agronomic traits, phenotypes, bioactivity, and functional ingredient content [2,3]. Approximately 19 mutant lentil (*Lens culinaris*; Fabaceae) cultivars have been officially registered in the Mutant Variety Database of the Food and Agriculture Organization/International Atomic Energy Agency (FAO/IAEA), with the majority being developed through the use of gamma irradiation [4]. Our research group generated hundreds of lentil mutant lines by applying different doses of gamma irradiation (70, 100, 200, and 300 kGy), aiming to develop functionally improved crop varieties through radiation breeding [5].

Lentils are a nutrient-dense pulse crop, containing high levels of protein, minerals, vitamins, and dietary fibers [6]. It also possess health-promoting properties due to the presence of en-

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riched polyphenols, such as phenolic acids, flavonols, flavan-3-ols, and anthocyanidins. These polyphenols exhibit antioxidant, antidiabetic, and cardiovascular protective effects [6,7]. Other bioactive phytochemicals, such as carotenoids, tocopherols, and saponins, have also been reported in lentils [8]. Additionally, recent studies have highlighted the antioxidant and anti-inflammatory activities of lentil hulls, as a useful by-product [9,10]. In our previous study, we evaluated the dipeptidyl peptide (DPP)-IV inhibitory activity and metabolites of 100 mutant lines developed by breeding lentil (L8) exposed to 200 kGy of gamma irradiation, identifying the mutant line L8-200-11 with improved DPP-IV inhibitory activity and its distinctive compound, 18 : 0 lyso-phosphatidylcholine. Their anti-obesity effects was subsequently validated [5]. In this study, we focused on 53 lentil mutant lines bred with 70 kGy of gamma irradiation and selected the mutant line L8-70-74(2), which demonstrated enhanced DPP-IV inhibitory compared to the original cultivar, for further investigation. DPP-IV is an enzyme that regulates glucose metabolism and inflammation and is associated with obesity and type 2 diabetes [11,12]. It is expressed in adipose tissues such as visceral fat, and its expression level correlates with adipocyte size and metabolic syndrome indicators [11]. Clinical studies have reported that serum DPP-IV concentrations in obese individuals are higher than those in individuals with a normal body mass index [13].

Feature-based molecular networking (FBMN) is a widely used analytical method for untargeted metabolomics data based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) [14]. Molecular networking is a method for visualizing and annotating the chemical space of untargeted mass spectrometry data and is based on the Global Natural Products Society Molecular Networking (GNPS) infrastructure, which uses chromatographic feature detection and alignment tools [15]. Feature relationships enable quantitative analysis and separation of molecules by network connectivity of structurally related molecules, identification of unknown ion-ligand complexes, and enrichment of annotations within molecular networks [16,17].

In this study, 70% acetone extracts of L8 (the original lentil cultivar) and the active mutant line L8-70-74(2) were subjected to untargeted metabolite analysis using LC-MS/MS. Through FBMN analysis, the molecular class of amino acids was derived differently from the L8-70-74(2) extract compared to the L8 extract. Therefore, the anti-obesity effects of the 70% acetone extracts of L8 and L8-70-74(2), L-arginine, and D-arginine were examined by assessing obesity-related indicators and histology in an obese mouse model induced by a high fat diet (HFD).

2. Materials and Methods

2.1. Plant Materials

The seeds of original lentil cultivar (L8) was obtained from the Rural Development Administration (RDA)'s Genebank (RDA Genebank number IT229628) and were cultivated for one year in an experimental field at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (KAERI) in Jeongeup-si, Korea. The seeds were then exposed to gamma irradiation with a dose of 70 kGy using a labeled cobalt (^{60}Co) source (150 TBq capacity; AECL, Ottawa, ON, Canada) at the same institute. 53 Gamma-irradiated lines were planted and grown in the experimental field at KAERI over a period of two years (2016-2018). The mutant line seeds were harvested in July 2018, and voucher specimens were deposited at the Advanced Radiation Technology Institute, KAERI.

2.2. Sample Preparation

The original lentil cultivar (L8) and a gamma-irradiated mutant line (L8-70-74(2)) were processed for analysis. Seeds were ground using a Geno/grinder (SPEX, New York, NJ, USA). A 100 g portion of each lentil powder was extracted with 100 mL of 70% acetone in an ultrasonic bath for 60 min, followed by freeze-drying to obtain the 70% acetone extract. For LC-MS/MS analysis, 1 mg of this dried extract was dissolved in 100 μL of methanol, centrifuged at 15,000 × g, and filtered using a 0.45-μm polyvinylidene fluoride (PVDF) filter. The final concentration of the stock solution was adjusted to 500 μg/mL with methanol. L-Arginine, D-arginine, and metformin were obtained from Sig-ma-Aldrich (St. Louis, MO, USA).

2.3. LC–MS/MS Analysis and Feature–Based Molecular Networking

The samples were analyzed using a Thermo Fisher UltiMate HPLC system (Thermo Fisher Scientific Inc., Waltham, MA, USA) in conjugation with a Thermo Scientific Q Exactive Focus Orbitrap LC-MS/MS system (Thermo Fisher Scientific Inc.) at the Core Research Support Center for Natural Products and Medical Materials (CRCNM) in Yeungnam University. Separation was achived on a Phenomenex Kinetex C18 column (2.1 mm × 100 mm i.d., 2.6 μm; Phenomenex, Torrance, CA, USA) employing a 7-min gradient elution. The mobile phase consisted of water (A) and acetonitrile (B), each containing 0.1% formic

acid. The gradient program was as follows: 0-7.0 min, 10-45% B; 7.0-12.0 min, 45-100% B; 12.0-16.0 min, 100% B; 16.0-16.1 min, 100-10% B; 16.1-19.0 min, 10% B. A 3 μL sample was injected at a flow rate of 0.3 mL/min. In MS analysis, electrospray ionization was performed in positive mode with these parameters: spray voltage 3.5 kV; capillary temperature 320°C; S-lens RF level 50; auxiliary gas heater temperature 350°C; resolution 70,000; scan range m/z 150-2000; ACG target 1×106. Data dependent MS/MS analysis was performed on the three most intense peaks per cycle with the following settings: resolution 17,500; normalized collision energy 30 eV; AGC target 5×104.

The raw high-resolution mass spectrometry (HRMS) data were converted to mzXML format using ProteoWizard 3.0.9935 [16]. The processed data were analyzed using MZmine2 (version 2.53) [16]. The results was exported as .mgf files and a quantitation table in CVS format for FBMN on the GNPS web platform (https://gnps.ucsd.edu, access on 7, October 2021) [15]. The FBMN analysis was performed with the following parameters: precursor ion mass tolerance of 0.02 Da, fragment ion mass tolerance of 0.02 Da, mini-mum cosine of 0.6, and a minimum of three matched fragment ions (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=597832d6e10b4cf3aff8df4ad06b1752). The resulting molecular network was visualized using Cytoscape (version 3.5.1) [17].

2.4. Animal Experiment

Male C57BL/6 mice (n=36, 6 weeks old) were obtained from Orient Bio, Seongnam-si, Republic of Korea. The animals were housed in a controlled environment with temperature (22±15°C), humidity (55±15%), and lighting (12 h light/dark cycle), with unrestricted access to food and drink. The mice were fed either a normal diet (D12451) containing 10% of calories from fat or a high-fat diet (D12450B) containing 45% of calories from fat, as detailed in Table 1. The mice were randomly assigned to one of seven experimental groups, each containing six animals. The groups included: the normal diet (ND) group, the high-fat diet (HFD) group, a group receiving 100 mg/kg of 70% acetone extract of the original lentil cultivar L8, a group receiving 100 mg/kg of 70% acetone extract of the radiation-mutated cultivar L8-70-74(2), a group receiving 100 mg/kg of L-arginine, a group receiving 100 mg/kg of D-argi-

Table 1. The composition of a normal diet and high-fat diet

Ingredient		Experimental Diet	
		Normal Diet (D12451)	High-Fat Diet
Casein, 30 mesh		200 g	200 g
L-Cystine		3 g	3 g
Com starch		315 g	72.8 g
Maltodextrin 10		35 g	100 g
Sucrose		350 g	172.8 g
Cellulose, BW200		50 g	50 g
Soybean oil		25 g	25 g
Lard		20 g	177.5 g
Mineral Mix S10026		10 g	10 g
Dicalcium phosphate		13 g	13 g
Calcium carbonate		5.5 g	5.5 g
Potassium citrate, H2O		16.5 g	16.5 g
Vitamin mix V10001		10 g	10 g
Choline bitartrate		2 g	2 g
FD&C yellow dye #5		0.05 g	-
FD&C yellow dye #40		-	0.05 g
Calorie (kcal%)	Carbohydrate	70%	35%
	Protein	20%	20%
	Fat	10%	45%

nine, and a group receiving 100 mg/kg of metformin. Mice in both the ND and HFD groups received oral administrated with 0.05% Tween-20 in PBS, whereas the treatment groups were given their specific treatments dissolved in 0.05% Tween-20 in PBS via oral gavage, administered five times per week for eight weeks. Body weight was recorded weekly throughout the study. At the conclusion of the experiment, mice were fasted overnight and then euthanized under anesthesia. The liver and visceral fat pads were extracted, and blood samples were collected for further analysis. The experimental protocol was approved by the Ethics Committee of the Advanced Radiation Technology Institute, KAERI (protocol code KAERI-IACUC-2021-9).

2.5. Biochemical Analysis

Blood samples were processed by centrifugation at 5000 × g for 20 min at 4 °C to separate the serum. Serum analysis was performed using a Fully Automatically Chemical Analyzer (DriChem NK500i, Fuji, Tokyo, Japan) to determine glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol. The concentration of calculated-low-density lipoprotein (c-LDL) cholesterol was calculated by the Friedewald formula: TC – HDL – (TG/5). The levels of adiponectin and leptin were assessed using commercially available kits (R&D system, Minneapolis, MN, USA), while the measurement of monocyte chemoattractant protein 1 (MCP-1) was conducted using a commercially available kit (BD Biosciences, Franklin Lakes, NJ, USA).

2.6. Histological Analysis

Epididymal adipose tissue samples were fixed in 10 % formaldehyde in neutral buffer (Sigma-Aldrich, MO, USA). Following fixation, the tissues were embedded in paraffin wax and sectioned. The sections underwent H&E staining were examined using a Moticam 2300 system (Motic Instruments, Richmond, BC, Canada). To determine the average adipocyte size, 70 adipocytes from each sample were analyzed.

2.7. Statistical Analysis

The data were statistically analyzed to determine the significance of differences be-tween groups using a Student's t test with a two-tails test, and the results are presented as the mean ± standard deviation (SD).

3. Results

A non-targeted metabolite analysis on the 70% acetone extracts of 54 lentil samples, including the original lentil cultivar (L8) and its gamma-irradiated mutant lines, was performed with the GNPS web platform to organize similar spectra into molecular families (Fig. 1a) and 36 MS features successfully annotated in GNPS spectral library (Table 1). Among the lentil samples, a mutant line L8-70-74(2) exhibited DPP-IV inhibitory activity, achieving 42.26% inhibition at a concentration of 100 μg/mL, whereas L8 showed less than 10% inhibition. Targeted cluster annotation for L8-70-74(2) is presented in Fig. 1b. The L8-70-74(2) displayed numerous spectral nodes associated with molecular families of amino acid derivatives, including the cluster of F. The pie charts of this cluster clearly indicated the predominance of arginine in the L8-70-74(2), presenting only the blue segment. When comparing the peak areas corresponding to arginine in the L8 and L8-70-74(2), the L8, it was confirmed that L8 was devoid of arginine, thus the L8-70-74(2) was selected as an arginine-rich mutant line. Based on this information, further *in vivo* studies using an obese mouse model induced by the HFD were planned for both the original cultivar L8 and the arginine-rich mutant line L8-70-74(2). In addition, since arginine, identified from the FBMN-based untargeted metabolite analysis, exists in two isomers (L- and D-forms), we planned to compare the anti-obesity efficacy of these two arginine conformations.

To explore the efficacies of the 70% acetone extracts of L8 and L8-70-74(2), L-arginine, and D-arginine in an obese mouse model induced by the HFD, these samples, along with the positive control, metformin (each 100 mg/kg/day), were administrated to C57BL/6 mice fed the HFD for 8 weeks. Mice consuming the HFD showed characteristic signs of obesity. Compared to mice fed the ND, those in the HFD group showed significant increase in body weight, weight gain, and gonadal adipose tissue weight; however these increase was counteracted by the administration of all samples (Fig. 2a). The body weight gain due to HFD in the L-arginine treatment group was significantly reduced, which was more effective than the administration of the positive control, metformin. The treatments of L8, L8-70-74(2), and D-arginine showed a slight weight loss compared to the HFD group, similar to the level of the metformin

Table 2. GNPS-based cluster-specific molecular annotations of metabolites in the original lentil cultivar and its gamma-irradiated mutant lines.

Group	Observed m/z	Matched library ID	Class
A	277.2157	9,12-Octadecadiynoic Acid	Fatty acid derivative
	293.2108	9(*S*)-HpOTrE	
	279.2318	Linolenic acid	
	315.2531	12,13-DiHOME	
	277.2162	9-Oxo-10*E*,12*Z*-octadecadienoic acid	
	353.2685	Monolinolenin (9c,12c,15c)	
	300.2896	Palmitoyl ethanolamide	
	324.2898	Linoleoyl ethanolamide	
	295.2262	13-Keto-9*Z*,11*E*-octadecadienoic acid	
	297.2424	9,10-Dihydroxy-12*Z*-octadecenoic acid	
	326.3053	*N*-Oleoylethanolamine	
	331.2848	1-Hexadecanoyl-sn-glycerol	
B	786.6004	1-Stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
C	420.1866	Phenylmethyl-6-*O*-[(*2R*,*3R*,*4R*)-tetrahydro-3,4-dihydroxy-4-(hydroxymethyl)-2-furanyl]-beta-D-Glucopyranoside	Carbohydrate
	325.1130	4-*O*-beta-Galactopyranosyl-D-mannopyranose	
	360.1502	Isomaltulose	
D	518.3212	1-Hexadecanoyl-*sn*-glycero-3-phosphocholine	Phosphatidylcholine
	544.3371	1-(9Z-Octadecenoyl)-*sn*-glycero-3-phosphocholine	
E	291.0865	Catechin	Flavonoid
	453.1394	2-[[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-3,4-dihydro-2*H*-chromen-3-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol	
F	175.1188	Arginine	Amino acid
G	480.3086	1-(9*Z*-Octadecenoyl)-*sn*-glycero-3-phosphoethanolamine	Phosphatidylethanolamine
	478.2945	4-[5-[[4-[5-[Acetyl(hydroxy)amino]pentylamino]-4-oxobutanoyl]-hydroxyamino]pentylamino]-4-oxobutanoic acid	
	454.2930	1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine	
H	518.3328	LPC-18:3	Phosphatidylcholine
	482.3247	1-Pentadecanoyl-*sn*-glycero-3-phosphocholine	
	524.3717	1-Stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine	
	522.3559	1-(9Z-Octadecenoyl)-*sn*-glycero-3-phosphocholine	
	468.3089	1-Myristoyl-*sn*-glycero-3-phosphocholine	
I	247.1442	Lenticin	Aromatic amino acid derivative
J	757.2191	5,7-Dihydroxy-2-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-[3,4,5-trihydroxy-6-[[3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	Flavonoid glycoside
K	328.1392	*N*-(1-Deoxy-1-fructosyl)phenylalanine	Aromatic amino acid derivative
L	205.0972	L-Tryptophan	Aromatic amino acid derivative
M	387.2015	4-Hydroxy-3,5,5-trimethyl-4-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxybut-1-enyl]cyclohex-2-en-1-one	-
N	182.0813	L-Tyrosine	Aromatic amino acid derivative
O	579.1501	Procyanidin B3	Procyanidin

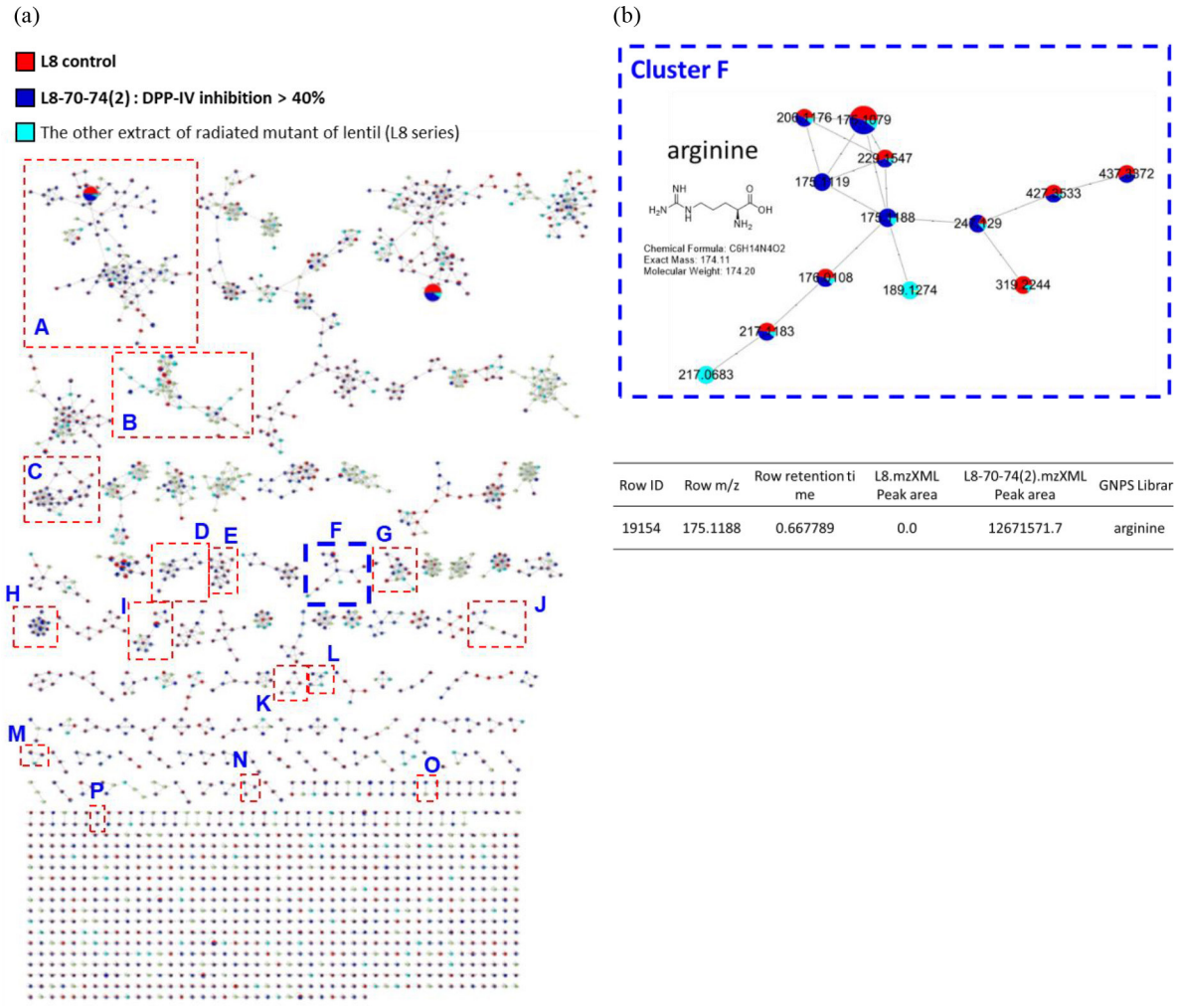

Fig. 1. Feature-based molecular networking of L8 and L8-70-74(2). (a) An entire molecular network of L8 and L8-70-74(2). (b) Targeted cluster annotation for L8-70-74(2) and annotated molecular node by comparison with Global Natural Product Social Molecular Networking library.

treatment group. By the end of the experiment, the HFD group had a significant 193% increase in the final body weight gain compared to that of the ND group (147%) (Fig. 2b). The L-arginine treatment group displayed a significantly lower weight gain rate by 162%. The administration of L8, L8-70-74(2), and D-arginine resulted in body weight gain rates of 178, 178, and 181%, respectively. At the conclusion of the study, liver and gonadal adipose tissues were extracted and weighed. The gonadal adipose tissue weight of the HFD group was more than doubled relative to the ND group, but this increase was significantly reduced by the administration of L8-70-74(2) and L-arginine (Fig. 2c). Moreover, L-arginine was more effective than the administration of the positive control, metformin.

Histological analysis of epididymis adipose tissue was conducted using Hematoxylin and Eosin (H&E) staining. The results revealed that mice in the HFD group exhibited enlarged adipocytes and a reduced number of cells per area compared to those in the ND group (Fig. 3a). The L-arginine treatment group demonstrated a significant reduction in adipocyte size, approximately half that of the HFD group (Fig. 3b), closely resembling the morphology of the ND group. Conversely, the L8, L8-70-74(2), and D-arginine treated groups showed a modest decrease in adipocyte size relative to the HFD group. These findings indicate that the L-arginine effectively mitigates adipocyte hypertrophy in HFD-induced obese mice, suggesting its potential therapeutic role in combating obesity.

The effects of the administrations of the samples on serum glucose level in mice fed the HFD were investigated. Compared with HFD group, which had elevated serum glucose level than the ND group, the administration of all samples reduced the

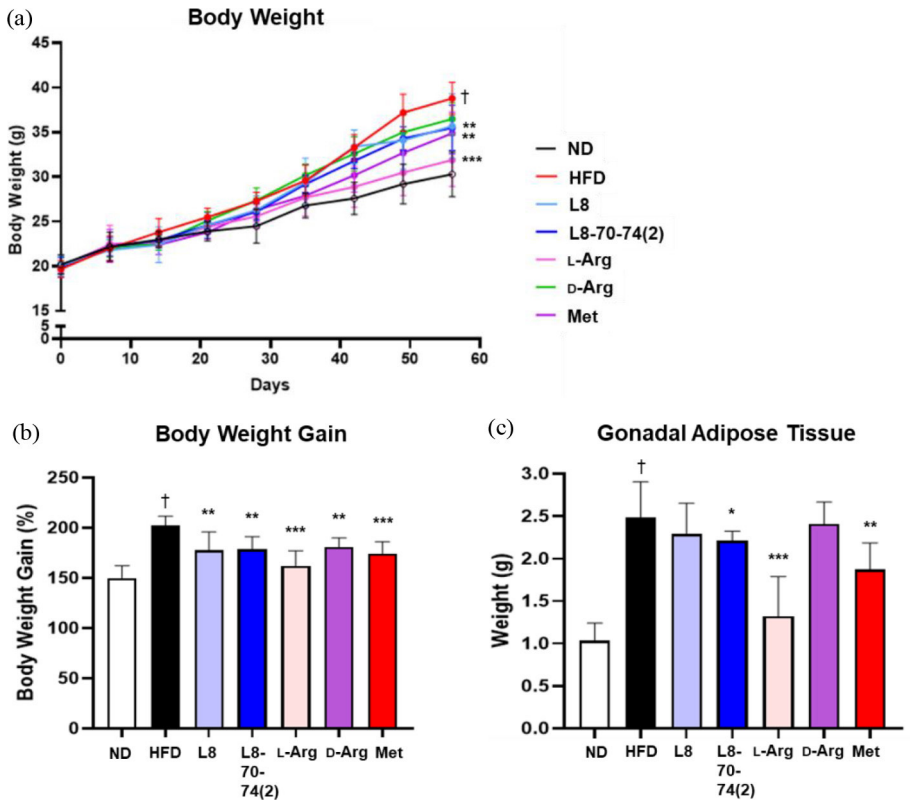

Fig. 2. Effects of samples on (a) body weight, (b) body weight gain, and (c) gonadal adipose tissue weight in mice fed with high-fat diet. ND, normal diet; HFD, high-fat diet; L8, the original lentil cultivar; L8-70-74(2), the lentil mutant line; L-Arg, L-arginine; D-Arg, D-arginine; Met, metformin. Data are presented as mean±SD (n=6). Significant differences were identified at $^{\dagger}p<0.001$, the ND group vs. the HFD group; $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$, the HFD group vs. the treatment group.

concentration of serum glucose, but this was significant only in the L8 and L-arginine groups (Fig. 4a). These findings suggest that the administration of 70% acetone extract of L8 and L8-70-74(2) and L-arginine can ameliorate glucose metabolism. To investigate whether the samples improved hyperlipidaemia in mice fed the HFD, the concentrations of serum TG, TC, HDL cholesterol were measured, and the levels of c-LDL cholesterol were calculated using the Friedewald formula (c-LDL = TC – HDL – (TG/5)), which input these values (Fig. 4b-e). Compared with the ND group, the HFD group displayed a significant increase in the levels of c-LDL cholesterol, which was significantly reduced by the treatment of L8-70-74(2) and L-arginine. TG levels significantly decreased by 21% in the L-arginine treated group compared to the HFD group. There was a significant decrease in the con-centration of TG in serum of all experimental groups. HDL levels significantly increased in the L8, L8-70-74(2), and D-arginine-treated groups. The observed decrease in TG levels and increase in HDL cholesterol levels in the L8-70-74(2) and L-arginine groups highlight the beneficial effects of these samples on lipid metabolism.

Obesity is associated with elevated levels of pro-inflammatory adipokines and reduced levels of anti-inflammatory adipokines, contributing to a chronic, low-grade inflammatory state [18]. This study investigated the effects of the samples on adipokine and chemokine levels in serum from mice fed the HFD. Leptin, a key adipokine produced by adipocytes, regulates pro-inflammatory cytokines and body weight [19]. The HFD group exhibited significantly increased serum leptin levels compared to the ND group. Bothe L8-70-74(2) and L-arginine treatments led to significant reductions in leptin concentration, outperforming the positive control, metformin. In contrast, L8 and D-arginine treatments showed only a mild decrease in serum leptin levels with no significant effect (Fig. 5a). Adiponectin, an anti-inflammatory adipokine secreted from adipose tissue, is known to be suppressed by pro-inflammatory cytokines, hypoxia, oxidative stress, and obesity-related conditions [20]. in the HFD group,

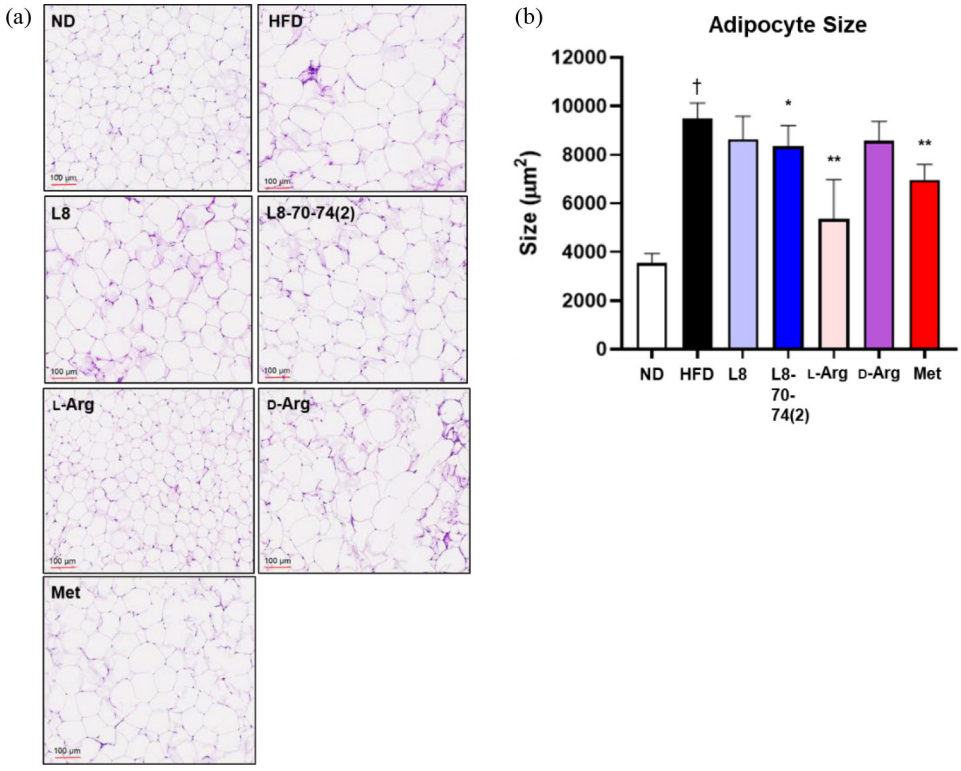

Fig. 3. Histological analysis of epididymal adipose tissue in high fat diet-fed mice. (a) Hematoxylin and eosin stained images of adipocytes from different groups (scale bar, 100 μm). (b) Distribution of adipocytes areas in adipose tissue (μm²). ND, normal diet; HFD, high fat diet; L8, the original lentil cultivar; L8-70-74(2), the lentil mutant line; L-Arg, L-arginine; D-Arg, D-arginine; Met, metformin. The results are presented as mean±SD (n=6). Significant differences were identified at †$p<0.001$, the ND group vs. the HFD group; *$p<0.05$ and **$p<0.001$, the HFD group vs. the treatment group.

serum adiponectin levels were significantly lower compared to the ND group. Administration of L8-70-74(2) and L-arginine significantly increased adiponectin levels compared to the HFD group, while L8 and D-arginine treatments had no effect on restoring adiponectin levels (Fig. 5b). MCP-1, known for recruiting monocytes and macrophages to inflammatory sites, is elevated in obesity-related disorders such as insulin resistance and type 2 diabetes [21,22]. The HFD group showed significantly elevated serum MCP-1 levels compared to the ND group. All experimental groups demonstrated a significant reduction in MCP-1 levels, with L8 and L8-70-74(2) extracts showing the most pronounced effect (Fig. 5c).

Macrophage infiltration occurs to remove dead adipocytes, forming crown-like structures (CLS) in gonadal adipose tissue of high fat diet-fed mice [23]. To evaluate the effects of sample treatment on alleviating macrophage infiltration of adipocytes, CLS, a visual marker of macrophage formation surrounding dead adipocytes, was measured in adipose tissue from mice fed a HFD (Fig. 6a). CLSs were observed in the HFD group, whereas they were hardly seen in ND group. The number of CLSs in HFD group was about 21, but administration of L8-70-74(2) and L-arginine significantly reduced them (Fig. 6b). The treatment of D-arginine did not affect the increase in the number of CLSs caused by the HFD. Therefore, these results suggest that L8-70-74(2) and L-arginine treatments could alleviate macrophage infiltration into adipose tissue.

4. Discussion

Obesity is a major global health issue, with nearly 2.5 billion adults aged 18 years and older classified as overweight or obese, contributing to the incidence of serious metabolic syndromes such as type 2 diabetes, hypertension, hyperlipidemia, cardiovascular diseases, and cancers [24,25]. Adipose tissue, acting as an active endocrine organ, plays a crucial role in regulating energy balance, metabolism, and inflammation [26]. It is consists of white adipose tissue (WAT), which stores energy and secretes adipokines such as leptin and adiponectin, and brown adipose

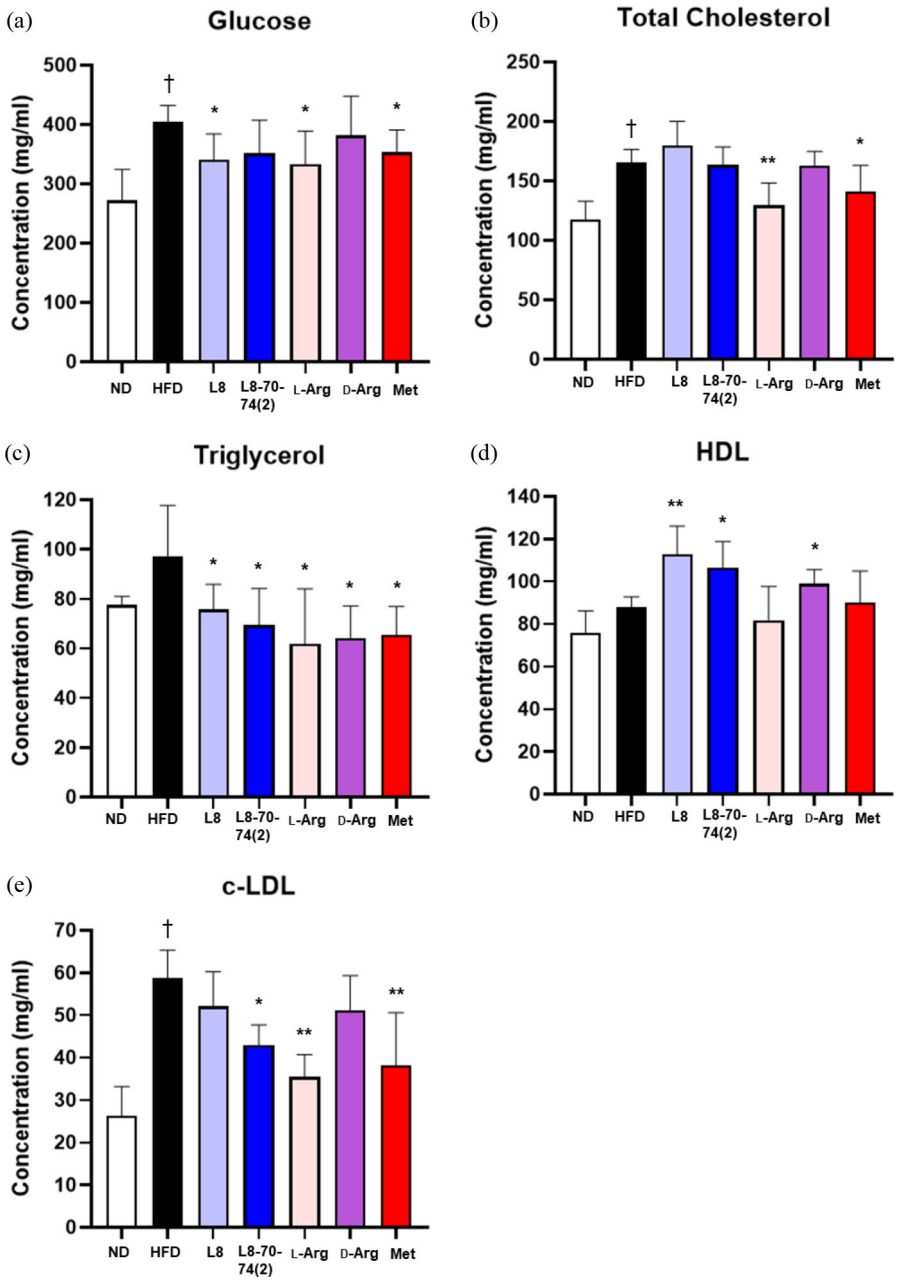

Fig. 4. Effects of Samples on biochemical parameters in serum of high-fat diet-fed mice: the concentrations of (a) glucose, (b) total cholesterol, (c) triglycerides, (d) high-density lipoprotein cholesterol, (e) calculated-low-density lipoprotein cholesterol. The results are presented as mean±SD (n=6). ND, normal diet; HFD, high fat diet; L8, the original lentil cultivar; L8-70-74(2), the lentil mutant line; L-Arg, L-arginine; D-Arg, D-arginine; Met, metformin. Significant differences were identified at $^{\dagger}p<0.01$, the ND group vs. the HFD group; $^{*}p<0.05$ and $^{**}p<0.01$, the HFD group vs. the treatment group.

tissue (BAT), which is involved in thermogenesis. Under obese conditions, adipose tissue undergoes pathological remodeling, leading to adipocyte death and immune cell infiltration, which is characterized by the formation of CLS.

Lentils are rich in prebiotic carbohydrates that serve as metabolic precursors for human microbiota activity, contributing to satiety, a low glycemic index (GI), and improved gut barrier function [27,28]. Previous studies have demonstrated the anti-diabetic effects of germinated lentils, showing improvements in blood glucose levels, serum-lipid profiles, and body weight in a streptozotocin-induced diabetic mouse models [29]. Additionally, cooked lentils have been shown to be more effective than

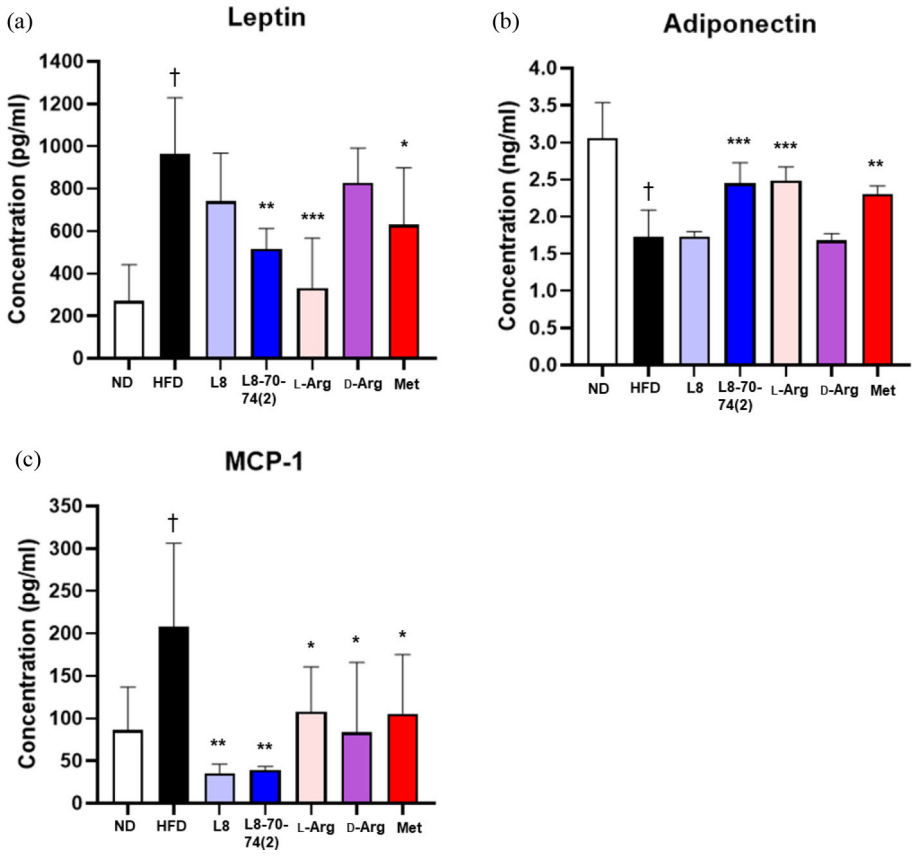

Fig. 5. Effects of samples on adipokines levels in serum of high-fat diet-fed mice. The concentrations of (a) leptin, (b) adiponectin, and (c) monocyte chemoattractant protein 1. ND, normal diet; HFD, high fat diet; L8, the original lentil cultivar; L8-70-74(2), the lentil mutant line; L-Arg, L-arginine; D-Arg, D-arginine; Met, metformin. The results are presented as mean±SD (n=6). Significant differences were identified at †p<0.001, the ND group vs. the HFD group; *p<0.05, **p<0.01, and ***p<0.001, the HFD group vs. the treatment group.

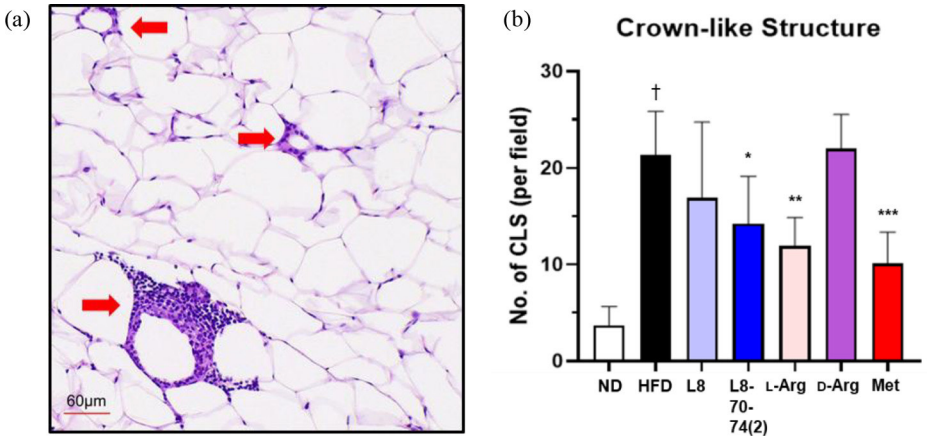

Fig. 6. The effects of samples on adipose tissue macrophage infiltration. (a) Hematoxylin and eosin (H&E) stained images for crown-like structure (CLS) from different group (scale bar, 100 μm), the arrows indicate CLS, (b) the quantification of CLS in adipose tissue (counts CLS per field; scale bar, 100 μm). The results are presented as mean±SD (n=6). ND, normal diet; HFD, high fat diet; L8, the original lentil cultivar; L8-70-74(2), the lentil mutant line; L-Arg, L-arginine; D-Arg, D-arginine; Met, metformin. Significant differences were identified at †p<0.005, the ND group vs. the HFD group; *p<0.005, **p<0.05, and ***p<0.5, the HFD group vs. the treatment group.

raw lentils in reducing the serum levels of glucose and lipids in diabetic rats [30]. In this study, we investigated the anti-obesity effects of lentils in a HFD mouse model for the first time. Our findings demonstrated improvements in body weight gain, serum glucose and lipid levels, and adipokine profiles. These results are supported by previous studies that have shown reductions in body weight, liver weight gain, and lipid deposition in a nonalcoholic fatty liver disease (NAFLD) model induced by dextran sulfate sodium (DSS) and HFD [31]. Our study also highlights the functional potential of the mutant line L8-70-74(2), as a source of anti-obesity agents. L8-70-74(2) exhibited significant improvements in adipokine profiles, characterized by reduced pro-inflammatory markers and increased anti-inflammatory markers. These findings suggest that L8-70-74(2) could serve as a promising functional ingredient for obesity management by modulating metabolic pathways and reducing inflammation associated with obesity.

Our study utilized FBMN analysis of the original and mutated lentils, combining it with DPP-IV inhibition screening to identify molecular families of amino acid derivatives as potential bioactive compounds. Among these, arginine emerged as a key bioactive component that significantly contributed to the enhanced activity observed in the mutant line compared to the original lentils. L-arginine is a prevalent isomer in nature and an essential amino acid serving as the primary precursor of nitric oxide (NO), a potent vasodilator and a neurotransmitter [32]. Numerous studies have documented the antioxidant, anti-inflammatory, cardiovascular-protective, and neuroprotective effects of L-arginine. Dietary L-arginine supplementation has demonstrated preventive effects against obesity by enhancing NO synthesis, reducing fat mass, and promoting the oxidation of energy substrates in skeletal muscle [33,34]. In contrast, D-arginine is considered metabolically inert during protein synthesis, and its biological activity has rarely been demonstrated, although studies have reported its neuroprotective properties and enrichment in the brain and breast milk [35]. Previous research has also elucidated the involvement of D-arginine in the L-arginine metabolic pathways [36]. To the best of our knowledge, this is the first study to compare the anti-obesity effects of L-arginine and D-arginine in an HFD mouse model. The study reconfirms the utility of L-arginine by demonstrating its superior anti-obesity effects over D-arginine. L-Arginine significantly reduced body weight gain, gonadal adipose tissue weight, and adipocyte size, whereas D-arginine showed no significant activities. In addition, while D-arginine did not exhibit any improvements, L-arginine produced alleviations in serum biochemical factors (glucose, total cholesterol, triglycerides, HDL, and c-LDL). When hypernutrition in adipocytes leads to increased leptin and decreased adiponectin, this imbalance promotes an inflammatory response and induces increased MCP-1 expression, leading to increased macrophage infiltration into adipose tissue. Furthermore, this inflammatory environment ultimately triggers the pathophysiological processes that lead to CLS formation [37]. L-Arginine drastically decreased the number of CLS compared with D-arginine. Although L-arginine has been reported to have the potential to suppress adipose tissue inflammation and CLS formation by modulating adipokine imbalance through the nitric oxide production pathway and reducing the expression of inflammatory chemokines such as MCP-1 [38], this comparative results underscore the importance of isomer specificity in nutritional interventions for obesity management. The enhanced metabolic effects observed with L-arginine treatment indicate its potential as a key bioactive compound in lentil-based functional foods for obesity management. The ability of L-arginine to modulate metabolic pathways and reduce inflammation makes it particularly valuable as a functional ingredient for combating obesity-related metabolic disorders.

5. Conclusion

This study provides scientific evidence for the anti-obesity effects of the mutant line L8-70-74(2) and bioactive compound derived therefrom. The comprehensive analysis demonstrates that this lentil mutant line exhibits significant metabolic improvements, including reduced body weight gain, improved lipid profiles, and modulation of adipokine levels. These effects are attributed to the enhanced content of L-arginine, which shows superior efficacy compared to its D-isomer counterpart. The study highlights L-arginine's multifaceted role in combating obesity through its ability to modulate metabolic pathways, reduce inflammation, and improve adipose tissue function. These findings suggest that the mutant line L8-70-74(2), with their enriched L-arginine content, hold significant promise as a functional food ingredient for obesity management. The comparative analysis between L- and D-arginine provides valuable insights into the importance of isomer specificity in nutritional interventions, reinforcing the therapeutic potential of L-arginine. Further research needs for developing practical applications of these findings, including the optimization of lentil processing methods to enhance L-arginine bioavailability and the develop-

ment of functional food products incorporating the mutant line L8-70-74(2) for obesity prevention and management.

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Ethical Approval

Ethical approval to report this case series was obtained from the Ethics Committee of Korea Atomic Energy Research Institute (approval number KAERI-IACUC-2021-009).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Ethics Committee of Korea Atomic Energy Research Institute (approval number KAERI-IACUC-2021-009) approved protocols.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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