Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Lactic acid bacteria-fermented product of green tea and *Houttuynia cordata* leaves exerts anti-adipogenic and anti-obesity effects

Li-Chun Wang [a,b], *Tzu-Ming Pan* [c,d,**], *Tsung-Yu Tsai* [a,e,*]

[a] *Ph.D. Program of Nutrition and Food Sciences, Fu Jen Catholic University, Taipei, Taiwan*
[b] *Continuing Education School, National Taitung Junior College, Taitung, Taiwan*
[c] *Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan*
[d] *R&D Division, Sunway Biotech Co., Ltd., Taipei, Taiwan*
[e] *Department of Food Science, Fu Jen Catholic University, Taipei, Taiwan*

ARTICLE INFO

Article history:
Received 18 August 2017
Received in revised form
19 November 2017
Accepted 25 November 2017
Available online 20 December 2017

Keywords:
Lactic acid bacteria
Green tea
Houttuynia cordata leaf
Anti-adipogenesis
Anti-obesity

ABSTRACT

Obesity is associated with higher risks of developing diabetes and cardiovascular disease. Green tea, rich in polyphenolic compounds such as epigallocatechin gallate (EGCG) and epigallocatechin (EGC), has been shown to display anti-obesity effects. *Houttuynia cordata* leaves have also been shown to exhibit anti-obesity effects due to their chlorogenic acid content. Lactic acid bacteria are able to increase the production of polyphenolic compounds. This study aims to develop a novel anti-obesity fermentation product by combining *H. cordata* leaf tea with green tea, using *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (NTU 101) for fermentation due to the advantages of bioconverting the polyphenolic compounds. The regulation of adipogenesis factors and the anti-obesity effect of the NTU 101-fermented tea were evaluated in an *in vitro* 3T3-L1 pre-adipocyte model and an *in vivo* obese rat model, respectively. The results show that the NTU 101-fermented tea, which contained higher EGCG, EGC, and chlorogenic acid levels than unfermented tea, was able to inhibit the lipogenesis of mature 3T3-L1 adipocytes by the stimulation of lipolysis. Furthermore, the body weight gain, body fat pad, and feeding efficiency of obese rats, induced with a high fat diet, were decreased by the oral administration of NTU 101-fermented tea. The significant anti-obesity effect was probably due to lipolysis. However, NTU 101 bacteria cells and EGCG may also act as functional ingredients to contribute to the anti-obesity effects of NTU 101-fermented products.

* *Corresponding author*. Department of Food Science, Fu Jen Catholic University, No. 510, Zhongzheng Rd., Xinzhuang Dist., New Taipei City, Taiwan. Fax: +886 2 2905 2540.
** *Corresponding author*. Department of Biochemical Science and Technology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei, Taiwan.
E-mail addresses: tmpan@ntu.edu.tw (T.-M. Pan), tytsai@mail.fju.edu.tw (T.-Y. Tsai).
https://doi.org/10.1016/j.jfda.2017.11.009

1. Introduction

The main cause of obesity is a greater intake than expenditure of energy, but there are various other causes including environmental factors (such as diet, lifestyle, lack of exercise, and medication), genetic factors, and psychological stresses. However, diet and lifestyle are considered the main causes of modern-day obesity. Obesity is believed to result from an increase in the number or size of adipocytes. The proliferation and differentiation of preadipocytes into mature adipocytes is a key stage in the development of obesity [1].

In *in vitro* studies, 3T3-L1 preadipocytes are mainly used to explore adipocyte differentiation and the associated molecular mechanisms. 3T3-L1 preadipocytes are a representative cell type for typical obesity and are the most widely used fat cells in studies on the differentiation of preadipocytes into adipocytes [2]. This differentiation results in triglyceride (TG) accumulation. During preadipocyte differentiation, substantial increases in gene expression of CCAAT/enhancer-binding protein α (*C/EBP*α) and peroxisome proliferator-activated receptor γ (*PPAR*γ) occur [3]. The mitogen-activated protein kinase (MAPK) pathway regulates the mRNA expression of *C/EBP*α and *PPAR*γ, which in turn inhibits the differentiation of 3T3-L1 preadipocytes into adipocytes [4]. When entering the differentiation stage, the characteristics of mature adipocytes become increasingly more apparent: externally, the cells change from fibrous to circular and intracellular lipid droplets accumulate [2,5].

Many natural products such as herbs and teas perform multiple health functions due to their functional compounds. However, these compounds in herbs and teas cannot be easily absorbed or used by the human body. These substances can undergo bioconversion mediated by microorganisms in which they are broken down into smaller molecules via enzymatic reactions to facilitate their utilization and health benefits [6–8]. For this reason, probiotics have been added to foods and Chinese herbal medicines for fermentation, to increase the nutrient content in foods and enhance the activity of the original ingredients, improve the nutritional value of the food products, and add new value [9]. It has been suggested that the addition of microorganisms into Chinese herbal medicine for fermentation results in the breakdown of the original ingredients, increasing the efficacy of the medicine [10], the alteration of the environmental factors for the original ingredients, increasing their bioavailability [9], or the synthesis of new active substances via microbial metabolism.

Lactobacillus paracasei subsp. *paracasei* NTU 101 (NTU 101), a strain isolated from infant feces, is resistant to stomach acid and bile in natural conditions. This study used NTU 101 to ferment herbal extracts (green tea and *Houttuynia cordata* leaves) and used bioconversion mechanisms to improve the levels of active ingredients in the fermented products. Green tea is rich in epigallocatechin gallate (EGCG), which was reported in a previous study to promote the expression of genes related to lipid oxidation in skeletal muscle [11]. *H. cordata* leaves are rich in chlorogenic acid, which has been shown to confer beneficial effects on lipid and glucose metabolism. It was suggested that the administration of chlorogenic acid to rats fed a high-fat diet will lead to the reduction of serum TG and cholesterol levels [12]. We used NTU 101 to ferment green tea and *H. cordata* leaf tea and aimed to increase EGCG and chlorogenic acid levels through NTU 101 bioconversion during the fermentation process. We thereby aimed to produce a fermented product, rich in NTU 101, EGCG, and chlorogenic acid. We further investigated the effects of the fermented product on body fat reduction, with the expectation that the results of this study may help to develop a new type of health food that contains lactic acid bacteria and can reduce body fat.

2. Materials and methods

2.1. Chemicals

LC grade acetonitrile, chloroform, methanol, and dimethyl sulfoxide were purchased from Merck Co. (Darmstadt, Germany). Tryptone, yeast extract, peptone, malt extract, potato dextrose agar, and Bacto-agar were purchased from Difco Co. (Detroit, MI, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). Dexamethasone, isobutylmethylxanthine, insulin, oil-red O, heparin, and *p*-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trypan blue stain was purchased from Gibco BRL Life Technologies Inc. (Gaithersburg, MD, USA).

2.2. Microorganism and seed cultures

L. paracasei subsp. *paracasei* NTU 101 (NTU 101; DSMZ 28047), originally isolated from an infant [13], was maintained on MRS agar at 37 °C for 48 h. Seed cultures were prepared by transferring a loopful of colony from MRS agar into a 500-mL glass bottle containing 500 mL MRS medium. The cultures were incubated at 37 °C for 48 h. After that, inoculum sizes of 5% were transferred to a submerged cultured substrate.

2.3. Bioconversion fermentation of herbal tea L. paracasei subsp. paracasei NTU 101

H. cordata leaf (36 g) and green tea (36 g) were mixed and extracted with RO water (2 L) at 95 °C for 1 h for the preparation of the herbal tea. This tea was defined as the unfermented herbal tea and a portion was set aside and used as a sample in the animal test after freeze-drying. After cooling, the herbal tea (2 L) was used as the bioconversion fermentation medium for NTU 101. The cultures were incubated at 37 °C for 10 h. After submerging the culture, the NTU 101-fermented tea was dried by freeze dryer. The dried product was analyzed for viable cell number, EGCG, epicatechin gallate (ECG), and chlorogenic acid respectively. It was also used as samples in the cell test and animal test for the evaluation of its anti-adipogenesis and anti-obesity effects.

2.4. Determination of EGCG, ECG, and chlorogenic acid

The NTU 101-fermented tea and the unfermented herbal tea (10%, w/v) were filtered with a 0.45 μm pore sized filter and analyzed by high-performance liquid chromatography (HPLC, Model L-2130, Hitachi Co., Tokyo, Japan) on a C_{18} column

(25 cm × 4.6 mm i.d., 5 μm, Kanto Chemical Co. Inc, Tokyo, Japan) using the gradient elution. HPLC was performed in triplicate according to methods described previously. EGCG, ECG, and chlorogenic acid were separated by gradient elution using a mobile phase with the composition of 9% acetic acid solution (solvent A) and methanol (solvent B) (0–4 min, 0% solvent A; 4–30 min, 0–0.1% solvent A). The flow rate was set at 0.8 mL/min. Cordycepin and adenosine were detected using a photodiode array detector (Model L-2455 DAD, Hitachi Co.) set at full wavelength (230–450 nm).

2.5. Cell culture and test substances treatment

3T3-L1 preadipocytes purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan) were cultured in DMEM containing 10% fetal bovine serum at 37 °C in 5% CO_2. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with 0.5 mM isobutylmethylxanthine, 1 mM dexamethasone, and 10 mg/mL insulin (MDI) added to a basal medium. On day 2, the MDI medium was replaced with basal medium containing insulin only. On day 4 and thereafter, the cells were cultured in basal medium, which was freshly changed every 2 days until the cells were analyzed.

The test substance (NTU 101-fermented tea, EGCG, or chlorogenic acid) was diluted to various concentrations with DMEM medium, and further used as the treatment medium in the cell experiments. The 3T3-L1 pre-adipocytes were treated with test substances during their differentiation stage (day 1–day 8). Differentiated 3T3-L1 cells on day 8 were analyzed using oil red O stain for adipocyte differentiation. The fully differentiated 3T3-L1 adipocytes (days 8–12 after differentiation induction) were treated with the test substances for the measurement of lipolysis effects and heparin-releasable lipoprotein lipase (HR-LPL) activity.

2.6. Animal experiments

Animal experiments followed the protocol described in our previous study which involved an evaluation of the anti-obesity effects of NTU 101-fermented tea [6]. Male Sprague Dawley (SD) rats at 6–8 weeks of age were purchased from the BioLasco Co. (Taipei, Taiwan). The animals were housed individually and allowed free access to a standard laboratory chow (Ralston Purina, St Louis, MO, USA) and water. Three weeks later, the rats were randomly assigned to one of the following diets for 8 weeks: standard chow (control group, NOR; 4.5% fat, 3.34 kcal/g), high-fat (HF) diet consisting of 26.7% butter powder (Gene Asia Biotech Co., Ltd., Nang-Tou, Taiwan) in standard chow (HF group; 30% fat, 4.17 kcal/g), HF diet plus 568 mg/day kg B.W. unfermented herb tea powder including 12.46 mg/day EGCG (T-1X group), HF diet plus 284 mg/day kg B.W. NTU 101-fermented tea including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101 (101T-0.5X group), HF diet plus 568 mg/day kg B.W. NTU 101-fermented tea including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101 (101T-1X group), HF diet plus 7.5×10^{10} CFU NTU 101/day 100 g B.W. (101 group), and HF diet plus 25.01 mg/day 100 g B.W. EGCG (EGCG group). The recommendation dosage of NTU 101-fermented tea for anti-obesity effects is suggested as 250 mL/day for a human. The doses of the test substances used in this study were calculated according to Boyd's formula for body surface area for adult humans (weight: 65 kg; height: 170 cm). Each sample was orally administrated to the rats by stomach tube.

Food consumption and body weight were recorded weekly. At the end of the study, the rats were deprived of food for 16 h before being scarified by CO_2 asphyxiation. Blood samples were collected from the posterior vena cava and centrifuged at 700 × *g* for 10 min; the serum was stored at −20 °C until analyzed. Perirenal and epididymal fat pads were removed and weighed. Portions of the adipose tissue were immersed in 10% formaldehyde for histological inspection; other portions were frozen immediately in liquid nitrogen and stored at −80 °C for analysis of lipolysis and HR-LPL activity. The liver was excised and stored at −20 °C for the measurement of lipids. The experiment was reviewed and approved by the Animal Care and Research Ethics Committee of the National Taitung University.

2.7. Biochemical analyses

The serum total cholesterol (TC), TG, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), creatinine, uric acid, Na, K, aspartate aminotransferase, and alanine aminotransferase levels, as well as ketone body (hydroxybutyrate) concentrations were measured using commercial kits (Randox Laboratories Ltd., Antrim, UK).

2.8. Adipose tissue histology

The adipose tissue samples were fixed in formaldehyde, embedded in paraffin, cut into 5-mm sections and stained with hematoxylin and eosin. The cross-sectional area of the adipocytes was calculated from the histogram according to Chen and Farese [14]. For the estimation of fat pads cell number, the lipid content of 0.3 g of fat tissue was extracted using the method described by Folch et al. [15]. The total cell number in the fat pads was calculated by dividing the lipid content of the fat pad by the mean weight of cell lipids. The lipid weight of the average fat cell was calculated from the mean cell volume, assuming a lipid density of 0.915 (the density of triolein).

2.9. Oil-red O staining

Differentiated 3T3-L1 cells on day 8 were fixed with 10% formaldehyde and then stained with oil-red O. Pictures were taken using a microscope (ECLIPSE TS100; Nikon Co., Tokyo, Japan) [16].

2.10. Lipolysis assay

The fully differentiated 3T3-L1 adipocytes (days 8–12 after differentiation induction) were treated with the test substances in Krebs Ringer bicarbonate (KRB) buffer (20 mM NaCl, 4.7 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and 2% BSA; pH 7.4) for 24 h. Adipose explants (0.1 g) of perirenal and epididymal fat pads from experimental rats were incubated in 1 mL of KRB buffer at

37 °C for 1 h [17]. Glycerol was determined enzymatically from the supernatant using a Randox kit.

2.11. Heparin-releasable lipoprotein lipase (HR-LPL) activity assay

After incubation of the 3T3-L1 mature adipocytes with the experimental medium for 24 h, the medium was discarded. The cells were rinsed with KRB buffer and then cultured in heparin-KRB (10 U/mL heparin) at 37 °C for 1 h. The conditioned heparin-KRB was collected from each well for the assay of HR-LPL activity. In the animal study, a sample of perirenal and epididymal adipose tissue weighing 0.1 g was placed in 1 mL of KRB buffer supplemented with 10 U/mL heparin at 37 °C for 1 h. LPL activity was measured on the basis of its esterase properties using *p*-nitrophenyl butyrate as a substrate, according to the methods described in our previous study [16].

The TG hydrolase activity of LPL with synthetic TG substrates is inhibited by molar sodium chloride, and this property has been used to distinguish LPL activity from the activities of other lipases in plasma. Thus, HR-LPL activity was calculated from the productivity of *p*-nitrophenol using the following equation [16].

$$C\ (\mu M) = [A_{400}\ (0.15\ M\ NaCl) - A_{400}\ (1\ M\ NaCl)]/0.012$$

where A_{400} (0.15 M NaCl) and A_{400} (1 M NaCl) were the absorbances of released *p*-nitrophenol at 400 nm in 0.15 M and in 1 M NaCl assay buffer, respectively, and 0.012 is the micromolar extinction coefficient of *p*-nitrophenol.

2.12. Determination of PPARγ and C/EBPα levels

The PPARγ and C/EBPα levels in the adipose tissue were determined with an ELISA kit from Mybiosource Inc. (San Diego, CA, USA), according to the manufacturer's protocol.

2.13. Statistics

Data are expressed as means ± standard deviation (n = 3 in the cell test; n = 8 in the animal test). Furthermore, the independent batches, non-parametric approach of one-way analysis of variance (ANOVA) by Duncan's test was conducted using SPSS version 10.0 software (SPSS Institute, Inc., Chicago, IL, USA). Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Comparison of functional compound production between unfermented leaf extract and co-fermentation product subsection

This study used *L. paracasei* subsp. *paracasei* NTU 101 to ferment a mixed medium of *H. cordata* leaf and green tea. The lactic acid bacteria were used to mediate the bioconversion of polyphenolic compounds in the tea to increase the contents of EGCG, ECG, and chlorogenic acid in the NTU 101-fermented product.

When NTU 101 was cultured in the green tea-*H. cordata* leaf mixture, the density reached 3×10^9 CFU/mL (Table 1). Compared to the unfermented medium, in the NTU 101-fermented mixture, the chlorogenic acid concentration increased from 38 mg/L to 46.9 mg/L, the EGCG concentration increased significantly from 482 mg/L to 968 mg/L, and the ECG concentration increased from 107.9 mg/L to 159 mg/L, which suggests that fermentation mediated by NTU 101 results in a substantial increase in polyphenols. An explanation for this may be that NTU 101 could convert the polyphenolic compounds in green tea and *H. cordata* leaves, thereby increasing the EGCG, ECG, and chlorogenic acid contents. Moreover, this study further explored the effects of the NTU 101-fermented product on the reduction of body fat and the inhibition of adipocyte differentiation.

3.2. Effect of fermented products on 3T3-L1 preadipocyte differentiation

During the process of conversion from preadipocytes to adipocytes, differentiation is the main step. Thus, inhibition of the differentiation step would help to reduce the production of adipocytes. This study explored whether the NTU 101-fermented product was able to effectively inhibit preadipocyte differentiation.

Oil Red O staining was performed to examine the effects of different concentrations of the NTU 101-fermented tea on 3T3-L1 preadipocyte differentiation. An increase in the concentration of the NTU 101-fermented solution gradually increased its inhibitory effects on cell differentiation (Fig. 1). The extent and degree of staining were reduced compared to those of the control group, which suggests that the NTU 101-fermented tea significantly inhibited 3T3-L1 preadipocyte differentiation; moreover, a higher dose resulted in a stronger inhibitory effect on differentiation. Further analysis of the amount of Oil Red O staining in adipocytes showed a trend similar to the staining pattern.

Table 1 – The comparison of functional compounds production between unfermented tea and NTU 101-fermented tea.

Components	Unfermented tea (Before fermentation)	NTU 101-fermented tea (After fermentation)
EGC (μg/mL)	107.9 ± 12.3	159.6 ± 12.6
EGCG (μg/mL)	482.4 ± 34.5	968.3 ± 59.5
Chlorogenic acid (μg/mL)	38.5 ± 2.7	46.9 ± 5.3
NTU 101 (10^9 CFU/mL)	0	3.3 ± 0.3
pH	6.5	3.2

The leaf of green tea and *Houttuynia cordata* were extracted with 95 °C for 1.5 h for the preparation of unfermented tea. After cooling, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (5×10^7 CFU in 10 mL) was inoculated in the 2 L unfermented tea (initial cell number: 2.5×10^4 CFU/mL), and further cultured at 37 °C for 10 h for the production of NTU 101-fermented tea.

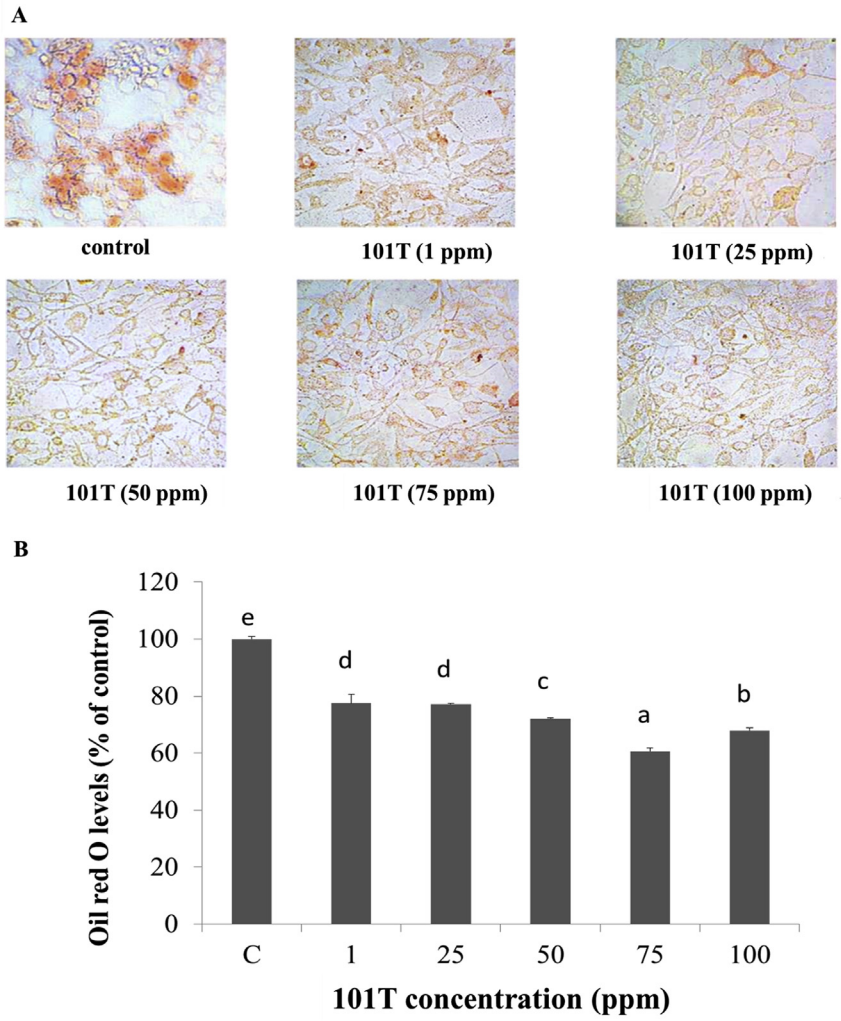

Fig. 1 – Effect of NTU 101-fermented tea on the lipid droplet accumulation (A) and oil red O levels (B) during the differentiation of 3T3-L1 preadipocyte.

3.3. *Effect of fermented products on lipogenesis of mature 3T3-L1 adipocytes*

After differentiation, the mature adipocytes undergo lipogenesis and increase in size. The lipases in adipocytes control the breakdown of lipids. Increased activity of lipases triggers a lipolytic effect, which results in the breakdown of TG lipid droplets in cells and the movement of the products out of the cells to be metabolized, resulting in smaller adipocytes. The results in Fig. 2A show that with increasing concentrations of the unfermented tea (T group) and the NTU 101-fermented tea, the lipolytic effects became increasingly significant ($p < 0.05$). The lipolytic effect of the NTU 101-fermented tea was significantly higher than that of the unfermented tea (T group) at all concentrations examined. In the comparison between the two functional ingredients, EGCG enhanced the lipolytic effect to a greater extent than that by chlorogenic acid. The results suggest that the promotion of the lipolytic effect by the NTU 101-fermented tea might be related to the higher levels of EGCG and chlorogenic acid in the fermented tea.

LPL is the key enzyme involved in lipid storage in adipose tissues. It breaks down TG into free fatty acids that enter the cells for re-synthesis into TG, resulting in lipid droplet accumulation and hence adipocyte hypertrophy [10]. The results in Fig. 3A show that the unfermented tea (T group), the NTU 101-fermented tea, and their constituents (EGCG and chlorogenic acid) did not significantly affect the activity of HR-LPL. Therefore, the inhibitory effect of the NTU 101-fermented tea on lipogenesis did not occur through the suppression of lipid droplet formation, but instead through enhancement of the lipolytic effect.

3.4. *Effect of NTU 101-fermented tea on body weight, weight gain, food intake, and feeding efficiency in high-fat diet-fed obese SD rats*

To explore the effect of the NTU 101-fermented tea on body fat reduction, this study further used high-fat diet-induced obese rats to evaluate the efficacy and main functions of the NTU 101-fermented tea, NTU 101, and the active ingredient EGCG in body-fat reduction.

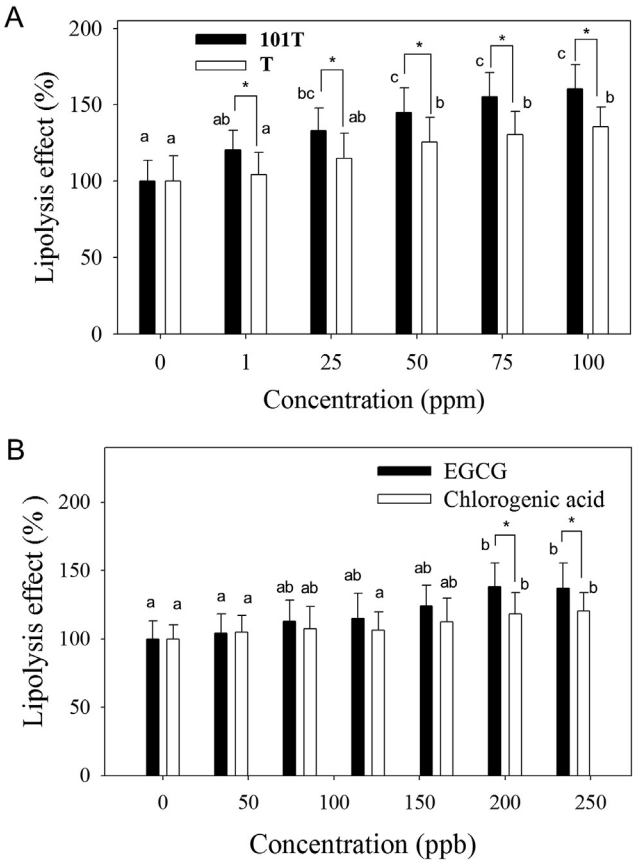

Fig. 2 – Comparisons of the effects between unfermented tea and NTU 101-fermented tea (A) and the effects between EGCG and chlorogenic acid (B) on lipolysis of mature 3T3-L1 adipocyte.

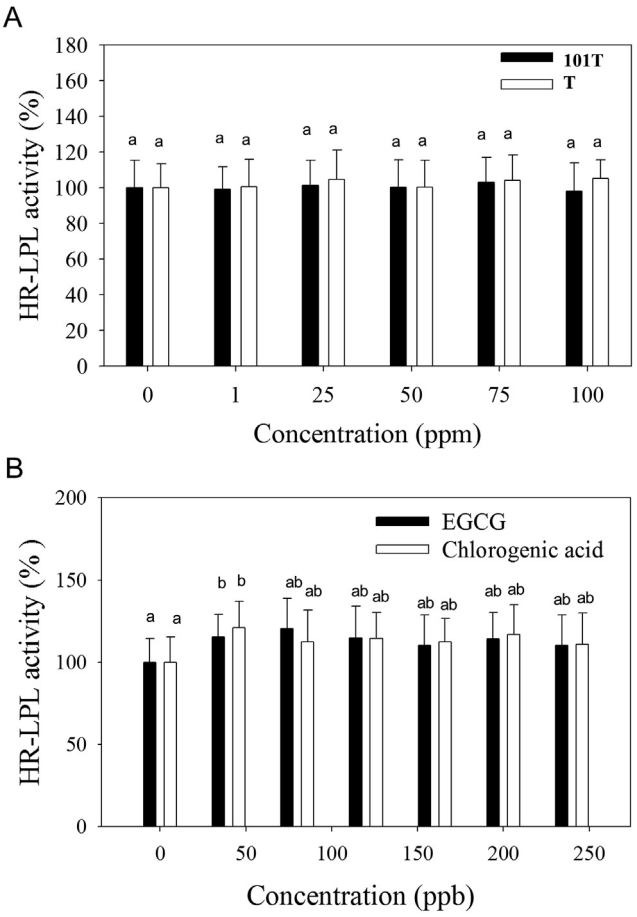

Fig. 3 – Comparisons of the effects between unfermented tea and NTU 101-fermented tea (A) and the effects between EGCG and chlorogenic acid (B) on lipogenesis effects (B) of mature 3T3-L1 adipocyte.

The effects of the NTU 101-fermented tea on the body weight gain of rats fed a high-fat diet are shown in Table 2. The starting body weights between groups were not significantly different ($p > 0.05$). After 8 weeks of feeding, their body weight significantly increased ($p < 0.05$). The 101T-0.5X and 101T-1X groups showed the greatest inhibitory effect on body weight gain, and their results were not significantly different from those of the normal diet group ($p > 0.05$). In addition, there was no significant difference in body weight inhibition between the T and HF groups ($p > 0.05$). The unfermented green tea and *H. cordata* leaf extracts were unable to reduce weight gain ($p > 0.05$), which indicated that only the co-fermentation and bioconversion of *H. cordata* leaves and green tea, mediated by NTU 101, could help to achieve the maximum inhibition of weight gain.

The effects of the NTU 101-fermented tea on the food intake and feeding efficiency of rats fed a high-fat diet are described in Table 3. The food intake of the high-fat diet group was lower than that of the control group. The explanation for this may be the higher calorie intake of the high-fat diet group, which in turn reduced food intake. In addition, the feeding efficiency of the high-fat diet group was significantly improved compared with that of the normal diet group ($p < 0.05$), suggesting that the intake of a high-fat diet resulted in greater body weight gain. As shown in Table 3, the values of feeding efficiency were lowest in the 101T-0.5X and 101T-1X groups treated with a high-fat diet (14.94 ± 1.70% and 14.99 ± 1.34%, respectively), and they were significantly lower than those of the HF, 101, and EGCG groups ($p < 0.05$). The results indicate that treatment with the NTU 101-fermented tea significantly increased food intake, but the feeding efficiency declined, suggesting that *H. cordata* leaves and green tea inhibit feeding efficiency. With regard to the pure substances, the NTU 101 bacteria and EGCG had no effects on food intake, but tended to decrease feeding efficiency ($p > 0.05$).

3.5. *Effect of NTU 101-fermented tea on perirenal fat pad weight and epididymal fat pad weight in high-fat diet-fed obese SD rats*

As shown in Table 4, the rat perirenal and epididymal fat pad weights increased significantly, owing to the long-term high-fat diet ($p < 0.05$). However, in the low-dose group (101T-0.5X group) and the high-dose group (101T-1X group) of the NTU 101-fermented tea treatment, significantly lower fat pad weights were observed ($p < 0.05$). The effect of the NTU 101 treatment on body-fat reduction was similar to that of the

Table 2 – Effect of NTU 101-fermented tea on body weight, and weight gain in obese SD rats fed with high fat diet.

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)
NOR	317.63 ± 6.91^{a}	504.00 ± 22.27^{a}	183.38 ± 19.21^{a}
HF	315.00 ± 6.14^{a}	545.00 ± 24.73^{b}	235.75 ± 26.8^{b}
T-1X	317.88 ± 6.10^{a}	547.38 ± 13.93^{b}	223.88 ± 12.70^{b}
101T-0.5X	317.75 ± 6.45^{a}	524.00 ± 29.21^{ab}	190.13 ± 20.44^{a}
101T-1X	315.25 ± 6.43^{a}	512.88 ± 30.06^{a}	184.25 ± 18.56^{a}
101	318.00 ± 6.16^{a}	551.88 ± 29.61^{b}	215.28 ± 26.38^{b}
EGCG	317.75 ± 6.27^{a}	548.88 ± 32.93^{b}	213.38 ± 25.05^{b}

NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

Table 3 – Effect of NTU 101-fermented tea on food intake and feed efficiency in obese SD rats fed with high fat diet.

Groups	Food intake (g)	Feed efficiency (%)
NOR	1711.50 ± 64.30^{d}	11.10 ± 0.74^{a}
HF	1357.88 ± 89.91^{b}	17.20 ± 1.66^{c}
T-1X	1438.63 ± 56.11^{c}	15.93 ± 0.81^{bc}
101T-0.5X	1297.50 ± 54.05^{ab}	14.94 ± 1.70^{b}
101T-1X	1235.38 ± 68.42^{a}	14.99 ± 1.34^{b}
101	1364.88 ± 83.33^{b}	16.68 ± 1.50^{c}
EGCG	1352.38 ± 67.30^{b}	16.30 ± 1.69^{bc}

NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

101T-0.5X treatment, which indicated that NTU 101 itself had a strong inhibitory effect on the growth of fat pads that was more significant than that of the unfermented green tea and *H. cordata* leaves (T group) and the EGCG treatment.

3.6. *Effect of NTU 101-fermented tea on the cross-sectional area and number of adipocytes in male SD rats fed a high-fat and high-cholesterol diet*

The cell cross-sectional area and cell number of the perirenal and epididymal fat pads of the HF group were significantly higher than those in the normal diet group ($p < 0.05$) (Table 5). After the administration of each test material, the cross-sectional area and cell number of adipocytes showed a significant decrease ($p < 0.05$). At the same dose, the effect of the NTU 101-fermented tea on the cell cross-sectional area and cell number was more significant than that of the unfermented tea (T group) ($p < 0.05$). NTU 101 and EGCG in the NTU 101-fermented tea also significantly decreased the cell cross-sectional area and cell number of adipocytes ($p < 0.05$). The results indicated that the NTU 101-fermented tea had a greater inhibitory effect on adipocyte proliferation and adipocyte enlargement than that of the unfermented tea (T group). This may be attributable to a synergistic effect of NTU 101 and EGCG.

Table 4 – Effect of NTU 101-fermented tea on perirenal fat pads weight and epididymal fat pads weight in obese SD rats fed with high fat diet.

Groups	Perirenal fat pads weight (g)	Epididymal fat pads weight (g)
NOR	6.21 ± 0.83^{a}	6.23 ± 1.21^{a}
HF	12.32 ± 2.96^{d}	10.57 ± 2.19^{d}
T-1X	10.24 ± 0.91^{cd}	9.51 ± 1.01^{cd}
101T-0.5X	8.97 ± 1.36^{bc}	7.54 ± 0.97^{ab}
101T-1X	8.16 ± 1.30^{b}	7.09 ± 0.95^{ab}
101	9.65 ± 1.43^{bc}	8.34 ± 1.67^{bc}
EGCG	11.71 ± 1.01^{cd}	10.8 ± 0.96^{d}

NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

3.7. *Effect of NTU 101-fermented tea on transcription factor expression of differentiation in high-fat diet-fed obese SD rats*

Both C/EBPα and PPARγ are important transcription factors in preadipocyte differentiation. The expression of transcription factors promotes the successful differentiation of preadipocytes into mature adipocytes [2,3]. Therefore, the inhibition of transcription factor expression can effectively suppress the differentiation process of preadipocytes. As shown in Fig. 4, a high-fat diet markedly promoted the expression of these two transcription factors, and treatment with the experimental materials in this study clearly resulted in their reduction. Compared with the unfermented tea (T group), the NTU 101-fermented tea more significantly reduced C/EBPα and PPARγ protein expression ($p < 0.05$). The examination of each active ingredient showed that NTU 101 had no significant effect on the expression of C/EBPα and PPARγ transcription

Table 5 – Effect of NTU 101-fermented tea on cell cross-sectional area and cell number of adipocyte in male SD rats fed with high-fat and high-cholesterol diet.

	Perirenal fat pads		Epididymal fat pads	
	Cell cross-sectional area (μm^2)	Cell number $\times 10^4$	Cell cross-sectional area (μm^2)	Cell number $\times 10^4$
NOR	16,456 ± 2089^{ab}	8.33 ± 0.81^{a}	16,443 ± 2493^{a}	7.92 ± 1.18^{a}
HF	25,355 ± 2405^{c}	17.42 ± 2.04^{c}	24,988 ± 1072^{c}	16.11 ± 1.82^{c}
T-1X	20,453 ± 3493^{b}	13.52 ± 1.66^{b}	19,030 ± 2494^{b}	12.02 ± 1.75^{b}
101T-0.5X	19,422 ± 3077^{b}	11.38 ± 1.90^{b}	18,381 ± 2361^{ab}	11.15 ± 1.20^{b}
101T-1X	15,882 ± 2850^{a}	8.39 ± 1.34^{a}	15,922 ± 1630^{a}	8.51 ± 0.90^{a}
101	18,385 ± 2455^{b}	12.45 ± 1.60^{b}	18,052 ± 2011^{ab}	12.07 ± 1.63^{b}
EGCG	18,650 ± 2047^{b}	12.30 ± 1.55^{b}	17,012 ± 2301^{ab}	12.38 ± 1.78^{b}

NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

factors ($p > 0.05$). In addition, because EGCG synthesized by NTU 101 bioconversion had a significant inhibitory effect on transcription factor expression ($p < 0.05$), the increased significance of the effect of the NTU 101-fermented tea compared with that of the unfermented tea (T group) is thought to result mainly from the higher content of EGCG.

3.8. *Effect of NTU 101-fermented tea on the lipolysis effect and HR-LPL activity in the adipose tissue of the high-fat diet-fed obese SD rats*

The lipases in adipose tissue can breakdown TG and subsequently elevate the lipase activity to reduce body fat gain [18]. As shown in Fig. 5A, the lipase activity of the HF group was significantly increased compared with that of the NOR group ($p < 0.05$). The increased body fat in the animal activated lipase activity. In both the low-dose and high-dose NTU 101-fermented tea groups, the lipase activity was significantly higher than that in the HF group ($p < 0.05$). However, the lipase activity of the unfermented tea group was not significantly different from that of the HF group ($p > 0.05$). Moreover, EGCG also demonstrated a significant lipase activity increasing effect. Thus, in the NTU 101-fermented tea, the increased EGCG level, owing to the bioconversion of the green tea polyphenolic compounds, further reduced body fat by increasing lipase activity.

In adipose tissues, LPL is the key enzyme for lipid storage [19]. High-fat and refined carbohydrate diets can increase LPL activity in the adipose tissue while decreasing LPL activity in the skeletal muscle; therefore lipids tend to move to adipose tissues for storage, which promotes the occurrence of obesity [20]. As shown in Fig. 5B, the materials examined did not cause an obvious change in HR-LPL activity; thus, it was concluded that these materials did not reduce lipid storage through the regulation of HR-LPL activity.

3.9. *Effect of NTU 101-fermented tea on liver triglycerides and total cholesterol levels in high-fat diet-fed obese SD rats*

A high-fat diet can also lead to the occurrence of a fatty liver, which results in the accumulation of cholesterol and TG in the liver. As shown in Table 6, the liver TG and TC levels were significantly elevated owing to the long-term intake of the high-fat diet. At equivalent doses, the administration of NTU 101-fermented tea significantly decreased the concentrations of TG and TC in the liver, compared to treatment with the unfermented tea ($p < 0.05$). Of the functional ingredients, both NTU 101 and EGCG had an effect on the improvement of liver lipids, although the effect was not as strong as that of the NTU 101-fermented tea after co-fermentation. The results suggested that, among the main functional ingredients of NTU 101-fermented tea for fatty liver prevention, NTU 101 and EGCG improved the condition of fatty liver, but the effect was not equivalent to that of the NTU 101-fermented tea.

3.10. *Effect of NTU 101-fermented tea on TBARS levels in the liver of high-fat diet-fed obese SD rats*

Thiobarbituric acid reactive substances (TBARS) are the main indicator of lipid peroxidation. Fig. 6 shows that, compared with the normal diet group, an 8-week period of high-fat diet administration caused lipid accumulation in the liver. The results showed that TBARS levels were significantly decreased in the 101T-1X group to values similar to the TBARS levels in the NOR group, indicating that 101T-1X could effectively inhibit lipid peroxidation. However, the lipid peroxidation caused by the high-fat diet in the 101, EGCG, and T groups did not improve.

4. Discussion

The fermentation of green tea and *H. cordata* leaf tea by *L. paracasei* subsp. *paracasei* NTU 101 significantly increased the levels of the EGCG, ECG, and chlorogenic acid via bioconversion. These ingredients formed only at the early stages of fermentation. After the NTU 101 entered the stationary phase, the content of these ingredients gradually decreased. Therefore, control of the fermentation time is a key element of this experiment. Studies have reported that these ingredients have the potential to reduce body fat [21,22]. This study aimed to improve the levels of these active ingredients to achieve body fat reduction.

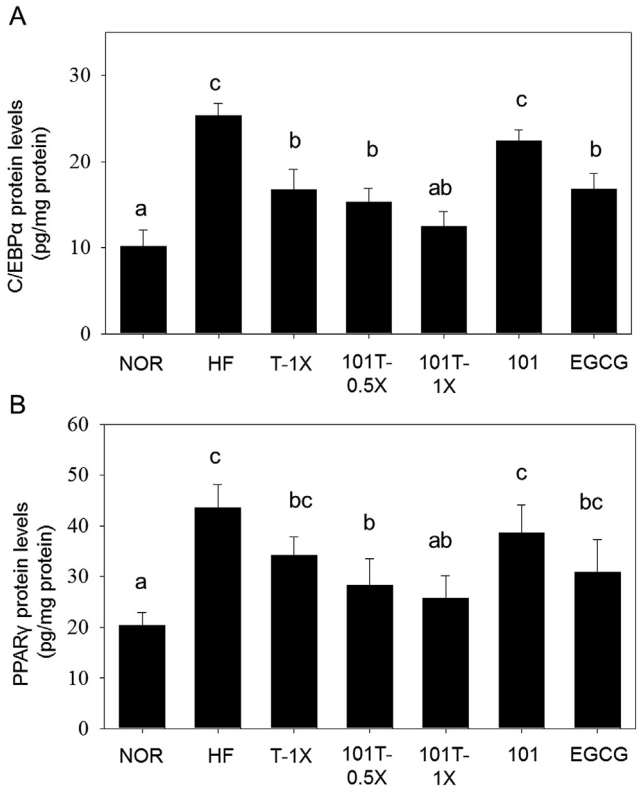

Fig. 4 – Effect of NTU 101-fermented tea on the protein expression of transcription factors C/EBPα (A) and PPARγ (B) in the adipose tissue of the obese SD rats fed with high fat diet.NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75 × 10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5 × 10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5 × 10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

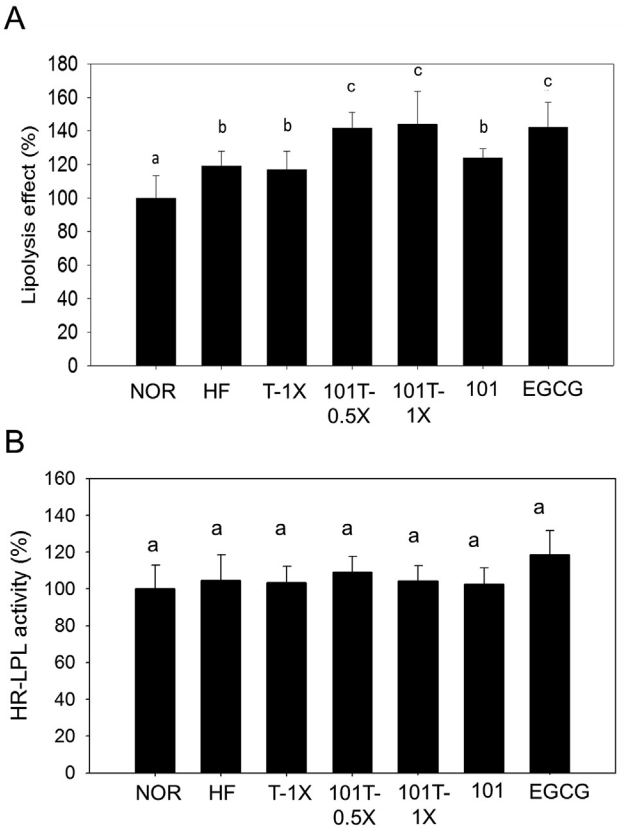

Fig. 5 – Effect of NTU 101-fermented tea on lipolysis (A) and lipogenesis effects (B) in the adipose tissue of the obese SD rats fed with high fat diet. NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75 × 10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5 × 10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5 × 10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

Various studies of NTU 101 have been conducted. In terms of its health effects, NTU 101 significantly reduced liver and serum TC levels [23]. In addition, by increasing the superoxidase dismutase (SOD) level and decreasing lipid peroxidation, NTU 101 effectively prevented and delayed oxidative stress and atherosclerosis caused by hyperlipidemia [24]. NTU 101 has also been found to have bioconversion capabilities, which increase the physiological activity of fermented products. In soymilk fermented by NTU 101, glucoside isoflavones are converted into substances with higher physiological activities; namely, aglycone isoflavones. Past studies have found that the NTU 101-fermented soymilk could reduce the formation of aorta lipid plaque in rats fed with a high-fat diet, as well as decreasing cell cross-sectional area and cell number, with the ability to inhibit lipogenesis and regulate adipocytes [25]. In addition, NTU 101-fermented soymilk and bitter gourd could delay the oxidative stress and inflammatory response caused by hyperlipidemia, and significantly decreased the LDL-C ratio and increased the HDL-C ratio, reducing atherosclerosis development [26]. In summary, NTU 101 have not only a variety of health effects, but also a capability for bioconversion.

Previous studies have also demonstrated that EGCG inhibits 3T3-L1 preadipocyte proliferation and differentiation and reduces lipid droplet accumulation [21]. In a high-fat diet-

Table 6 – Effect of NTU 101-fermented tea on liver triglyceride and total cholesterol levels in obese SD rats fed with high fat diet.

Groups	TG levels (mg/g liver tissue)	TC levels (mg/g liver tissue)
NOR	65.35 ± 10.53^{d}	15.66 ± 1.32^{a}
HF	80.64 ± 12.34^{b}	20.12 ± 1.34^{c}
T-1X	75.23 ± 10.43^{c}	18.64 ± 1.55^{bc}
101T-0.5X	76.46 ± 13.48^{ab}	18.35 ± 1.75^{b}
101T-1X	69.53 ± 12.95^{a}	16.35 ± 1.62^{b}
101	72.45 ± 14.60^{b}	16.42 ± 1.26^{b}
EGCG	73.42 ± 10.50^{b}	17.43 ± 1.77^{bc}

NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

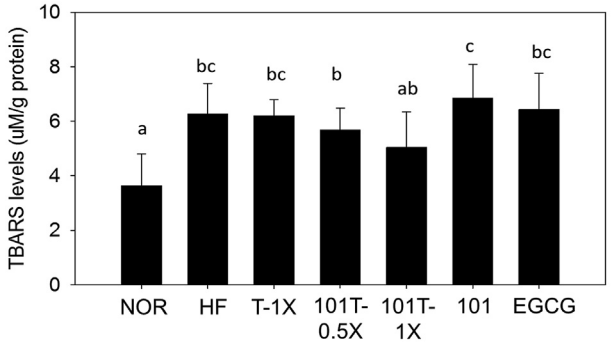

Fig. 6 – Effect of NTU 101-fermented tea on the TBARS levels in the liver of the obese SD rats fed with high fat diet. NOR group, normal diet (3.34 kcal/g); HF group, high-fat diet (4.17 kcal/g); T-1X group, Tea powder (1X, 568 mg/day kg B.W. including 12.46 mg/day EGCG) and high-fat diet; 101T-0.5X group, NTU 101-fermented tea (0.5X, 284 mg/day kg B.W. including 12.51 mg/day EGCG and 3.75×10^{10} CFU/day NTU 101) and high-fat diet; 101T-1X group, NTU 101-fermented tea (1X, 568 mg/day kg B.W. including 25.01 mg/day EGCG and 7.5×10^{10} CFU/day NTU 101) and high-fat diet; 101 group, *Lactobacillus paracasei* subsp. *paracasei* NTU 101 (7.5×10^{10} CFU NTU 101/day 100 g B.W.) and high-fat diet; EGCG group, EGCG (1X, 25.01 mg/day 100 g B.W.) and high-fat diet; Data are presented as means ± SD (n = 8). Mean values within each column with different superscripts are significantly different ($p < 0.05$).

induced model, prolonged feeding with EGCG reduced body fat formation and the occurrence of metabolic syndrome and fatty liver [22]. The polyphenol extracts in green tea, black tea, and oolong tea had similar effects. EGCG can act as a pro-oxidant, which enhances the production of reactive oxygen species (ROS) and activates the AMP-activated protein kinase (AMPK), further inhibiting lipogenesis [27–29]. During this process, the increased activity of AMPK causes elevated gene and protein expression of lipases, which suppresses the expression of transcription factors and subsequently achieves body-fat reduction. Transition metal ions, such as Fe and Cu, can promote EGCG-induced ROS production and activity. EGCG can also decrease the levels of antioxidants such as glutathione, SOD, catalase, and thioredoxin reductase, which results in an increase in ROS levels [30–32].

This study used the 3T3-L1 preadipocytes as an experimental material to examine whether the adipocyte differentiation process was inhibited by NTU 101-fermented tea. The results of this study showed that NTU 101-fermented tea could inhibit preadipocyte differentiation. Adipocytes transform into mature adipocytes by a process of differentiation. The fatty acids cleaved from the mature lipids by LPL are imported into adipocytes and converted into TG for storage; this process, which is called lipogenesis [18], gradually results in enlarged adipocytes. The lipogenesis of mature adipocytes is the main cause of obesity. The results of this study suggested that the NTU 101-fermented green tea inhibits the accumulation of lipid droplets by the promotion of lipase activity in adipocytes, which thereby improve the lipolytic effect.

The second phase of this study evaluated the effects of the compounds in high-fat diet-induced obese rats. The results showed that the NTU 101-fermented green and *H. cordata* leaf tea had a significant effect on the reduction of body weight gain. The subsequent efficacy experiments also discovered that feeding efficiency, perirenal fat pads, and epididymal fat pads were significantly decreased after administration of the NTU 101-fermented tea, which produced stronger effects than those of the unfermented tea. The more significant effects may arise from differences in the functional ingredients such as EGCG, chlorogenic acid, and NTU 101. The bioconversion during the fermentation process resulted in significantly different active ingredients and led to different effects on the overall reduction in body fat.

The results of this study showed that the lactic acid bacterium itself had a clear effect on body fat reduction, but the effect was not as strong as that of the NTU 101-fermented tea. The main effect on body fat reduction may result from the improvement of intestinal bacterial types and the promotion of digestion and absorption, and not from lipogenesis inhibition, as NTU 101 did not show a significant effect on promoting lipase activity. Another functional ingredient examined in this study was EGCG. The results suggested that EGCG could reduce body weight gain. However, EGCG contributed by significantly increasing lipolysis. EGCG and NTU 101, respectively, contributed to the body fat reduction capability of the fermented tea; however, when they were used separately, their effects were not significant. The combination of both in the NTU 101-fermented tea resulted in a very significant reduction of body fat. The results indicate that these two materials had synergistic or complementary effects. Although previous studies have indicated that these two materials showed significant effects, their efficacy might be affected by the concentrations used. This is the first study to indicate that

the use of NTU 101 in the fermentation process of green tea and *H. cordata* leaves could increase the levels of EGCG and chlorogenic acid, as well as provide highly active probiotic activities in the fermented products.

The experimental results showed that in the *in vitro* experiments, the NTU 101-fermented tea inhibited the adipocyte differentiation into mature adipocytes. In addition, the differentiation process of preadipocytes was mainly regulated by the expressed transcription factors. Among them, C/EBPs belong to the basic leucine zipper family of transcription factors, and the members of this family can bind to each other to form homodimers or heterodimers and exert transcriptional activity. The C/EBP family includes six members that are distributed in liver cells, adipocytes, hematopoietic cells, and in the spleen, kidney, and brain. C/EBPα, C/EBPβ, and C/EBPδ are present in white and brown adipocytes and play a very important role in adipocyte differentiation. C/EBPβ and C/EBPδ are the first transcription factors to be expressed, which can induce PPARγ expression. Activated PPARγ can further induce the expression of C/EBPα, thereby allowing preadipocyte differentiation into adipocytes [2]. The PPARs are transcription factors that belong to the nuclear hormone receptor superfamily. Members of this family need to form a complex with another nuclear hormone receptor; they have transcriptional activity only after binding to the ligand. PPARγ is necessary for adipocyte differentiation. Based on different transcription start sites and alternative splicing, the compounds can be distinguished as PPARγ1 and PPARγ2. The PPARγ1 level is low in adipocytes, while it is expressed in various other types of cells and tissues, such as in macrophages and colon epidermal cells and in the bladder, chest, and prostate. PPARγ2 is mainly present in adipocytes and regulates adipocyte-specific gene expression [3,33,34]. Further *in vivo* experiments in this study confirmed that in the NTU 101-fermented tea, the increased level of EGCG inhibited the expression of transcription factors during the differentiation process and that the inhibitory effect was mainly observed at the early stage of adipocyte differentiation.

In conclusion, a comparison between the NTU 101-fermented tea and the unfermented tea (T group) suggested that in terms of metabolite synthesis, the fermented tea has not only the added advantage of active NTU 101, but also higher levels of EGCG, ECG, and chlorogenic acid produced during bioconversion. Hence, the fermentation process could lead to more functional ingredients in the final fermented products. These functional ingredients caused the NTU 101-fermented tea to have a more significant effect on the reduction of body fat than the unfermented tea in the final *in vitro* and *in vivo* tests. In the *in vitro* test, the NTU 101-fermented tea mainly promoted lipolysis in the mature adipocytes, due to higher EGCG and chlorogenic acid contents. In the *in vivo* study, NTU 101-fermented tea had a more significant reducing effect on body weight gain and body-fat content than the unfermented tea, which was attributed to the presence of NTU 101 and to a higher EGCG content in the NTU 101-fermented tea. Regarding the mechanism of body-fat reduction, NTU 101-fermented tea and its functional ingredients, NTU 101 and EGCG, decreased mature adipocyte size by stimulating lipolysis and blocking pre-adipocyte differentiation by repressing transcription factor expression. NTU 101 and EGCG promoted lipolysis and, during the process, inhibited the expression of transcription factors.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We thank National Taitung University for providing the space and facility of animal experiments.

REFERENCES

[1] Marcos A. The impact of overweight and obesity on adolescence: consequences on metabolic biomarkers. J Nutr Sci Vitaminol 2015;61(Suppl.):S211.
[2] Ntambi JM, Young-Cheul K. Adipocyte differentiation and gene expression. J Nutr 2000;130:3122S–6S.
[3] Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. Gene Dev 2000;14:1293–307.
[4] Prusty D, Park BH, Davis KE, Farmer SR. Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem 2002;277:46226–32.
[5] Cornelius P, MacDougald OA, Lane MD. Regulation of adipocyte development. Annu Rev Nutr 1994;14:99–129.
[6] Saravanan R, Shubethar S, Narayanan S, Jain M, Lade S, Jadhav D, et al. A novel process for the production of high-purity galactooligosaccharides (GOS) using consortium of microbes. Prep Biochem Biotechnol 2017;47:245–53.
[7] Cooperstone JL, Goetz HJ, Riedl KM, Harrison EH, Schwartz SJ, Kopec RE. Relative contribution of alpha-carotene to postprandial vitamin A concentrations in healthy humans after carrot consumption. Am J Clin Nutr 2017;106:59–66.
[8] Raimondi S, Roncaglia L, De Lucia M, Amaretti A, Leonardi A, Pagnoni UM, et al. Bioconversion of soy isoflavones daidzin and daidzein by Bifidobacterium strains. Appl Microbiol Biotechnol 2009;81:943–50.
[9] Rekha CR, Vijayalakshmi G. Bioconversion of isoflavone glycosides to aglycones, mineral bioavailability and vitamin B complex in fermented soymilk by probiotic bacteria and yeast. J Appl Microbiol 2010;109:1198–208 [in Eng].
[10] Bhushan B, Trott S, Spain JC, Halasz A, Paquet L, Hawari J. Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a rabbit liver cytochrome P450: insight into the mechanism of RDX biodegradation by Rhodococcus sp. strain DN22. Appl Environ Microbiol 2003;69:1347–51 [in Eng].
[11] Sae-Tan S, Grove KA, Kennett MJ, Lambert JD. (-)-Epigallocatechin-3-gallate increases the expression of genes related to fat oxidation in the skeletal muscle of high fat-fed mice. Food Funct 2011;2:111–6 [in Eng].
[12] Balzan S, Hernandes A, Reichert CL, Donaduzzi C, Pires VA, Gasparotto Jr A, et al. Lipid-lowering effects of standardized extracts of Ilex paraguariensis in high-fat-diet rats. Fitoterapia 2013;86:115–22 [in Eng].
[13] Lin FM, Chiu CH, Pan TM. Fermentation of a milk-soymilk and *Lycium chinense* Miller mixture using a new isolate of

Lactobacillus paracasei subsp. *paracasei* NTU101 and *Bifidobacterium longum*. J Ind Microbiol Biotechnol 2004;31:559–64.
[14] Chen HC, Farese Jr RV. Determination of adipocyte size by computer image analysis. J Lipid Res 2002;43:986–9 [in Eng].
[15] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509 [in Eng].
[16] Kusunoki M, Hara T, Tsutsumi K, Nakamura T, Miyata T, Sakakibara F, et al. The lipoprotein lipase activator, NO-1886, suppresses fat accumulation and insulin resistance in rats fed a high-fat diet. Diabetologia 2000;43:875–80 [in Eng].
[17] Berger JJ, Barnard RJ. Effect of diet on fat cell size and hormone-sensitive lipase activity. J Appl Physiol 1999;87:227–32 [in Eng].
[18] Steinberg GR, Kemp BE, Watt MJ. Adipocyte triglyceride lipase expression in human obesity. Am J Physiol Endocrinol Metabol 2007;293:E958–64.
[19] Frayn KN, Coppack SW, Fielding BA, Humphreys SM. Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue in vivo: implications for the control of fat storage and fat mobilization. Adv Enzyme Regul 1995;35:163–78.
[20] Roberts CK, Barnard RJ, Liang KH, Vaziri ND. Effect of diet on adipose tissue and skeletal muscle VLDL receptor and LPL: implications for obesity and hyperlipidemia. Atherosclerosis 2002;161:133–41.
[21] Moon HS, Chung CS, Lee HG, Kim TG, Choi YJ, Cho CS. Inhibitory effect of (-)-epigallocatechin-3-gallate on lipid accumulation of 3T3-L1 cells. Obesity 2007;15:2571–82.
[22] Klaus S, Pultz S, Thone-Reineke C, Wolfram S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes (Lond) 2005;29:615–23.
[23] Chiu CH, Lu TY, Tseng YY, Pan TM. The effects of Lactobacillus-fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. Appl Microbiol Biotechnol 2006;71:238–45.
[24] Tsai TY, Chu LH, Lee CL, Pan TM. Atherosclerosis-preventing activity of lactic acid bacteria-fermented milk-soymilk supplemented with Momordica charantia. J Agric Food Chem 2009;57:2065–71 [in Eng].
[25] Cheng MC, Tsai TY, Pan TM. Anti-obesity activity of the water extract of Lactobacillus paracasei subsp. paracasei NTU 101 fermented soy milk products. Food Funct 2015;6:3522–30.
[26] Chiang SS, Pan TM. Beneficial effects of Lactobacillus paracasei subsp. paracasei NTU 101 and its fermented products. Appl Microbiol Biotechnol 2012;93:903–16.
[27] Yang CS, Zhang J, Zhang L, Huang J, Wang Y. Mechanisms of body weight reduction and metabolic syndrome alleviation by tea. Mol Nutr Food Res 2016;60:160–74.
[28] Li GX, Chen YK, Hou Z, Xiao H, Jin H, Lu G, et al. Pro-oxidative activities and dose-response relationship of (-)-epigallocatechin-3-gallate in the inhibition of lung cancer cell growth: a comparative study in vivo and in vitro. Carcinogenesis 2010;31:902–10.
[29] Suh KS, Chon S, Oh S, Kim SW, Kim JW, Kim YS, et al. Prooxidative effects of green tea polyphenol (-)-epigallocatechin-3-gallate on the HIT-T15 pancreatic beta cell line. Cell Biol Toxicol 2010;26:189–99.
[30] Zhang H, Cao D, Cui W, Ji M, Qian X, Zhong L. Molecular bases of thioredoxin and thioredoxin reductase-mediated prooxidant actions of (-)-epigallocatechin-3-gallate. Free Radic Biol Med 2010;49:2010–8.
[31] Manohar M, Fatima I, Saxena R, Chandra V, Sankhwar PL, Dwivedi A. (-)-Epigallocatechin-3-gallate induces apoptosis in human endometrial adenocarcinoma cells via ROS generation and p38 MAP kinase activation. J Nutr Biochem 2013;24:940–7.
[32] Tao L, Forester SC, Lambert JD. The role of the mitochondrial oxidative stress in the cytotoxic effects of the green tea catechin, (-)-epigallocatechin-3-gallate, in oral cells. Mol Nutr Food Res 2014;58:665–76.
[33] Morrison RF, Farmer SR. Insights into the transcriptional control of adipocyte differentiation. J Cell Biochem 1999;(Suppl. 32–33):59–67.
[34] Morrison RF, Farmer SR. Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis. J Biol Chem 1999;274:17088–97.