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Kefir Peptides Prevent Hyperlipidemia and Obesity in High-Fat-Diet-Induced Obese Rats via Lipid Metabolism Modulation

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Scope: Obesity has reached epidemic proportions worldwide. Obesity is a complex metabolic disorder that is linked to numerous serious health complications with high morbidity. The present study evaluated the effects of kefir peptides on high fat diet (HFD)-induced obesity in rats.
Methods and results: Kefir peptides markedly improved obesity, including body weight gain, inflammatory reactions and the formation of adipose tissue fat deposits around the epididymis and kidney, and adipocyte size. Treating high fat diet (HFD)-induced obese rats with kefir peptides significantly reduced the fatty acid synthase protein and increased the p-acetyl-CoA carboxylase protein to block lipogenesis in the livers. Kefir peptides also increased fatty acid oxidation by increasing the protein expressions of phosphorylated AMP-activated protein kinase, peroxisome proliferator-activated receptor-α, and hepatic carnitine palmitoyltransferase-1 in the livers. In addition, administration of kefir peptides significantly decreased the inflammatory response (*TNF-α*, *IL-1β*, and *TGF-β*) to modulate oxidative damage.
Conclusion: These results demonstrate that kefir peptides treatment improves obesity via inhibition of lipogenesis, modulation of oxidative damage, and stimulation of lipid oxidation. Therefore, kefir peptides may act as an anti-obesity agent to prevent body fat accumulation and obesity-related metabolic diseases.

1. Introduction

Obesity has reached epidemic proportions globally, especially in developing and developed countries. Obesity in children under the age of 5 years and adults was estimated at over 42 million and 1.9 billion, respectively, in 2013 and 2014.[1] Obesity is a complex metabolic disorder that is linked to numerous serious health complications with high morbidity, including type 2 diabetes mellitus, coronary atherosclerotic heart disease, and cardiovascular diseases, such as hypertension and hyperlipidemia.[2,3] Obesity is characterized by excessive fat weight and an expansion of adipose tissue resulting from adipocyte hyperplasia and hypertrophy, which causes weight gain.[4] Obesity is an imbalance between energy intake and expenditure and occurs because of the interaction between genetic, environmental, and psychosocial factors.[5] Available pharmacological approaches to the treatment of obesity include several side effects. Therefore, numerous scientists are interested in anti-obesity agents from natural products.[6,7]

Kefir originates in the Caucasian Mountains, and it is traditionally produced by the symbiotic fermentation of milk by lactic acid bacteria and yeasts contained within an exopolysaccharide and protein complex called a kefir grain.[8] Kefir is associated with broad health benefits, such as for treatment of high cholesterol, gastrointestinal disease, allergy and asthma, antimicrobial

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activity, tumor suppression, increased speed of wound healing, and prevention of hypertension and ischemic heart disease.[8–10] Our previous study first demonstrated that kefir peptides prevented high fructose corn syrup-induced non-alcoholic fatty liver disease in a mouse model.[11] In addition, kefir contains abundant natural bioactive peptides and exhibits a variety of biological activities, such as angiotensin-converting enzyme inhibitory, antithrombotic, mineral binding, opioid, immunomodulating, antimicrobial, and anti-oxidative effects.[12–15]

The present study evaluated the anti-obesity effect of kefir peptides. We fed animals a moderately high energy diet containing 8% soybean oil and 44% sweetened condensed milk supplemented with kefir peptides for 8 weeks. The expression of several proteins involved in the lipogenesis pathway and the lipid oxidative pathway were measured after kefir peptides treatment. The expression levels of inflammatory genes (*TNF-α*, *IL-6*, *IL-1β*, and *TGF-β*) were also evaluated to monitor the oxidative damage status after kefir peptides treatment.

2. Experimental Section

2.1. Kefir Peptides Preparation

Kefir peptides powder (KEFPEP) was purchased from Phermpep Co. (Taichung, Taiwan). Kefir peptides powder was produced via kefir grain fermentation in sterilized milk. After the grains were filtered, the fermented products were spray-dried as the kefir peptides powder according to the previous preparation methods.[16,17] Chen et al.[11] shows the quality controls of kefir peptides powder for peptide separation and reproducibility. The content of peptides in the kefir peptides powder (Phermpep Co.) was calculated as a triglycine equivalent in grams per 100 g, which was 23.1 g 100 g^{-1} within the sample. Supporting Information Table S1 shows the compositions of commercial milk powder and kefir peptides powder.

2.2. Isolation and Identification of Kefir Peptides

The kefir peptides powder were further separated into three fractions (>30 kD, 3–30 kD, and <3 kD) by 30-kD and 3-kD size exclusion centrifugal filter devices (Millipore, Billerica, MA, USA) and then identified their peptides profile by semipreparative HPLC. On a model PU-980 pump (Jasco, Japan) equipped with a UV detector and a 300 × 7.8 mm id, 5-μm particles TSK-GEL G2000SWXL column (Sigma-Aldrich, St Louis, MO, USA). The mobile phase was 100 mM KH_2PO_4, 1 M NaCl, and 1 mM EDTA (pH = 6.5) at a flow rate of 0.5 mL min^{-1}, and the wavelength was detected at 215 nm. The fractions of kefir-fermented peptides (KFPs) are shown in the Supporting Information Figure S1.

2.3. Animals and Study Design

Six-week-old male Sprague Dawley rats were purchased from BioLASCO (A Charles River Licensee Corp., Yi-Lan, Taiwan) and maintained on a 12-hour light/dark cycle at 22 ± 2 °C. This study was performed according to institutional guidelines and approved by the Institutional Animal Care and Utilization Committee of Da-Yeh University, Taiwan (IACUC No. DYU99015). The experimental animals were acclimated to the environment and diet for 2 weeks. Animals were fed a standard chow diet ($n = 8$) or an HFD ($n = 24$) for 6 weeks after acclimatization. Standard chow (No. 5001) contained 3.35 kcal g^{-1} with 28.5% protein, 13.4% fat and 58.1% carbohydrates. The HFD contained 8% (wt/wt) soybean oil, 44% (wt/wt) sweetened condensed milk (Original, Eagle Brand, Nestle) and 48% (wt/wt) standard chow, for 3.76 kcal g^{-1} with 15.5% as protein, 33.4% as fat, and 51.1% as carbohydrates. Animals were divided into four treatment groups after 6 weeks: (1) rats on a normal diet + Mock group (ND/Mock; $n = 8$); (2) rats on a HFD + Mock group (HFD/Control; $n = 8$); (3) rats on a HFD + Milk powder group (HFD/MP; $n = 8$); and (4) rats on a HFD + Kefir peptides group (HFD/KP; $n = 8$). Milk powder and kefir peptides were dissolved in double-distilled water and orally administered at 164 mg kg^{-1} of body weight (BW) daily for 8 weeks. Rats were sacrificed at 22 weeks of age after 8 weeks of double-distilled water (Mock), milk powder (MP), or kefir peptides (KPs) administration. Liver, kidney, fat around epididymis, and fat around kidney tissues were collected, weighed, and pathological examined. Total RNA and protein of liver tissue were extracted according to previously described protocols.[18–20]

2.4. Detection of Serum Biochemical Markers

Serum was collected, and triglyceride (TG), total cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), free fatty acids (FFA), uric acid (UA), and creatinine (CRE) contents were measured colorimetrically using kits (IDEXX Vet Tests, IDEXX Lab. Inc., Westbrook, ME, USA) in an automatic analyzer.[21,22]

2.5. Hematoxylin and Eosin (H&E) Staining

Fat bodies around the epididymis and kidney tissues were collected and fixed in 10% buffered formaldehyde and examined using hematoxylin and eosin (H&E) staining as described previously.[23–25]

2.6. Western Blot Analysis of Protein Expressions

Liver tissue protein expression was measured using Western blotting. Liver tissues from different groups of rats were homogenized in 500 μL of RIPA buffer (5 mM Tris–HCl pH 7.4, 0.15 M NaCl, 1% NP40, 0.25% sodium deoxycholate, 5 mM EDTA, and 1 mM ethylene glycol-bis(2-aminoethyl-ether)-*N, N, N, N*-tetraacetic acid). Homogenates were centrifuged at 12 000 × *g* for 30 min at 4 °C. Protein (40 μg) was separated on a 10% SDS–PAGE and electrotransferred onto polyvinylidene difluoride membranes as described previously.[22] Membranes were incubated in a blocking solution (5% BSA) at room temperature for 2 h and incubated with a primary antibody (SREBP-1c, fatty acid synthase (FAS), p-ACC (p-acetyl-CoA carboxylase), p-AMPK, PPAR-α (peroxisome proliferator-activated receptor-α),

CPT1, or β-actin) overnight at 4 °C. Membranes were washed and incubated with a goat anti-rabbit IgG peroxidase-conjugated secondary antibody. Protein bands on membranes were developed using an enhanced chemiluminescence Western blot detection system as described previously.[22,26]

2.7. Cell Culture and Treatment

The HepG2 cell line purchased from ATCC was cultured in DMEM supplemented with 10% fetal bovine serum. The HepG2 cells were seeded into the 6-well plates in triplicate and incubated in a 5% CO_2 incubator at 37 °C for 24 h. After cell adherence, 0.66 mM of oleate, FFA, was added to the medium for 24 h. Then, fresh medium containing 100 μg mL^{-1} test samples (kefir peptides powder, >30 kD KFPs, 3–30 kD KFPs, and <3 kD KFPs) was added into the cultures for 24 h, followed by Oil red O and hematoxylin staining and gene expression analysis.[11]

2.8. Real-Time RT-PCR Analysis of mRNA Expression

Total RNA was extracted from liver tissue or cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) as specified by the manufacturer. Briefly, 2 μg of total RNA was resuspended in 9 μL of diethylpyrocarbonate-treated water. First strand cDNA was synthesized using random primers and the ImProm-II RT (Promega, Madison, WI, USA) in a total volume of 20 μL. The reaction was performed at 42 °C for 1 h. An aliquot (1:10) of the RT product was adjusted to contain 0.1 μg of each primer for further PCR amplification (Supporting Information Table S2), and additional buffer was added to a total volume of 20 μL.[22] RT-PCR was performed for five genes (*TNF-α*, *IL-6*, *IL-1β*, *TGF-β*, and *β-actin*) from liver tissues or five genes (*ACC*, *FAS*, *AMPK*, *CPT1*, and *β-actin*) from cells using cDNA to evaluate gene expression. β-actin cDNA was used as an internal control for RT-PCR quantification.

2.9. Statistical Analysis

Experimental values are expressed as the means ± standard error (SE). Significant differences were analyzed using one-way ANOVA with Duncan's test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of Kefir Peptides on Liver, Kidney, and Fat Bodies around the Epididymis and Kidney Masses in HFD-Induced Obese Rats

Liver, kidney, fat bodies around epididymis and kidney tissues were weighed at the end of the study (**Figure 1**). Fat bodies around epididymis and kidney were white adipose tissues in the body. The HFD/Mock group exhibited significantly increased liver, kidney, fat bodies around epididymis, and fat bodies around kidney weights (Figure 1A and B) at the end of the study ($p < 0.05$). Initial BW of the HFD/Mock group did not significantly differ from the HFD groups, but BW gain was lower for the HFD/KP group as compared to the HFD/Mock group ($p < 0.05$) (Supporting Information Table S3). Fat around the epididymis in the ND/Control, HFD/Mock, HFD/MP, and HFD/KP groups were 1.1 ± 0.2, 7.2 ± 0.6, 5.6 ± 0.3, and 2.6 ± 0.3 g, respectively. Fat around the kidneys of the ND/Control, HED/Mock, HED/MP, and HED/KP groups were 0.2 ± 0.1, 8.3 ± 0.6, 5.6 ± 0.1, and 2.8 ± 0.6 g, respectively. Fat body masses around the epididymis and kidneys were greater for HFD/Mock group than the ND/Control group by 5.55-fold ($p < 0.05$) and 40.50-fold ($p < 0.05$), respectively. However, the HFD/KP group did significantly decrease fat bodies around the epididymis and kidney by 64% ($p < 0.05$) and 66% ($p < 0.05$), respectively, as compared to the HFD/Mock group (Figure 1B).

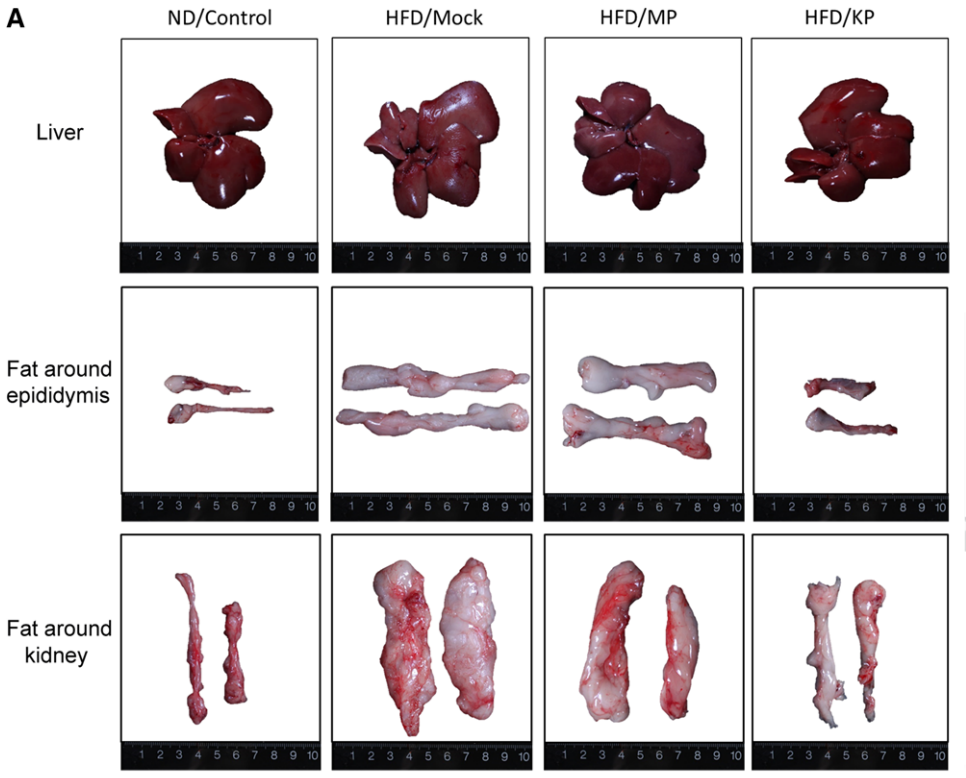
3.2. Effects of Kefir Peptides on Serum Biochemical Parameters in HFD-Induced Obese Rats

Table 1 shows serum TG, CHOL, HDL, LDL, FFA, UA, and CRE levels of the different treated rat groups. An HFD in Sprague Dawley rats significantly increased serum levels of TG, CHOL, LDL, FFA, and UA by 44% ($p < 0.05$), 18% ($p < 0.05$), 78% ($p < 0.05$), 150% ($p < 0.05$), and 71% ($p < 0.05$), respectively, as compared to the ND. Kefir peptides supplementation to the treated group (HFD/KP) significantly decreased serum levels of LDL (44%), FFA (40%), and UA (33%) as compared to the HFD/Mock group ($p < 0.05$).

3.3. Effects of Kefir Peptides on the Expression of Proteins Involved in Lipogenesis and Lipid Oxidation in HFD-Induced Obese Rats

The expression patterns of proteins involved in lipogenesis (SREBP-1c, FAS, and p-ACC) and lipid oxidation (p-AMPK, PPAR-α, and CPT1) were assessed in the four experimental rat groups using Western blot analysis. **Figure 2** shows the molecular mechanisms that mediate the effects of kefir peptides in the regulation of lipogenesis in the liver, and the protein expressions of SREBP-1c, FAS, and p-ACC were analyzed (Figure 2A). No significant difference in SREBP-1c expression was observed between the four groups. However, FAS expression increased significantly by 43% in the HFD/Mock group as compared to the ND/Control group ($p < 0.05$). The HFD/KP group exhibited a significant reduction in FAS expression by 61% as compared to the HFD/Mock group ($p < 0.05$). Levels of phosphorylated ACC (p-ACC) were reduced by 45% and 52% in the HFD/Mock and HFD/MP groups, respectively, as compared to the ND/Control group, but the HFD/KP group exhibited a significant increase in p-ACC level as compared to the HFD/MP group ($p < 0.05$).

Figure 3 shows the effect of KPs on the expression of proteins involved in lipid oxidation (p-AMPK, PPAR-α, and CPT1). The HFD/Mock group exhibited a significant decreased in p-AMPK of 76% as compared to the ND/Control group ($p < 0.05$), and the HFD/KP group exhibited a significant increase in p-AMPK of 224% as compared to the HFD/Mock group ($p < 0.05$). The



B

	ND/Control	HFD/Mock	HFD/MP	HFD/KP
Liver (g)	8.7 ± 0.2^{b}	15.8 ± 0.5^{a}	16.9 ± 0.9^{a}	$13.0 \pm 0.5^{a,b}$
Kidney (g)	1.6 ± 0.04^{b}	2.8 ± 0.19^{a}	2.8 ± 0.05^{a}	$2.3 \pm 0.1^{a,b}$
Fat around epididymis (g)	1.1 ± 0.2^{b}	7.2 ± 0.6^{a}	5.6 ± 0.3^{a}	2.6 ± 0.3^{b}
Fat around kidney (g)	0.2 ± 0.08^{c}	8.3 ± 0.57^{a}	$5.6 \pm 0.12^{a,b}$	$2.8 \pm 0.6^{b,c}$

Figure 1. Effects of kefir on A) the exterior of the liver, fat around the epididymis, and fat around the kidney, and B) the organ weights of liver, kidney, fat around the epididymis, and fat around the kidney of high fat diet-induced obesity in rats. The animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Mock); (2) rats on a HFD + Mock group (HFD/Control); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir powder group (HFD/KP). The data are expressed as the means ± SE ($n = 8$). Different letters indicate significant differences at $p < 0.05$ using one-way ANOVA.

HFD/KP group exhibited a significant increase in p-AMPK concomitant with p-ACC, which suggests an adaptive mechanism to inhibit fatty acid synthesis.

No significant difference in PPAR-α and CPT1 was observed between ND/Control and HFD/Mock groups. However, the expression of PPAR-α and CPT1 proteins was significantly upregulated in the HFD/MP and HFD/KP groups as compared to the HFD/Mock group ($p < 0.05$). The HFD/KP group exhibited increased p-AMPK and p-ACC, which reduces TG accumulation in the liver. These results indicate that KPs affected fat bodies deposition via stimulation of lipid oxidation and inhibition of the lipogenesis pathway.

3.4. Effects of Kefir Peptides on the mRNA Levels of *TNF-α*, *IL-6*, *IL-1β*, and *TGF-β* in HFD-Induced Obese Rats

The gene expression levels of *TNF-α*, *IL-6*, *IL-1β*, and *TGF-β* were analyzed using real-time RT-PCR (**Figure 4**). The mRNA expression levels of *TNF-α*, *IL-6*, *IL-1β*, and *TGF-β* increased

Table 1. Effects of kefir peptides on serum biochemical parameters in HFD-fed rats[a)].

	ND/Control	HFD/Mock	HFD/MP	HFD/KP
TG [mg dL^{-1}]	110.5 ± 3.9	158.6 ± 5.8*	142.1 ± 8.2	164.3 ± 4.7
CHOL [mg dL^{-1}]	45.3 ± 1.7	53.6 ± 0.9*	56.6 ± 0.9	61.3 ± 2.4
HDL [mg dL^{-1}]	16.1 ± 0.7	19.1 ± 0.5	20.5 ± 0.5	19.1 ± 0.8
LDL [mg dL^{-1}]	6.3 ± 0.3	11.2 ± 6.1*	6.9 ± 0.3#	6.3 ± 0.2#
FFA [mg dL^{-1}]	0.4 ± 0.01	1.0 ± 0.04*	0.9 ± 0.03	0.6 ± 0.01#
UA [mg dL^{-1}]	0.7 ± 0.03	1.2 ± 0.08*	0.8 ± 0.04#	0.8 ± 0.03#
CRE [mg dL^{-1}]	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01

a) Animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Control); (2) rats on a high fat diet (HFD) + Mock group (HFD/Mock); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir peptides powder group (HFD/KP). Data are expressed as the means ± SE ($n = 8$). *$p < 0.05$ versus ND/Control group. #$p < 0.05$ versus HFD/Mock group.

markedly by 142%, 110% ($p < 0.05$), 68% ($p < 0.05$), and 55%, respectively, in the HFD/Mock group as compared to the ND/Control group. However, mRNA levels of *TNF-α*, *IL-1β*, and *TGF-β* decreased markedly in the HFD/KP group by 85% ($p < 0.05$), 76% ($p < 0.05$), and 48% ($p < 0.05$), respectively, as compared to the HFD/Mock group. Ho et al.[20] demonstrated that pretreatment with kefir fractions decreased *TNF-α* mRNA expression as compared to untreated control cells. Chen et al.[15] also suggested that high fructose intake from drinking water resulted in inflammatory activity, and kefir peptides administration improved lipid peroxidation and reduced the expression of 4-HNE and IL-6, TNF-α, and IL-1β in liver.

3.5. Effects of Kefir Peptides on Histochemical Staining of the Fat Bodies around the Epididymis and Kidney Tissues in HFD-Induced Obese Rats

Large adipocytes (>100 μm) of the fat bodies around the epididymis (**Figure 5**A and B) and kidney (Figure 5C and D) increased markedly by 27% ($p < 0.05$) and 40% ($p < 0.05$), respectively, in the HFD/Mock group as compared to the ND/Control group. However, large adipocytes (>100 μm) in epididymal adipose tissue decreased significantly in the HFD/KP group (23%; $p < 0.05$) as compared to the HFD/Mock group (Figure 5B). The percentage of large adipocytes (>100 μm) in the fat around kidney was also lower in the HFD/KP group (24%; $p < 0.05$) as compared to the HFD/Mock group (Figure 5D). Histological analyses revealed larger adipocytes in the HFD/Mock group as compared to the ND/Control group. However, the HFD/KP group exhibited reduced adipocyte size as compared to the HFD/Mock group ($p < 0.05$). Our previous study[22] also demonstrated that the treatment of *ob/ob* mice with kefir peptides (140 mg kg^{-1} BW) significantly reduced the formation of adipose tissues.

4. Discussion

Kefir is a fermented milk beverage made with kefir grain. Kefir grain is a microbial symbiont mixture that contains lactic acid bacteria, yeasts, and acetic acid bacteria.[27,28] Kefir exhibits numerous biological activities, including immune system modulation, reduction of lactose intolerance symptoms, lowering of cholesterol, and anti-oxidative, anti-inflammatory, pro-digestive,

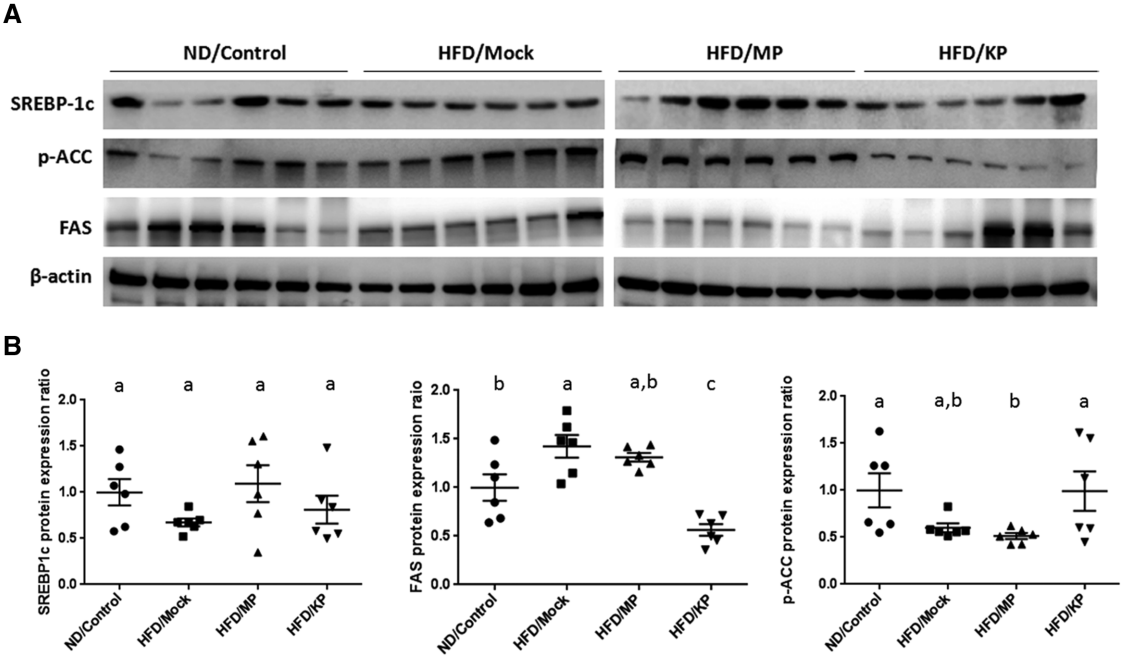


Figure 2. Effects of kefir on the expression of proteins involved in lipogenesis (SREBP-1c, FAS, and p-ACC) in HFD-induced obesity in rats. The animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Mock); (2) rats on a HFD + Mock group (HFD/Control); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir powder group (HFD/KP). β-actin was used as an internal control. The data are expressed as the means ± SE ($n = 8$). Different letters indicate significant difference at $p < 0.05$ using one-way ANOVA.

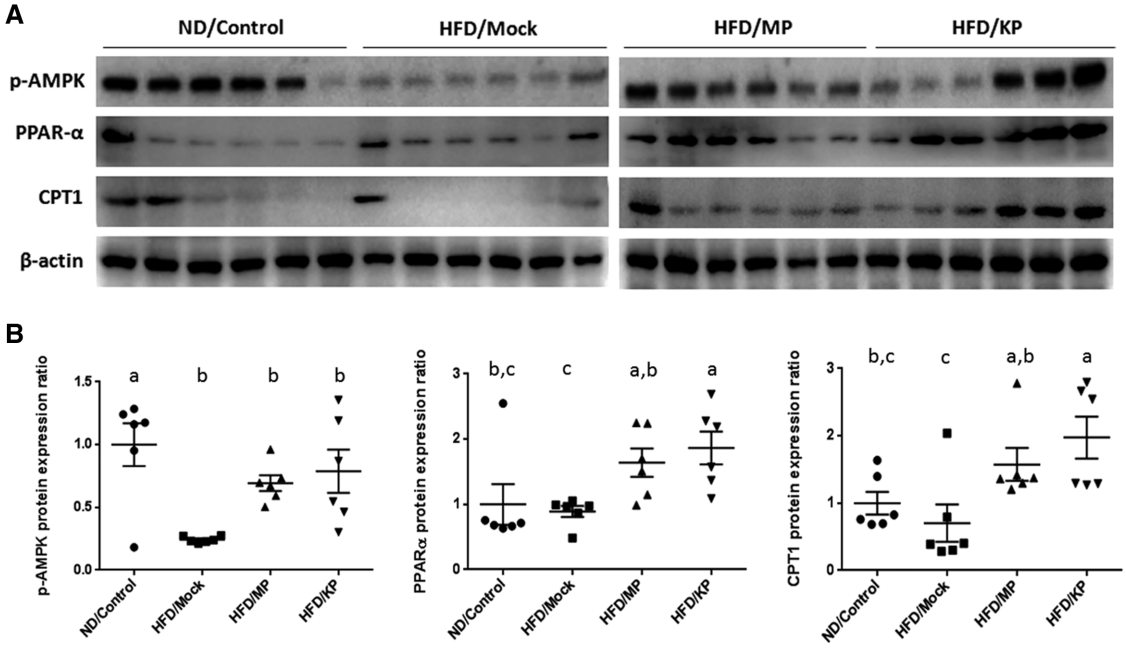


Figure 3. Effects of kefir on the expression of proteins involved in lipid oxidation (p-AMPK, PPARα, and CPT1) in high fat diet-induced obesity in rats. The animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Mock); (2) rats on a HFD + Mock group (HFD/Control); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir powder group (HFD/KP). β-actin was used as an internal control. The data are expressed as the means ± SE ($n = 8$). Different letters indicate significant difference at $p < 0.05$ using one-way ANOVA.

and anticarcinogenic functions.[28–31] Ebner et al.[12] identified 236 casein-derived unique peptides from bovine kefir including 16 peptides with angiotensin-converting enzyme-inhibitory, antimicrobial, immunomodulating, opioid, mineral binding, antioxidant, and antithrombotic effects. The current in vitro data demonstrated that kefir inhibited 3T3-L1 adipocyte differentiation via down-regulation of adipogenic transcription factor expression.[28] Our previous in vivo animal study demonstrated that treating *ob/ob* mice with kefir significantly improved non-alcoholic fatty liver disease on BW, energy expenditure, and the basal metabolic rate via inhibition of the lipogenesis pathway.[22] Chen et al.[15] also demonstrated that KPs improved some symptoms of non-alcoholic fatty liver diseases, including BW, energy intake, inflammatory reaction, and the formation of fatty liver, via activation of JAK2 signal transduction through the JAK2/STAT3 and JAK2/AMPK pathways in a high fructose-induced fatty liver animal model. The present study demonstrated, for the first time, that KPs improved obesity in HFD-induced obese rats.

In addition, to elucidate the clear function of KPs on fatty acid metabolism, we also established an oleate-induced fatty liver cell model and treated with different fractions of KFPs. Data from Oil red O staining analysis showed that the kefir peptides powder as well as three different KFPs fractions (>30 kD, 3–30 kD, and <3 kD) significantly reduced fatty acid accumulation in liver cells after 0.66 mM oleate treatment (Supporting Information Figure S2). In the gene expression levels, we also demonstrated treatment with kefir peptides or three different KFPs fractions in oleate-induced fatty liver cells, the mRNA levels of *ACC* and *FAS* (lipid synthesis-related genes) were significantly reduced and *AMPK* and *CPT1* (lipid oxidation-related genes) were significantly increased, especially in the <3 kD KFPs treated group (Supporting Information Figure S3).

Obesity is defined as an abnormal or excessive fat accumulation and may impair health in developing and developed countries.[32] Proliferation of preadipocytes increases the adipocyte number during the period of weight gain, and preadipocytes differentiate into mature adipocytes, which accumulate TG and become enlarged.[33] The pathogenesis of HFD-induced obese visceral cavity in a rat model is similar to humans.[34] The present study investigated the effect of KPs, a mixture of sole peptides from kefir, on rats with HFD-induced obesity. KPs were administered as a daily supplement (164 mg kg^{-1}) for 8 weeks. Treatment with KPs improved HFD-induced BW gain, adipose tissue weights of peritoneal and epididymal fat bodies, and adipocyte size (Figures 1 and 5). Visceral cavity body fat is a major contributor of multiple risk factors for diabetes, hyperlipidemia, hypertension, and arteriosclerosis.[35] Previous studies demonstrated that obesity adversely affected plasma lipids, including an increase in TG, CHOL, and LDL and decrease in HDL.[36] The present study demonstrated that obese rats actually exhibited elevated TG, CHOL, LDL, FFA, and UA and that KPs decreased the levels of LDL, FFA, and UA, which may partially inhibit adipocyte differentiation (Table 1). Treatment with kefir peptides slightly increased serum TG because TG was not hydrolyzed into MG or FFA, which are required for absorption (Table 1). Kefir may effectively prevent non-alcoholic fatty liver disease because its peptides inhibit digestion and fat absorption in the liver. Our previous study[22] demonstrated that treating *ob/ob* mice with 140 mg kg^{-1} BW of kefir significantly reduced hepatic TG and CHOL (26 and 27%, respectively) as

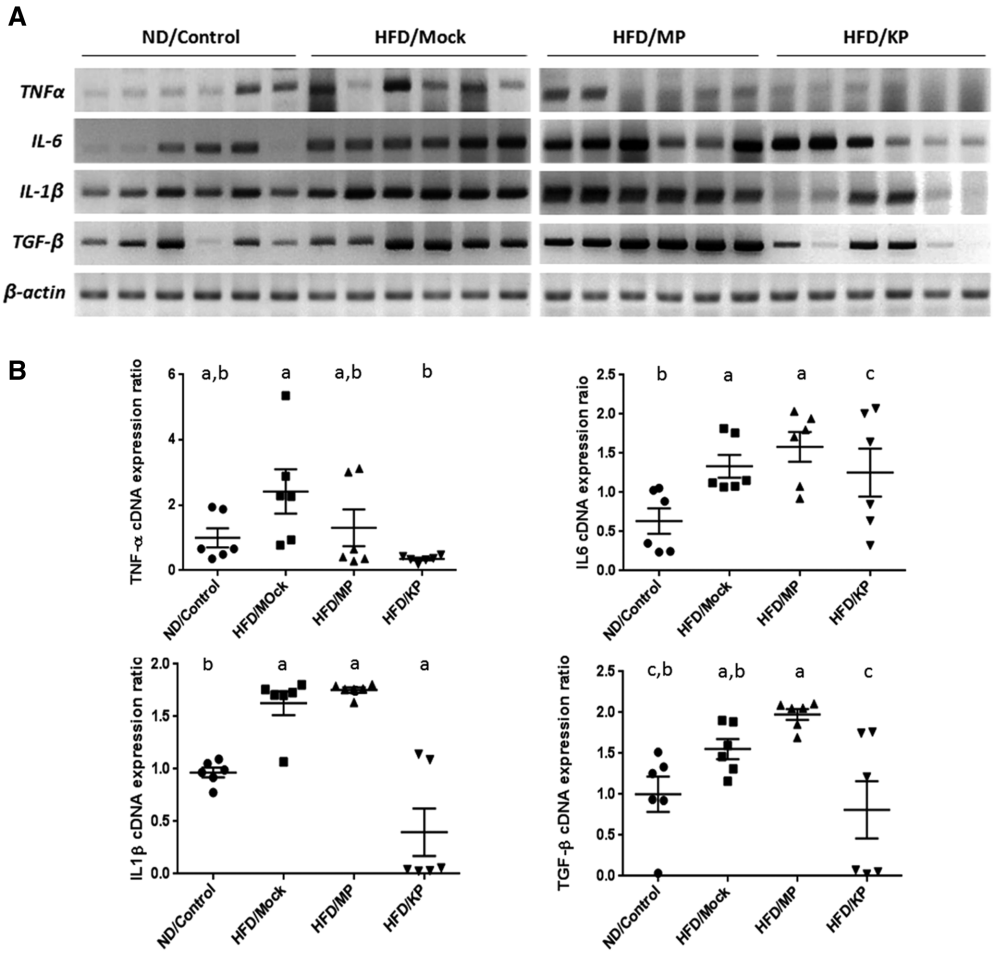


Figure 4. Effects of kefir on the expression of proteins involved in anti-inflammatory activity (*TNF-α*, *IL-6*, *IL-1β*, and *TGF-β*) in HFD-induced obesity in rats. The animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Mock); (2) rats on a HFD + Mock group (HFD/Control); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir powder group (HFD/KP). *β*-actin was used as an internal control. The data are expressed as the means ± SE ($n = 8$). Different letters indicate significant difference at $p < 0.05$ using one-way ANOVA.

compared to mock-treated *ob/ob* mice. However, the serum TG and CHOL levels were not significantly different between kefir- and mock-treated *ob/ob* mice. Hyson et al.[37] demonstrated that the blood levels of LDL and its oxidation were related to cardiovascular risk and that LDL is a major index of health. Therefore, treatment with kefir peptides may reduce the LDL content to protect the development of cardiovascular disease.

FAS is a major enzyme in fatty acid synthesis in the liver.[38] Obese rats in the present study exhibited elevated FAS protein, and treatment with KPs significantly decreased the FAS content. These results suggest that KPs reduced the FFA levels via lipogenesis.[28] p-AMPK regulates fatty acid metabolism via inhibition of ACC and activation of p-ACC, which eventually stimulates fatty acid *β*-oxidation and down-regulates fatty acid biosynthesis.[39] Increased p-AMPK in the liver stimulates PPAR-*α* and CPT1 production, which increases fatty acid oxidation.[40] FAS was significantly decreased in the present study, and KPs treatment significantly increased p-ACC, p-AMPK, PPAR-*α*, and CPT1 levels. These results demonstrate that KPs improved lipid accumulation via inhibition of lipogenesis and stimulation of lipid oxidation in HFD-induced obese rats.

In conclusion, kefir peptides could mediate the lipid metabolism pathway in HFD-induced obesity. First, we observed that treatment of HFD-induced obese rats with KPs significantly reduced the FAS enzyme and increased the p-ACC protein to inhibit lipogenesis. Second, KPs increased fatty acid oxidation via increased expression of p-AMPK, PPAR-*α*, and CPT1. Third, administration of KPs significantly decreased the inflammatory response (*TNF-α*, *IL-1β*, and *TGF-β*) to modulate oxidative damage. These results supposed that KPs clearly improved obesity, including BW gain, inflammatory reaction and formation of adipose tissue fat deposits around the epididymis and kidney, and adipocyte size via inhibition of lipogenesis and stimulation of lipid oxidation.

Abbreviations

ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; BW, body weight; CHOL, total cholesterol; CPT1, hepatic carnitine palmitoyltransferase-1; CRE, creatinine; FAS, fatty acid synthase; FFA, free fatty acids; H&E, hematoxylin and eosin; HDL, high-density

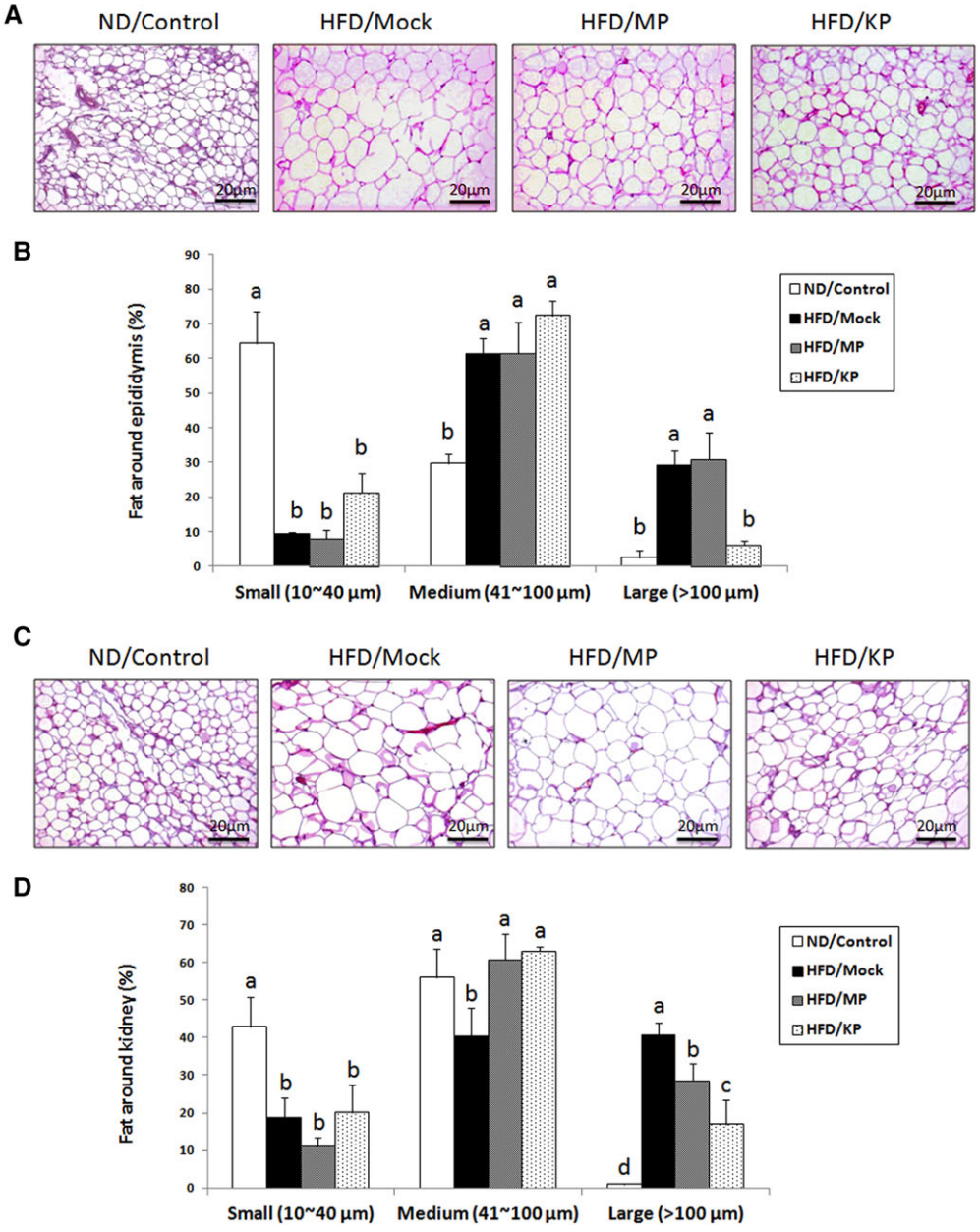


Figure 5. Effects of kefir on the hematoxylin-eosin (H&E) staining of histologically sectioned A) fat around epididymis and B) fat around kidney in high fat diet-induced obesity in rats. The animals were divided into four treatment groups: (1) rats on a normal diet + Mock group (ND/Mock); (2) rats on a HFD + Mock group (HFD/Control); (3) rats on a HFD + Milk powder group (HFD/MP); and (4) rats on a HFD + Kefir powder group (HFD/KP). The data are expressed as the means ± SE ($n = 8$). Different letters indicate significant differences at $p < 0.05$ using one-way ANOVA. A) Photomicrographs of H&E staining of fat around the epididymis and kidney. Scale bar = 20 μm at 200X magnification.

lipoprotein; HFD, high fat diet; KFP, Kefir-fermented peptide; KP, kefir peptide; LDL, low-density lipoprotein; MP, milk powder; PPAR-α, peroxisome proliferator-activated receptor-α; SE, standard error; TG, triglycerides; UA, uric acid

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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the research. Y.T.T., H.L.C. and C.M.C. analyzed the data and drafted the manuscript.

Conflict of Interest

The authors have declared no conflict of interest.

Keywords

high fat diet (HFD), kefir peptides, lipid oxidative pathway, lipogenesis, obesity, oxidative damage

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