Effects of Yogurt Containing Fermented Pepper Juice on the Body Fat and Cholesterol Level in High Fat and High Cholesterol Diet Fed Rat

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Abstract

This experiment investigated whether yogurt containing fermented pepper juice (FPJY) affects cholesterol level in high fat and high cholesterol diet (HFCD) fed rat. Twenty five Sprague-Dawley male rats of 7 wk were divided into 5 groups, and fed following diets for 9 wk; CON (control diet), HFCD (HFCD), PY (HFCD supplemented with 2% of plain yogurt), LFY (HFCD supplemented with 2% of FPJY), and HFY (HFCD supplemented with 5% of FPJY). In the LFY group, hepatic total lipid level decreased significantly compared to the HFCD group (p<0.05). Serum HDL cholesterol level tended to increase and hepatic total cholesterol level decreased and were comparable to the CON group (p>0.05). In HFY group, body weight and hepatic total lipid level significantly decreased over the HFCD group (p<0.05). Serum and hepatic total cholesterol level, kidney, and body fat weights decreased, and were compared to the CON group (p>0.05). Liver weight decreased as FPJY content was increased. Results suggested FPJY would inhibit organ hypertrophy and accumulation of body fat, hepatic lipid, and cholesterol in HFCD fed rat.

Keywords: fermented pepper juice, yogurt, high fat and high cholesterol diet, Bacillus licheniformis, body fat accumulation

Introduction

High cholesterol in blood can cause cardiovascular disease (Wardlaw et al., 2005), piles up and creates plaque at vessel walls, possibly leading to atherosclerosis. The blood vessel can narrow and blood circulation can be restricted so oxygen in the blood cannot reach the heart or brain, causing makes heart attacks (Wardlaw et al., 2005; Xiao et al., 2003).

Yogurt contains lactic acid bacteria. It is a probiotic food which can cause beneficial effects in the intestinal tract of humans (Odamaki et al., 2011; Tang et al., 2014). Yogurt is proven to remove cholesterol as well as decrease the serum cholesterol and low density lipoprotein (LDL) cholesterol (Akalin et al., 1997; Anderson et al., 1999; Kapila et al., 2006; Pereira et al., 2003). Therefore, yogurt can be a beneficial food for patients with cardiovascular disease.

Meanwhile, Korean pepper, especially chung-yang pepper, is famous for its pungency which results from capsaicin. Capsaicin and its several homologues are categorized as capsaicinoids. Most of the pungency results from capsaicin and dihydrocapsaicin (Iwai and Watanabe, 1999). Capsaicin inhibits body fat accumulation and promotes energy expenditure, pain relief, antibacterial activity, antioxidative activity, anti-stress activity, and inhibition of tumor cell growth (Chatteriee et al., 2010; Chen et al., 2014; Dou et al., 2011; Gerner et al., 2008; Iwai and Watanabe, 1999; Luo et al., 2011; Reinbach et al., 2009; Shin and Moritani et al., 2007). Although capsaicin has various effects, its application in the food industry is limited because of its pungency (Kim et al., 2011).

Capsaicinoids can be degraded by several microorganisms (Cho et al., 2014; Flagan and Leadbetter, 2006; Kim et al., 2011; Lee et al., 2008). In a previous experiment, the amount of capsaicin and dihydrocapsaicin decreased as pepper was fermented by Bacillus licheniformis (Yeon et al., 2013). Fermented pepper inhibited body fat accumu-
ulation in mice fed a high fat diet even if it lost pun-
gency. It might have lost pungency because the food intake
between the control diet fed group and the fermented pep-
per diet fed group showed no significant difference (Yeon
et al., 2013). On the other hand, a group fed the same
concentration of normal pepper diet showed significantly
lowered total food intake (Yeon et al., 2013).

There is little information about the application of fer-
mented pepper on yogurt. Yogurt is popular probiotic de-
sert which can drink easily. It is important whether fer-
mented pepper act as functional effect even if it is in yo-
gurt. In this experiment, pepper juice was fermented and
added to yogurt to identify the effect of degraded capsai-
cin in yogurt on decreasing cholesterol concentration and
body fat accumulation in rats fed a high fat and high cho-
lesterol diet (HFCD).

Materials and Methods

Preparation of fermented pepper juice
Pepper juice was extracted from chung-yang pepper that
was purchased from a local market in Seoul, Korea by a
juicer. This sample was mixed with bacillus minimal me-
dium of double concentration (Bacillus minimal medium
×2 : NaCl 1 g/L, NaH2PO4 9.52 g/L, KH2PO4 6 g/L, NH4Cl
2 g/L, MgSO4 0.48 g/L, CaCl2 0.02 g/L, glucose 3.6 g/L)
without glucose (1:1, v/v). After adjustment to pH 7, it
was sterilized and 5% of Bacillus licheniformis SK1230
was inoculated after cooling at 37°C. It was then incu-
bated at 37°C for 5 d.

Preparation of yogurts
The composition of yogurt mix is expressed in Table 1. Milk, skim milk powder, pectin, sugar, distilled water and fer-
mented pepper juice were mixed using a homogenizer
(Homogenizer T25, Janke and Kunkel type, Germany) for
10 min. Then, it was heated at 85°C for 30 min. After
cooling at 42°C, the starter was inoculated and the mix-
ture was incubated at 42°C until it reached pH 4.5. This
yogurt was stored at 4°C for 24 h and then freeze dried to
add to experimental diets. Ten milliliter of distilled water
was added to the premix of plain yogurt as a substitute for
fermented pepper juice. Starter (Lyofast YAB 450 AB,
Sacco srl., Codarago, Italy) contained Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus and Bifidobacterium animalis ssp. lacti.

Animal experiment
Seven wk old 25 Sprague-Dawley male rats (Central Lab.

Table 1. The composition of yogurt premix

| Ingredients            | Fermented pepper juice | Plain |
|------------------------|------------------------|-------|
| Milk (mL)              | 850                    | 850   |
| Skim milk powder (g)   | 40                     | 40    |
| Pectin (g)             | 2                      | 2     |
| Sugar (g)              | 15                     | 15    |
| Distilled water (mL)   | 95                     | 105   |
| Fermented pepper juice (mL) | 10                   | -     |
| Starter (mg)           | 20                     | 20    |

1Starter: Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus and Bifidobacterium animalis ssp. lacti. (Lyofast YAB 450 AB, Sacco srl., Codarago, Italy).

Table 2. The ingredients of the experimental diets

| Groups | Ingredients (g) | CON1 | HFCD2 | PY3 | LFY4 | HFY5 |
|--------|----------------|------|-------|-----|------|------|
| Casein | 200.0          | 233.0| 228.4 | 228.9| 222.9|      |
| Corn starch | 397.5 | 84.8 | 73.4  | 72.4 | 53.4 |      |
| Sucrose | 100.0          | 201.4| 201.4 | 201.4| 201.4|      |
| Dextrose | 132.0          | 116.5| 116.5 | 116.5| 116.5|      |
| Celulose | 50.0           | 58.3 | 58.3  | 58.3 | 58.3 |      |
| Soybean oil | 70.0           | 29.1 | 25.6  | 25.8 | 20.9 |      |
| Lard    | 0.0            | 206.9| 206.9 | 206.9| 206.9|      |
| Mineral mixture | 35.0 | 52.4 | 52.4  | 52.4 | 52.4 |      |
| Vitamin mixture | 10.0         | 11.7 | 11.7  | 11.7 | 11.7 |      |
| L-cystine | 3.0            | 3.0  | 3.0   | 3.0  | 3.0  |      |
| Choline | 2.5            | 2.5  | 2.5   | 2.5  | 2.5  |      |
| Cholesterol | -              | 10.0 | 10.0  | 10.0 | 10.0 |      |
| Cholin acid | -              | 5.0  | 5.0   | 5.0  | 5.0  |      |
| Plain yogurt | -              | 20.0 | -     | -    | -    |      |
| Total (g) | 1000.0         | 1015.0| 1015.0| 1015.0| 1015.0|      |
| Total (kcal) | 4000.0         | 4865.5| 4865.5| 4865.5| 4865.5|      |

1CON: control diet fed group.
2HFCD: high fat and high cholesterol diet fed group.
3PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
4LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
5HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.

Animal Inc., Korea) were individually housed in plastic
cages and were acclimated for one wk. Then, animals were
randomly assigned into five groups (five rats/group); the
weights of each group were not significantly different (p>
0.05). The room was maintained at 22 ± 2°C, 50 ± 5% hu-
midity, and a 12 h light/dark cycle during the 9 wk ex-
perimental period. The animal experiment was approved
by the Institutional Animal Care and Use Committee
(IACUC approval no. KU13090).

Experimental groups were as follows; CON (control
diet), HFCD (45% kcal high fat and high cholesterol diet;
HFCD), PY (HFCD supplemented with plain yogurt diet),

Animal experiment
Seven wk old 25 Sprague-Dawley male rats (Central Lab.
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LFY (HFCD supplemented with 2% fermented pepper juice yogurt), and HFY (HFCD supplemented with 5% fermented pepper juice yogurt). The ingredients of feed are represented in Table 2. The feeding amount was restricted to 20.5 g/mouse/1 d and tap water was available ad libitum.

The body weight was measured once a week and food intake was weighed at the same time each day using a balance (Adventurer AR2140, OHAUS crop, USA). Food intake was calculated as the difference of the feed ration of the day before and remains of the day. Feed efficiency ratio (FER) was calculated as follows; FER (%) = body weight gain (g) / total food intake (g) × 100.

All animals had an adaptation period of 1 wk and each experimental diet was offered to each group for 9 wk. After fasting of 24 h, dissection was performed. Anesthesia was accomplished by diethyl ether. Blood was gathered from the abdominal vein and the spleen, liver, cecum, kidney, epididymal adipose tissue (EAT), and perirenal adipose tissue (PAT) were weighed.

Serum analysis

After standing at 25°C for 3 h, serum was separated from blood and centrifuged (VS-550, Vision Scientific, Korea) at 3,000 rpm for 20 min at 4°C. Serum aspartate aminotransferase (AST), total cholesterol, high density lipoprotein cholesterol (HDL cholesterol) and glucose were analyzed by Green Cross Corp. (Korea).

Liver analysis

Total lipid content was analyzed by modified Folch’s method (Folch et al., 1956). The liver was homogenized with 0.85% NaCl, and the residue was washed with chloroform/methanol (2:1, v/v). After centrifuging at 3,000 rpm for 20 min, the supernatant and floating matter were removed. Residue was transferred to previously weighed tube and concentrated with N₂ gas. Total lipid content was calculated as concentrated tube weight minus previous weight of the tube.

Total cholesterol content was analyzed using a kit (Asan Pharm. Co., Korea). 2 mL of ethanol was added to the concentrated total lipid sample. Then, 20 µL was mixed with 3 mL of enzyme solution. After storage at 37°C for 5 min, absorbance was measured at 500 nm (Optizen 2120 UV, Mecasys Co. Ltd., Korea).

Statistical analysis

All data were expressed as mean ± standard deviation, and analyzed by Tukey’s multiple-range test using SAS ver. 8.2 software (SAS Institute, USA). Significance was declared at p<0.05. Extremely different value was excluded.

Results and Discussion

Body weights, food intake, and FER during the experimental period are summarized in Table 3. Initial body weight in each group was not significantly different (p>0.05). Final body weight in the HFY group significantly decreased comparing to the HFCD group (p<0.05). FER in the high fat diet fed groups were significantly higher than that of the CON group (p<0.05), but the HFY group showed lower FER as compared to other high fat diet fed groups (p>0.05). Total food intake was same except for the HFCD group but it was not significantly lower than the other groups (p>0.05). Kim et al. (2010) also reported lower food intake in high fat and high cholesterol diet fed groups.

| Item                        | CON (n=5) | HFCD (n=5) | PY (n=5) | LFY (n=5) | HFY (n=5) |
|-----------------------------|-----------|------------|----------|-----------|-----------|
| Initial body weight (g)     | 270.6±9.29<Superscript NS> | 270.4±5.86 | 270.4±5.86 | 270.3±5.94 | 270.3±5.91 |
| (n=5)                       |           |            |          |           |           |
| Final body weight (g)       | 477.5±6.25<Superscript a> | 541.5±14.27<Superscript b> | 536.5±2.38<Superscript b> | 535.5±8.02<Superscript b> | 518.8±4.35<Superscript b> |
| (n=4)                       |           |            |          |           |           |
| Total food intake (g)       | 1433.0±0.00<Superscript NS> | 1424.2±13.42 | 1433.0±0.00 | 1433.0±0.00 | 1433.0±0.00 |
| (n=5)                       |           |            |          |           |           |
| FER (%)                     | 14.7±1.25<Superscript b> | 18.7±0.83<Superscript b> | 18.7±0.73<Superscript b> | 18.5±0.39<Superscript b> | 17.9±0.73<Superscript b> |
| (n=5)                       |           |            |          |           |           |

<Superscript NS>: Superscripts with different letters indicate significant difference (p<0.05).

<Superscript 1>: CON: control diet fed group.
<Superscript 2>: HFCD: high fat and high cholesterol diet fed group.
<Superscript 3>: PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
<Superscript 4>: LFY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
<Superscript 5>: HFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
<Superscript 6>: FER: food efficacy ratio.
<Superscript 7>: NS: not significant.
compared to the CON group.

Weights of liver, spleen, kidney, cecum, PAT and EAT in animals are shown in Table 4. Weights of liver in high fat diet fed groups were significantly higher than CON group (p<0.05) possibly because of the existence of the depot fat in liver. There were no significant differences in liver weights in high-fat diet groups, which showed decreasing tendency as fermented pepper juice concentrate in yogurt was increased (p>0.05). Spleen weights in high fat diet fed groups were significantly higher than in the CON group (p<0.05), whereas the weight of the cecum was not significantly different weight among the 5 groups (p>0.05). Kidney weights in the HFCD and LFY groups were significantly higher than the CON group (p<0.05). Weights of PAT and EAT in the HFCD, PY, and LFY groups were significantly higher than the CON group (p<0.05), but HFY group was not (p>0.05). This might bring about the significantly lower body weight in HFY group than HFCD group (p<0.05). Lipid such as high body fat and cholesterol is absorbed to liver and it can cause fatty liver, moreover other hepatic diseases, which are associated with obesity (Tessari et al., 2009). HFY group showed significantly lower body fat (p<0.05), therefore fermented pepper might lower occur of hepatic diseases.

Table 4. Weights of organs and body fats in CON, HFCD, PY, LFY, and HFY group after 9 wk of experiment

| Item          | CON\(^1\) | HFCD\(^2\) | PY\(^3\) | LFY\(^4\) | HFY\(^5\) |
|---------------|-----------|------------|----------|-----------|-----------|
| Liver (g) (n=5) | 10.4±0.32\(^b\) | 25.1±1.95\(^c\) | 25.0±1.70\(^a\) | 23.6±2.22\(^a\) | 22.8±1.17\(^a\) |
| Spleen (g) (n=5) | 0.7±0.03\(^c\) | 1.0±0.19\(^ab\) | 1.1±0.05\(^a\) | 1.0±0.13\(^ab\) | 0.9±0.14\(^a\) |
| Kidney (g) (n=5) | 2.5±0.09\(^b\) | 2.7±0.12\(^a\) | 2.7±0.04\(^ab\) | 2.8±0.16\(^a\) | 2.6±0.18\(^ab\) |
| Cecum (g) (n=5) | 3.1±0.49\(^NS\) | 3.7±0.31 | 3.4±0.36 | 3.3±0.23 | 3.5±0.60 |
| PAT\(^6\) (g) (n=4) | 11.9±3.29\(^b\) | 16.7±0.65\(^a\) | 18.0±1.71\(^a\) | 18.8±1.33\(^a\) | 16.0±1.21\(^ab\) |
| EAT\(^7\) (g) (n=4) | 9.5±1.07\(^b\) | 14.8±2.15\(^a\) | 14.7±1.81\(^a\) | 14.7±0.98\(^a\) | 12.7±3.09\(^ab\) |

\(^{a-b}\)Superscripts with different letters indicate significant difference (p<0.05).
\(^1\)CON: control diet fed group.
\(^2\)HFCD: high fat and high cholesterol diet fed group.
\(^3\)PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
\(^4\)LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
\(^5\)HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
\(^6\)PAT: perirenal adipose tissue.
\(^7\)EAT: epididymal adipose tissue.
\(^8\)NS: not significant.

Table 5. Level of serum glucose, aspartate aminotransferase, total cholesterol, HDL cholesterol in CON, HFCD, PY, LFY, and HFY group after 9 wk of experiment

| Item          | CON\(^1\) | HFCD\(^2\) | PY\(^3\) | LFY\(^4\) | HFY\(^5\) |
|---------------|-----------|------------|----------|-----------|-----------|
| GLU\(^6\) (mg/dL) (n=5) | 78.4±11.0\(^NS\) | 63.8±13.48 | 81.6±16.40 | 72.0±5.48 | 83.8±19.07 |
| AST\(^7\) (U/L) (n=4) | 329.3±16.56\(^b\) | 644.0±80.94\(^a\) | 606.8±125.23\(^a\) | 704.5±46.62\(^a\) | 572.0±132.77\(^a\) |
| TC\(^8\) (mg/dL) (n=4) | 70.8±2.87\(^b\) | 106.5±18.12\(^a\) | 113.3±11.09\(^a\) | 111.5±9.98\(^a\) | 88.0±16.99\(^ab\) |
| HDL-C\(^9\) (mg/dL) (n=4) | 68.0±9.20\(^a\) | 51.0±4.24\(^b\) | 61.0±1.15\(^ab\) | 62.0±7.26\(^ab\) | 54.75±4.57\(^ab\) |

\(^{a-b}\)Superscripts with different letters indicate significant difference (p<0.05).
\(^1\)CON: control diet fed group.
\(^2\)HFCD: high fat and high cholesterol diet fed group.
\(^3\)PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
\(^4\)LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
\(^5\)HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
\(^6\)GLU: glucose.
\(^7\)AST: aspartate aminotransferase.
\(^8\)TC: total cholesterol.
\(^9\)HDL-C: high density lipoprotein cholesterol.
\(^10\)NS: not significant.
(1996) reported that feeding a high fat diet to rat increases serum glucose concentration even after a 24 h fasting for 4 wk. However, in this experiment, serum glucose content showed no significant difference ($p>0.05$). This difference might be resulted from different strain and feeding duration (Wirth-Dzięciołowska et al., 2009). AST exist in almost all organ such as heart, liver, skeletal muscle, and kidney and it would be released to blood, if the organs are damaged (Rappaport, 1993). In this research, AST content in serum was measured, and the high fat diet fed groups were significantly higher than CON group ($p<0.05$), maybe because of the damage from depot fat in the liver. Total cholesterol concentration in the HFY group was decreased compared to other high-fat diet groups, and it was not significantly different from the CON group ($p>0.05$). According to reports of Tani et al. (Tani et al., 2004) and Srinivasan and Satyanarayana (Srinivasan and Satyanarayana, 1987), total cholesterol level in capsaicin supplemented diet fed group significantly decreased ($p<0.05$), and cholesterol excretion through fecal showed increasing tendency than control group (Tani et al., 2004). In this study, fermented product such as cleavage of capsaicin might act as a role of capsaicin in report of Tani et al. It means fast lipid metabolism in HFY group than HFCD group. HDL cholesterol amount in the HFCD group was the lowest and showed significantly lower amounts compared to that of the CON group ($p<0.05$). On the other hand, the PY and LFY groups showed insignificant differences with the CON group ($p>0.05$).

Amounts of total lipid and total cholesterol in liver are represented in Table 6. The HFCD group showed the highest amount of hepatic total lipid among the 5 groups, significantly ($p<0.05$). Level of total lipid in the PY, LFY, and HFY groups were decreased significantly compared to the HFCD group ($p<0.05$). The PY and LFY groups showed no significant differences with the CON group ($p>0.05$). In total cholesterol, the HFCD and PY groups were significantly higher than the CON group ($p<0.05$) and the LFY and HFY groups showed decreased levels compared to the HFCD group and had no significant differences with the CON group ($p>0.05$). This result means that fermented pepper powder might inhibit the formation of cholesterol in liver.

Generally, absorbed capsaicin is metabolized to vanillylamine, and subsequently vanillin, vanillyl alcohol, and vanillic acid (Kawada and Iwai, 1985). These bind with transient receptor potential vanilloid 1 (TRPV1) and it secretes catecholamine and stimulates the sympathetic nerve (Iwai and Watanabe, 1999). It results in lipolysis, body heat by energy metabolism, and inhibition of body fat accumulation (Iwai and Watanabe, 1999; Iwasaki et al., 2011).

Fermentation products, such as cleavages of degraded capsaicin, in fermented pepper juice would be transferred and added to yogurt. When it is absorbed, this also would stimulate TRPV1, as capsaicin metabolites, but without pungency.

In this study, fermented pepper juice supplemented yogurt fed group showed anti-obesity, and improvement in lipid metabolism. Therefore, the final fermentation product made from fermented pepper juice might act as an inhibitor of both hypertrophy in organ and accumulation of body fat. It might effect on accumulation of hepatic lipid and high cholesterol in high fat and high cholesterol diet fed rats. Further study would be needed about the fermentation product of pepper and the identification of mechanism.

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### Table 6. Hepatic total lipid and total cholesterol amounts in CON, HFCD, PY, LFY and HFY group after 9 wk of experiment

| Item | CON$^{a}$ | HFCD$^{a}$ | PY$^{b}$ | LFY$^{b}$ | HFY$^{b}$ |
|------|------------|------------|----------|----------|----------|
| TL$^{c}$ (g) (n=3) | 95.5±0.47$^{a}$ | 124.5±2.29$^{a}$ | 94.9±5.33$^{a}$ | 100.4±3.88$^{a}$ | 110.9±1.14$^{b}$ |
| TC$^{d}$ (mg/dL) (n=3) | 43.1±9.11$^{b}$ | 137.5±35.96$^{a}$ | 122.0±45.46$^{a}$ | 110.2±8.55$^{ab}$ | 106.5±27.16$^{ab}$ |

$^{a,b}$Superscripts with different letters indicate significant difference ($p<0.05$).

1) CON: control diet fed group.
2) HFCD: high fat and high cholesterol diet fed group.
3) PY: high fat and high cholesterol diet supplemented with 2% plain yogurt powder.
4) LFY: high fat and high cholesterol diet supplemented with 2% fermented pepper juice yogurt powder.
5) HFY: high fat and high cholesterol diet supplemented with 5% fermented pepper juice yogurt powder.
6) TL: total lipid.
7) TC: total cholesterol.
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