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Anti-obese and anti-diabetic effects of Kaempferol glycosides from unripe soybean leaves in high-fat-diet mice

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Abstract

The present study investigated the anti-obese and anti-diabetic effects of kaempferol glycosides fraction (KG) which was composed by four kaempferol glycosides and purified from unripe Jindai-soybean (Edamame) leaves in C57BL/6J mice. High fat-fed mice treated with 0.15% dietary KG for 92 days had reduced body weight, adipose tissue and TG levels compared to high fat-fed control group. KG-treatment also decreased fasting blood glucose, serum HbA1c (hemoglobin A1c) levels and improved insulin resistance. Gene expression analysis of liver showed that KG decreased peroxisome proliferators-activated receptor (PPAR-γ) and sterol regulatory element-binding protein (SREBP-1c) expression. These results suggest that KG reduced the accumulation of adipose tissue, improve haperlipidemia as well as diabetes in obese mice by increasing lipid metabolism through the downregulation of PPAR-γ and SREBP-1c. Thus, KG may have an anti-obese and anti-diabetic potential.

Key words: kaempferol glycosides; obesity; diabetes; high-fat-diet; C57BL/6J mice

Abbreviations

HbA1c hemoglobin A1c
OGTT glucose tolerance test
HMW adiponectin, high molecular weight adiponectin
T-Chol total-cholesterol
HDL-Chol high density lipoprotein- cholesterol
TG triglyceride
PL phospholipids
NEFA non-esterified fatty acids
T-BA total bile acid
FAS fatty acid synthase
CPT carnitine palmitoyl transferase
AUC the area under the curve
TNF-α tumor necrosis factor
PPAR-γ proliferators-activated receptor
SREBP-1 sterol regulatory element-binding protein
Introduction

The prevalence of obesity and type 2 diabetes has increased worldwide in both developed countries and developing countries during the last century \(^1\). The global prevalence of diabetes in 2010 was 280 million people worldwide (6.2% of the total population), and it has been predicted that by 2030 the number will reach more than 7.5% of the world’s total population \(^2\). The increase of type 2 diabetes is closely linked to upsurge in obesity. About 90% of type 2 diabetes is attributable to excess weight. Further, approximately 197 million people worldwide have impaired glucose tolerance, related to obesity and the associated metabolic syndrome \(^3\).

The flavonoid kaempferol is commonly found in plant-derived foods and in plants used in traditional medicine \(^4\). Studies have shown that the presence of a double bond at C2-C3 in conjugation with an oxo group at C4, and the presence of hydroxyl groups at C3, C5 and C4’, are important structural features involved in the antioxidant activity of kaempferol \(^5, 6\). Numerous reports have shown diverse properties of kaempferol, including antioxidant activity, anti-inflammatory activity, anticancer activity, anti-obese activity, and anti-diabetic activity \(^7-12\). Some kaempferol glycosides have also been proved to have anti-diabetic effects \(^13-15\), especially activities of kaempferitrin which is one of the diglycosyl kaempferol derivative has also been fully studied and proved \(^16, 17\). However, to our knowledge, the data on the bioactivities of the present four kaempferol glycosides, especially the comprehensive effects of anti-obese and anti-diabetes \(in vivo\) is limited.

The dietary flavonoids in nature exist as aglycon and glycosides, but almost all of them exist as their glycosides \(^18\). In the past, it was strongly believed that flavonoid glycoside could not be absorbed but only after hydrolysis by bacterial flora in the lower part of the intestine \(^19\). Later researchers proved that flavonoid glycosides can be absorbed intact efficiently \(^20\). Studies showed that aglycon form flavonoids are more efficient than their glycosides \(^21\). Other studies proved that flavonoid glycosides have similar or higher bioactivities than their flavonoid aglycones \(^18\). This indicates that there is a lack of data that would make it possible to make broad generalizations concerning the influence of glycosylation on the benefits of flavonoids for human health.

In this study, kaempferol glycosides-rich fraction prepared from unripe soy bean leaves and its anti-obese and anti-diabetic effects were determined. The four kaempferol glycosides are kaempferol 3-O-\(\beta\)-D-glucopyranosy(1→2)-O-[\(\alpha\)-L-rhamnopyranosyl(1→6)]-\(\beta\)-D-galactopyranoside (1), kaempferol 3-O-\(\beta\)-D-glucopyranosy(1→2)-O-[\(\alpha\)-L-rhamnopyranosyl(1→6)]-\(\beta\)-D-glucopyranoside (2), kaempferol 3-O-\(\beta\)-D-(2-O-\(\beta\)-D-glucopyranosy) galactopyranoside (3) and kaempferol 3-O-\(\beta\)-D-(2,6-di-O-\(\alpha\)-L-rhamnopyranosyl) galactopyronoside (4), which were identified in the authors’ previous study \(^[22]\).
The HPLC chromatogram and chemical structure of the four compounds are shown in Fig. 1. The amounts of the four major compounds in the KG were 244, 241, 99, and 49 mg/g dry weight, respectively. On markers of obesity, body weight, adipose tissue were observed, serum and liver lipid levels were also measured. Furthermore, the anti-diabetic effects were determined by measuring the relevant markers of diabetes as glucose tolerance, HbA1c, insulin, fasting glucose and some adipocytokine levels. In order to further study the mechanism, we also measured some relative enzyme activities and gene mRNA expression concerned with lipid metabolism.

Materials and methods

Materials

The kaempferol glycoside-rich fraction (KG) was prepared from Jindai-Soybean (Glycine max. L. Merr. ‘Jindai’) leaves which were collected at the Mogami area of Yamagata Prefecture, Japan. The dried leaves were extracted with 70% methanol at 60-70 °C under reflux for 3 h. The evaporated extract was dissolved with MeOH, followed by addition of CHCl3 and H2O to become the mixture of CHCl3/MeOH/H2O (1:1:2). The upper phase of the mixture was applied to Diaion-exchange resin column after its concentration. After washing the column with H2O, phenolic compounds were eluted with aqueous 20%, 50% and 100% MeOH solution, successively. KG obtained by applying the aqueous 20% MeOH solution to Silica gel column chromatography, using EtOAC/MeOH system as developing solvent. The detail of identification and extraction methods were showed in our previous study 22. The HPLC chromatogram of KG and chemical structure of the four compounds were showed in figure 1.

Animals

Male C57BL/6J mice, average weighing 19 g (6 weeks old) were purchased from Clea (Tokyo, Japan) and housed individually under a 12:12 h light-dark cycle at 22 ± 2 °C and 40-60% humidity. After acclimatizing for 5 d, the C57BL/6J mice were randomly divided into 3 groups of 7 each, and fed on either the basal diet control group (CON) or high fat diet (HF) or high fat diet containing kaempferol glycosides fraction (HFKG) at 0.15%. The composition of each experimental diet is shown in Table 1. The diets and water were given for 92 d ad libitum.

The mice were anesthetized with Nembutal (Dainippon Pharmaceutical Co. Osaka, Japan) after 10 h of fasting, and the blood was collected by cardiac puncture, followed by detaching the liver. The detached live was stored at -80 °C until needed for analysis. The left kidney, left pararenal adipose tissues, left epididymal adipose tissue and
mesenteric adipose tissue were also detached and stored at -80 °C until needed for analysis. Serum was prepared by centrifuging the collected blood at 1,000 × g for 15 min.

Mice were taken care of according to the institutional guidelines of Yamagata University.

Measurements of fasting blood glucose levels, HbA1c and blood glucose tolerance test

Fasting blood glucose levels were measured with a Medesafe GR-102, 20-600 mg/dl measuring range (Termo Co., Tokyo, Japan) on the day 1, 42 and 92 after 10 h of fasting. HbA1c levels were measured with a Micromat II (Bio-Rad Laboratories, California, USA) at 10:00-11:00 am after 10 h of fasting on the day 42 and 92 of the feeding period. An oral glucose tolerance test (OGTT) was performed on day 42 after 10 h of fasting.

Measurements of Insulin, adiponectin, and tumor necrosis factor (TNF-α) levels

The serum level of insulin, adiponectin, high molecular weight (HMW) adiponectin, TNF-α, and leptin were measured with commercial ELISA kits (each, Mouse insulin (S-Type) Kit, Shibayagi Co., Gunma, Japan; Mouse adiponectin kit, Otsuka Co., Tokyo, Japan; Mouse high molecular weight adiponectin kit, Shibayagi Co., Gunma, Japan; Mouse TNF-α kit, Biosource International, California, USA; Mouse leptin Kit, Morinaga Co., Tokyo, Japan).

Lipid analysis

Total-cholesterol and HDL-cholesterol (T-Chol and HDL-Chol), triglyceride (TG), phospholipid (PL), and total non-esterified fatty acids (NEFA) in the serum and liver, and feces T-Chol, TG and total bile acid (T-BA) levels were measured with commercial kits (cholesterol E test, HDL-cholesterol E test, triglyceride E test, phospholipid B test, NEFA C test and total bile acid test; Wako pure Chemical Industries, Osaka, Japan). Liver and feces lipids were measured by using the isopropyl alcohol-soluble fraction of the lipids, which was prepared by removing the solvent in the lipid fraction obtained by the method of Folch et al23.

Liver enzyme activities

Each sample used for measurements of liver enzyme activities was prepared by homogenizing the liver in a 3 mM Tris-HCl buffer (PH 7.2) containing 0.25 M sucrose and 1mM EDTA. The supernatant of the homogenate
obtained by centrifuging at 500 × g for 10 min at 4 °C was re-centrifuged at 9,000 × g for 10 min at 4 °C, and further centrifuged at 105,000 × g for 60 min, respectively. The FAS activity was determined in the terms of malonyl-CoA- and acetyl-CoA-dependent oxidation of NADPH according to the methods of Kumer et al. and Carey et al. The reaction mixture was composed of a 0.1 M phosphoric acid buffer (pH 7.0) containing 0.2 mM malonyl-CoA. The rate of decrease in the absorbance at 340 nm was measured. To measure the CPT activity, the reaction mixture was composed of a 58 mM Tris-HCl buffer (pH 8.0) containing 0.25 mM DTNB, 0.04 mM palmitoyl-CoA, 1.25 mM EDTA and 1.25 mM L-carnitine. The CPT activity was determined from the rate of change in absorbance at 412 nm.

**Fecal lipid analyses**

Feces were collected on day 86-88 for 72 h. Fecal total lipids were extracted by the method of Folch et al. The lipids in the extracts were measured with commercial kits as described above.

**Quantitative real-time RT-PCR analysis**

The RNA was isolated from the liver using the RNeasy Mini kit (Qiagen N.V., Hilden, Germany), and its quantity and quality were assessed spectrophotometrically using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). The isolated RNA was converted to cDNA using the WT Sence Target Labeling kit (NuGen Technologies, Inc., San Carlos, CA, USA). The primer sets for each target gene, peroxisome proliferators-activated receptor (PPAR)-γ, sterol regulatory element-binding protein (SREBP)-1c were designed with the Primer3 Out program.

**Statistical analysis**

Each value is given as the mean ± SEM. The homogeneity of variance between treatments was verified by Bartlett’s test. Data were statistically analyzed by a one-way analysis of varist-hoc analysis of significance was made by using Fisher’s PLSD test, with significant difference at $p < 0.05$.

**Results**
Body weight and Adipose Tissue

Body weight, body weight gain and feed efficiency of the HFKG group were significantly lower than those of the HF group, but there were no significant differences between CON and HFKG groups (Figure 2). The liver and kidney weight did not differ among the three groups, the pararenal adipose tissue, epididymal adipose tissue, mesenteric adipose tissue and visceral adipose tissue weights of the HFKG group were significantly lower than those of the HF group (Figure 3, Table 2).

Fasting blood glucose levels, HbA1c and oral glucose tolerance test

The fasting blood glucose levels on day 1 did not differ among the CON, HF and HFKG groups, but those on day 42 tended to be decreased in the HFKG group compared with the HF group, and those on day 92 showed significantly lower levels in the HFKG group than in those in the CON group (Figure 4). Serum HbA1c levels measured on day 42 and 92 were significantly lower in the HFKG and CON groups compared with the HF group (Figure 5). Blood glucose levels on the OGTT carried out on day 42 tended to be lower in the HFKG group than the other two groups, but no statistic differences were found (Figure 6A). The area under the curve (AUC) on the OGTT also showed lower tendency in the HFKG group than in the CON and HF groups (Figure 6B).

Serum lipid levels and cytokine

As shown in Table 3, the serum TG was significantly lower, serum TC tended to be declined in the HFKG group compared with the HF group, but HDL-Chol, LDL-Chol and NEFA levels did not differ among the CON, HF and HFKG groups. The serum adiponectin and HMW adiponectin levels also did not differ among the three groups, even HMW adiponectin of the HFKG group was higher than that of the HF group. Leptin and Insulin levels of the HFKG group were significantly lower than those of the HF group. The TNF-α level tended to be lower in the HFKG group than in the HF group.

Liver lipid and fecal lipid levels

Liver TG was significantly decreased in the HFKG group than that in HF group. There were no differences on the liver TC and fecal total lipid, T-Chol, TG and total bile acids between the HFKG and HF groups (Table 3).
Live enzyme activities

The activities of the liver FAS and CPT enzymes are shown in Figure 7. The CPT and FAS activities in the HFKG group showed significantly higher and lower levels respectively, than those in the HF group.

Adipogenesis-related gene mRNA expression

We performed RT-PCR to examine the expression of PPAR-γ and SREBP1 mRNA expression in the liver. Both of them were suppressed by KG administration (Figure 8).

Discussion

Obesity is defined as an accumulation of excess adipose tissue, and decrease of body weight is recommended as first-line treatment to reduce it. In this study, the body weight of mice in the HFKG group decreased significantly in the 3rd week of the feeding period (Fig. 2). The adipose tissue weights were significantly higher in the HF group compared to the CON group. However, the dietary KG significantly suppressed the high fat diet-induced increase in the tissue weight deposits (Fig. 3). It suggests that the dietary KG has potential for anti-obesity. Previous studies also reported that the kaempferol and some glycosides may reduce body weight gain and visceral fat-pad weights caused by high fat diet.

TGs are involved in the accumulation of lipid stores in the liver and are associated with a number of diseases such as metabolic syndrome and type 2 diabetes. TG levels decreased significantly on both serum and liver by dietary of KG compared to the HF group. There was a decline on serum TC in the HFKG group in comparison with that of the HF group. No difference was found on liver TC among the groups. A higher serum HDL-Chol level and a lower serum LDL-Chol level was found in the KG group, but no statistical difference with HF group was found.

The results indicate an improvement of lipids accumulation by dietary of KG. Chang et al. proved that dietary of kaempferol resulted a positive effects on both serum and hepatic lipid levels on rats, and Yu et al. reported that the supplementation of kaempferol glycoside can significantly reduce serum TG and increase serum TC level on rats. It seems that the opinions were conflict. In the present study, the lipids lower effects of kaempferol glycosides on TGs are better than that on TCs (Table. 2).

Obesity is highly associated with insulin resistance and it is the biggest risk factor for non-insulin-dependent diabetes mellitus. The serum insulin level increased significantly in the HF group, as it has been reported that
feeding of a high fat diet could increase the serum insulin level, inducing insulin resistance on C57BL/6J mice, the lower insulin levels in the HFKG group indicate that KG is available to improve insulin resistance. One possibility for the improvement is the decrease of the visceral adipose tissue. Lower levels of fasting blood glucose and HbA1c in the feeding period of HFKG group may prove the improvement of insulin resistance by dietary of KG as well as the lower tendency of the OGTT (Figs. 4 and 5). A previous study also found that kaempferol improved diabetes.

It is considered that leptin is an indication of the state of energy stores in the body, and when the energy stores enlarge, leptin acts on the central nervous system stimulating satiety and energy expenditure. However, in most cases, human obesity is accompanied by a blunt central response to leptin action, and this leads to a situation of leptin resistance and compensatory hyperleptinaemia. In the present study, serum leptin level in the HF group was significantly increased when compared with that of the CON group (Table 3), suggesting that HF diet induced leptin resistance as has been previously reported. The lower serum leptin level in the HFKG group in comparison with the HF group suggests that the leptin resistance has been improved by KG consumption. Adiponectin, including high molecular weight (HMW) adiponectin is the most abundant hormone produced by adipose tissue, which has been suggested to be a better predictor of metabolic disturbances and insulin resistance associated with obesity. But changes in adiponectin and HMW adiponectin in this study, were less than those in the insulin and leptin levels (Table 3).

Obesity, insulin resistance and type 2 diabetes are closely associated with chronic ‘inflammation’ characterized by abnormal cytokine production, increased acute-phase reactants, and activation of a network of inflammatory signaling pathways. A previous study shows that TNF-α is over-expressed in the obese mice provided the first clear link between obesity, diabetes and chronic inflammation. In this study, lower tendency of the serum TNF-α level in the HFKG group was observed (Table 3), as well as the significantly decline of serum insulin and HbA1c compared with the HF group, suggesting that this inflammatory response has a critical role in the regulation of insulin action in obesity. The HFKG group mice deficient in TNF-α was protected from the development of obesity-induced insulin resistance.

The lower level of FAS activity of liver suggests that the reduction in fatty acid synthesis and enhance glycolysis by KG were closely related to the activity of mitigating increases in the serum and liver TG levels. On the other hand, the liver CPT activity in the HFKG group was significantly increased than that in the HF group. It is considered that the enhanced β-oxidation by KG was closely related to its stronger activity in suppressing the TG levels.
PPAR-γ and SREBP-1c are critical regulators of hepatic lipid metabolism. The factors stimulate expression of several enzymes involved in liver fatty-acid synthesis and glucose transport, gluconeogenesis, and lipolysis. The increased PPAR-γ and SREBP-1c expression in obese patients is strongly associated with fatty liver disease.

In the present study, the PPAR-γ and SREBP-1c expression were decreased in the HFKG group (Fig. 7), we assume that the decline of hepatic TG level is related to this effect, but it could also be linked to the inhibition of adipose tissue accumulation and body weight gain. It is reported that adenosine monophosphate-activated protein kinase (AMPK) inactivates PPAR-γ and SREBP-1c transcription and inhibits hepatic steatosis in HFD-induced animal models. A previous study showed that kaempferol significantly activated hepatic AMPK in mice, and AMPK is considered as a potential therapeutic target in the treatment of diabetes and obesity. It is assumed that anti-obese and anti-diabetic effects of KG are mediated by SREBP-1c and PPAR-γ regulation through AMPK activation.

The most abundant flavonoid glycosides in plants are flavone O/C-glycosides and flavonol O-glycosides. In some plants, especially in fruits, flavonol glycosides frequently occur at C-3 position as 3-O-glycosides. The four kaempferol glycosides in the present study are all flavonol 3-O-glycosides, compound 1, 2, and 4 are kaempferol triglycosides, and compound 3 is diglycoside. It is reported that flavonoid glycosides are commonly hydrolyzed to aglycone and produce effects in vivo. In this study, we supposed that the four kaempferol glycosides were hydrolyzed partly to aglycone, and produce activities in the form of aglycone, glycosides and conjugated forms, the metabolites of the compounds were not analyzed. The glycosides moiety of the compounds may perform the biological activities of improving the net absorption by the intestinal wall, as a result the transport through brush border of kaempferol 3-O-glycosides was more efficient than its aglycone. We hypothesize that the activities of kaempferol glycosides should be higher than their aglycone kaempferol under the equimolar administration, and thus, further investigation is required to prove it.

Conclusion

Dietary of KG (kaempferol glycosides) can improve obesity by decreasing the deposit of adipose tissue and lipid levels, accompanied with the beneficial effects of diabetes through the improvement of insulin and leptin resistance on C57BL/6J mice. Our findings provide evidence that the soybean leaves might be used as a material of the functional food. More study should be done on the mechanism in the future.
Acknowledgement

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Notes

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Table 1. Ingredient composition of the diet (%)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>CON</th>
<th>HF</th>
<th>HFKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>19.85</td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>53</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Tallow</td>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Mineral mixture$^1$</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture$^2$</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Kaemperol glycosides-rich fraction</td>
<td>—</td>
<td>—</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Total: 100 100 100

$^1$ AIN-93G-MX and $^2$ AIN-93-VX were purchased from Clea Japan (Tokyo, Japan).
Table 2. Effects of dietary kaempferol glycosides on the total food intake, body weight and feed efficiency

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>CON</th>
<th>HF</th>
<th>HFKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>4.95±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.50±0.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.33±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>10.1±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.8±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.6±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed efficiency (%)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.23±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.00±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.25±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver (% of body weight)</td>
<td>3.54±0.05</td>
<td>3.38±0.06</td>
<td>3.31±0.11</td>
</tr>
<tr>
<td>Kidney (% of body weight)</td>
<td>0.652±0.023</td>
<td>0.581±0.026</td>
<td>0.646±0.020</td>
</tr>
</tbody>
</table>

<sup>1</sup>(body weight gain (g)/food intake (g)). Each value is the mean ± SEM. n = 6 for each group. Values without a common letter differ significantly (p < 0.05).
Table 3. Effects of dietary kaempferol glycosides on the serum, liver, faeces lipids, and serum leptin, insulin and TNF-α levels

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>CON</th>
<th>HF</th>
<th>HFKG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>106±3</td>
<td>116±7</td>
<td>102±3*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>50.8±3.5 b</td>
<td>62.4±3.3 a</td>
<td>45.6±1.1 b</td>
</tr>
<tr>
<td>NEFA (mEq/dl)</td>
<td>0.69±0.03</td>
<td>0.72±0.03</td>
<td>0.65±0.04</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>91.0±9.0</td>
<td>68.9±8.0</td>
<td>79.1±14.2</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>43.1±7.5</td>
<td>49.7±6.6</td>
<td>37.0±8.2</td>
</tr>
<tr>
<td>Adiponectin (ug/ml)</td>
<td>1.96±0.07</td>
<td>1.77±0.07</td>
<td>1.86±0.05</td>
</tr>
<tr>
<td>HMW Adiponectin (ug/ml)</td>
<td>0.31±0.07</td>
<td>0.17±0.00</td>
<td>0.22±0.05</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>13.4±4.6 b</td>
<td>81±26.8 a</td>
<td>25.1±8.5 b</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>181±7</td>
<td>252±49</td>
<td>169±10*</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/g of liver)</td>
<td>2.71±0.24</td>
<td>2.48±0.20</td>
<td>2.72±0.18</td>
</tr>
<tr>
<td>TG (mg/g of liver)</td>
<td>38.3±1.0 ab*</td>
<td>45.7±2.5 a</td>
<td>35.6±2.8 b</td>
</tr>
<tr>
<td>Faeces</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipid (mg/3 days of feces)</td>
<td>50.5±2.1 b</td>
<td>65.2±4.8 a</td>
<td>73.6±3.3 a</td>
</tr>
<tr>
<td>T-Chol (mg/3 days of feces)</td>
<td>5.92±0.31</td>
<td>5.11±0.59</td>
<td>5.97±0.7</td>
</tr>
<tr>
<td>TG (mg/3 days of feces)</td>
<td>1.30±0.19 b</td>
<td>3.98±0.63 a</td>
<td>3.38±0.63 a</td>
</tr>
<tr>
<td>Total bile acid (mg/3 days of feces)</td>
<td>0.89±0.07 b</td>
<td>1.75±0.21 a</td>
<td>1.56±0.18 a</td>
</tr>
</tbody>
</table>

*\(\text{airy} - \text{HDL-Chol}/\text{HDL-Chol}\). Each value is the mean ± SEM. n = 3-7 for each group.

Values without a common letter differ significantly \((p < 0.05)\). *Compare with HF group \((0.05 < p < 0.1)\).
Fig. 1 HPLC chromatogram of kaempferol glycosides from unripe soybean (Jindai-mame) leaves, and the chemical structure of the four compounds.

R1: 3-O-β-D-glucopyranosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside;
R2: 3-O-β-D-glucopyranosyl(1→2)-O-[α-L-rhamnopyranosyl(1→6)]-β-D-galactopyranoside;
R3: 3-O-β-D-(2-O-β-D-glucopyranosyl) galactopyranoside;
R4: 3-O-β-D-(2,6-di-O-α-L-rhamnopyranosyl) galactopyranoside.
Fig. 2 Effects of dietary kaempferol glycosides on body weight.

Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05).
**Fig. 3** Effects of dietary kaempferol glycosides on adipose tissue. Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05). *Compare with HF group (0.01 < p < 0.05).
Fig. 4 Effects of dietary kaempferol glycosides on the fasting blood glucose (A), HbA1c (B) and Insulin (C) levels.

Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05).
**Fig. 5** Effects of dietary kaempferol glycosides on the blood glucose levels and AUC during the glucose tolerance test.

A: loading was carried out on day 42 of the feeding period; B: AUC (area under the curve). Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05). *Compare with HF group (0.05 < p < 0.1).
Fig. 6 Effects of dietary kaempferol glycosides on the hepatic activities of FAS and CPT. A: Activity of FAS; B: Activity of CPT. Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05).
Fig. 7 Effects of dietary kaempferol glycosides on the hepatic PPAR-γ and SREBP1 expression. A: PPAR-γ expression; B: SREBP1 expression. Each value is mean ± SEM. n = 6-7 for each group. Values without a common letter differ significantly (p < 0.05).