Pycnogenol Supplementation Promotes Lipolysis via Activation of cAMP-Dependent PKA in ob/ob Mice and Primary-Cultured Adipocytes

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Summary This study investigated the PKA-dependent inhibitory effect of pycnogenol (Pyc) on lipolysis using ob/ob mice and primary mouse adipocytes. Supplementation of Pyc at 30 mg/kg significantly reduced body weight gain and visceral fat mass. The serum and hepatic triglyceride (TG) and total cholesterol (TC) levels were reduced by Pyc supplementation, and high density lipoprotein (HDL)-cholesterol level significantly increased. In addition, hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) mRNA levels increased with Pyc supplementation in adipose tissue of ob/ob mice. The treatment of primary cultured adipocytes with Pyc at 100 μg/mL significantly increased glycerol release, cAMP level by reduction of phosphodiesterase-3B (PDE3B), and HSL levels, but decreased protein levels of perilipin A and fatty acid synthetase (FAS). The PKA inhibitor (H89) clearly blocked the cellular levels of perilipin A and HSL, suggesting that Pyc promotes lipolysis of adipocytes through activation of cAMP-dependent PKA, resulting in induction of HSL and reduction of perilipin A. Therefore, this study may elucidate the possible mechanism of Pyc, which is a candidate for weight loss through stimulation of lipolysis.

Key Words pycnogenol, C57BL/6J ob/ob mouse, primary adipocytes, PKA pathway

Obesity is a serious public health problem throughout the world and a major risk factor of chronic diseases such as hypertension, cancer, type-2 diabetes, and hyperlipidemia (1, 2). Its prevalence is increasing rapidly worldwide (3). Obesity stems from a positive mismatch between energy intake and energy expenditure, and is the pathological accumulation of triglycerides (TG) in adipose and other tissues (4, 5). The World Health Organization (WHO) estimates that by 2015 approximately 2.3 billion adults will be overweight and more than 700 million will be obese (6). Excessive energy intake, especially dietary fat intake, and reduced physical activity appear to be the major causes of obesity (7).

Adipose tissue is the largest energy storage site and releases hormones and adipokines that regulate metabolic homeostasis and insulin resistance (2, 8, 9). Excess triglyceride (TG) accumulation in adipose tissue leads to obesity (10). TG stored in lipid droplets is hydrolyzed to fatty acids (FAs) and glycerol via lipolysis (11). Lipolysis in adipocytes is controlled by hormones, neurotransmitters and other effector molecules (11, 12). The best known mechanism mediating lipolysis is the cAMP pathway, wherein increased levels of cAMP activate cAMP-dependent protein kinase A (PKA). In this pathway, hormone sensitive lipase (HSL) is phosphorylated by PKA and then translocates from the cytoplasm to the lipid droplet surface where it interacts with perilipin A, the result of which is a subsequent release of free fatty acids (12, 13). HSL is the most important lipase in lipolysis and is subject to hormonal regulation (14). In addition to HSL, adipose triglyceride lipase (ATGL) is expressed predominantly in adipose tissue and is considered to be the rate-limiting lipolytic enzyme in adipocytes (15, 16).

Pycnogenol (Pyc), a standardized extract from the bark of the French maritime pine (Pinus maritima), is widely used in dietary supplements, multi-vitamins and health products. Pyc is primarily composed of proanthocyanidins, flavonoids, and polyphenol (17, 18). It has strong antioxidant, anti-inflammatory, anti-cancer, anti-diabetes and anti-hypertension characteristics (17–19). Several studies reported that Pyc stimulates lipolysis through activation of β-receptor and HSL and inhibits lipid accumulation through suppression of adipogenic gene expression in 3T3-L1 adipocytes (20–22). However, the inhibitory effect of Pyc on PKA-dependent lipolysis has not been clearly determined. Therefore, the present study examined the lipolysis effect of Pyc on body weight gain, adipose tissue weight, lipid profiles, and mRNA expression of lipases in ob/ob mice as well as investigated cAMP-activated PKA-dependent lipolysis in primary cultured adipocytes.

MATERIALS AND METHODS

Reagents and preparation of pycnogenol. Pyc was
obtained from Jupiter International Co. (sole Korean agent of Horphag Research Ltd., Seoul, Korea). Pyc was dissolved in DMSO (final concentration: 0.1%) and stored at −20°C until assay. PKA inhibitor H89 was obtained from Calbiochem (La Jolla, CA). Anti-perilipin A and HSL. antibodies were purchased from Cell Signaling (Beverly, MA), and anti-β-actin antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Free glycerol detection reagents and all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Animals and diets. Male leptin-deficient (ob/ob) obese mice and lean C57BL/6J wild type (WT) mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The mice were housed in standard cages and placed in a room with a temperature of 22 ± 2°C, humidity of 50 ± 5%, and 12 h light/dark cycles. Following the adaptation period, mice were randomly divided into four groups of six mice each. The wild type control (WT control) and obese control (ob/ob control) groups were fed a normal basal diet (Purina Rodent Chow 38057), and two ob/ob groups (Pyc-10 and Pyc-30) were fed diets equivalent to 10 or 30 mg of Pyc per kg body weight daily for 9 wk. The amount of food consumption by each group was recorded daily and body weight was recorded weekly for 9 wk. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Kyung Hee University.

Adipose tissue, serum and liver analysis. At the end of the study, animals were fasted overnight and then sacrificed. Blood samples were collected to measure serum triglyceride (TG), total cholesterol (TC) and high-density lipoprotein (HDL)-cholesterol. Serum TG and TC concentrations were colorimetrically measured by reaction of lipase and esterase using commercial kits (BioVision, San Francisco, CA). HDL-cholesterol concentrations were also measured using commercial kits (Abcam, Cambridge, MA). Heart, kidney, liver, spleen and adipose tissues were removed and weighted. Liver tissues were homogenized and hepatic lipids were extracted according to the procedure described in Folch et al. (23). Hepatic TG and TC concentrations were analyzed with the same commercial kits used for serum analysis.

Quantitative real-time PCR. Adipose tissue was disrupted and homogenized using rotor-stator homogenizers with RLT buffer (lysis buffer, Qiagen, Valencia, CA) including β-mercaptoethanol. Total RNA was isolated from adipose tissue lysate using the RNaseasy Mini kit (Qiagen). Briefly, 1 μg of total RNA obtained from adipose tissue was incubated with reverse-transcript II reverse transcriptase (Invitrogen, Carlsbad, CA) to produce cDNA. Real-time PCR was performed using the selective primer sets with Universal SYBR Green PCR Master Mix according to the manufacturer’s instructions (Qiagen). The sequences of the sense and antisense primers used for amplification were as follows: GAPDH, (F) 5′-CCATGAGAAATGATCACAACGCC-3′ and (R) 5′-TTCGAGCCTTCTCAGACCAGGG-3′; HSL, (F) 5′-ACC-GAGCAGGGCTGCTGTTG-3′ and (R) 5′-GAATGGGCTGCGAGGCG-3′; adipose triglyceride lipase (ATGL), (F) 5′-AACACCGCATCCGTTCAA-3′ and (R) 5′-GGTCGATAGGCCATTCC-3′; lipoprotein lipase (LPL), (F) 5′-ACTCGCTCTCAAGATGCCCTA-3′ and (R) 5′-TGTGTGCTGTCATTCCCTC-3′. Data analysis was performed with 7500 System SDS software version 1.3.1 (Applied Biosystems Inc., Carlsbad, CA).

Isolation and culture of primary adipocytes. Primary cultured adipocytes were obtained from male C57BL/6 mice (purchased at 6 wk old from Japan SLC, Inc.) fed high-fat diets (45% fat content) for 12 wk. First, mouse intra-abdominal adipose tissues were dissected immediately after sacrifice and minced in 5 mL of DMEM supplemented with 1 mg/mL type I collagenase (Sigma-Aldrich) and 1% BSA for 30 min at 37°C while shaking at 100 cycles/m. The cells were filtered through a 100 μm nylon mesh, and washed three times in pre-warmed serum-free DMEM (f-DMEM). The floating adipocytes in the tube were centrifuged at 1,600 rpm for 5 min. Packed adipocytes were diluted in 1% BSA f-DMEM to generate a cell suspension. Thereafter, primary cultured adipocytes were seeded onto 24-well or 6-well plate for each experimental purpose and incubated with Pyc (0, 50 or 100 μg/mL) to measure glycerol release, intracellular cAMP level and lipolytic proteins.

Measurement of free glycerol release. The incubation medium for the primary cultured adipocytes was transferred to another set of tubes and heated at 70°C for 10 min to inactivate any enzymes released by the cells. Then, the incubation medium was analyzed for free glycerol using the glycerol detection reagent in 1 mL disposable cuvette and the absorption was measured at 540 nm. In order to compensate for the cell number, protein content was determined using a Bradford assay.

Measurement of intracellular cAMP concentrations. In primary cultured adipocytes, intracellular cAMP concentrations were measured using a commercially available enzyme immunoassay kit (Amersham Pharmacia Biotech, Piscataway, NJ), according to the manufacturer’s recommendations. Results were corrected for cellular protein content and expressed as femtomoles of cAMP per milligrams of protein.

Western blot analysis. Primary mouse adipocyte lysates were prepared using RIPA buffer containing 50 mM phosphate buffer, pH 7.4, 0.5% Nonidet P-40, 0.1% SDS and protease inhibitor cocktail (Sigma). Equal amounts of proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Millipore, Bedford, MA). Membranes were blocked with 5% skim milk and then incubated with primary antibodies overnight at 4°C. After washing and binding with horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for 1 h, the reaction products were visualized with chemiluminescent reagents (ECL, Amersham).

Statistical analysis. Data are presented as the mean ± standard deviation (SD). Data was analyzed by ANOVA followed by the Bonferroni test for multiple comparisons and Student’s t-test using SAS software. Differences were considered statistically significant at p<0.05.
Effect of Pyc on PKA-Dependent Lipolysis

RESULTS

Body weights and food intake

The body weight gain (g/d) in the ob/ob control group was 2.3-fold greater than in the WT control group (Table 1). The body weight gain in the Pyc-30 group decreased significantly by 45% compared to the ob/ob control group. Supplementation of Pyc did not influence food intake in the treated mice. However, the food efficiency ratio (FER) in the Pyc-30 group was significantly lower than in the ob/ob control group. There was no significant difference in weight gain, food intake or FER with supplementation of Pyc-10.

Weight of organs and adipose tissue

There were no significant differences in the heart, kidney or spleen weights among the groups (Table 2). However, liver weight in the ob/ob control group significantly increased by 2.9-fold compared to the WT control. The liver weight in mice supplemented with Pyc-30 decreased by 37% compared to the ob/ob control group. The visceral adipose tissue was measured in two separate compartments, retroperitoneal adipose tissue (RAT) and epididymal adipose tissue (EAT). The weights of RAT and EAT in the ob/ob control group significantly increased by 4.1-fold and 2.5-fold compared to the WT control group. But Pyc-30 supplementation significantly decreased RAT and EAT weight by 48% and 42%, respectively, compared to the ob/ob control group.

Lipid profiles in serum and liver

The effect of Pyc supplementation on triglyceride (TG) and total cholesterol levels in both serum and liver was determined (Table 3). Serum TG and TC levels were sig-

<table>
<thead>
<tr>
<th>Group 1</th>
<th>WT control</th>
<th>ob/ob control</th>
<th>Pyc-10</th>
<th>Pyc-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>20.5±0.7b</td>
<td>32.3±3.1a</td>
<td>33.1±2.8a</td>
<td>33.0±1.9a</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>32.6±3.0c</td>
<td>60.1±4.5a</td>
<td>56.3±5.4a</td>
<td>48.2±6.1b</td>
</tr>
<tr>
<td>Weight gain (g/d)</td>
<td>0.19±0.1b</td>
<td>0.44±0.1a</td>
<td>0.37±0.1a</td>
<td>0.24±0.1b</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>3.5±0.3b</td>
<td>5.3±0.5a</td>
<td>4.8±0.6a</td>
<td>4.80±0.7a</td>
</tr>
<tr>
<td>FER</td>
<td>5.4±0.6b</td>
<td>8.3±0.9a</td>
<td>7.7±1.0a</td>
<td>5.0±0.8b</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6) and different letters indicate a significant difference (p<0.05) as determined by ANOVA followed by the Bonferroni test for multiple comparisons.

1 WT control group; ob/ob control group; Pyc 10 mg/kg supplemented group; Pyc 30 mg/kg supplemented group.

Table 2. Effects of Pyc on organ weight and adipose tissue mass in ob/ob mice.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>WT control</th>
<th>ob/ob control</th>
<th>Pyc-10</th>
<th>Pyc-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.11±0.01a</td>
<td>0.12±0.01a</td>
<td>0.11±0.01a</td>
<td>0.11±0.01a</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.24±0.02a</td>
<td>0.26±0.03a</td>
<td>0.24±0.03a</td>
<td>0.24±0.04a</td>
</tr>
<tr>
<td>Liver</td>
<td>1.72±0.27c</td>
<td>4.93±0.46a</td>
<td>4.51±0.61a</td>
<td>3.13±1.07b</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.06±0.01a</td>
<td>0.07±0.01a</td>
<td>0.06±0.01a</td>
<td>0.07±0.01a</td>
</tr>
<tr>
<td>RAT</td>
<td>0.48±0.14c</td>
<td>1.97±0.42a</td>
<td>1.74±0.55a</td>
<td>1.03±0.37b</td>
</tr>
<tr>
<td>EAT</td>
<td>1.61±0.29c</td>
<td>3.94±1.03a</td>
<td>3.23±0.72a</td>
<td>2.29±0.48b</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6) and different letters indicate a significant difference (p<0.05) as determined by ANOVA followed by the Bonferroni test for multiple comparisons.

RAT: retroperitoneal adipose tissues, EAT: epididymal adipose tissue.

Table 3. Effects of Pyc on serum and hepatic lipid contents in ob/ob mice.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>WT control</th>
<th>ob/ob control</th>
<th>Pyc-10</th>
<th>Pyc-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>137.2±27.5d</td>
<td>343.1±64.8a</td>
<td>267.3±47.5b</td>
<td>192.7±31.5c</td>
</tr>
<tr>
<td>TC</td>
<td>117.3±18.7b</td>
<td>194.0±29.4a</td>
<td>174.7±24.8a</td>
<td>144.7±19.1b</td>
</tr>
<tr>
<td>HDL-C</td>
<td>59.0±9.4b</td>
<td>51.2±13.3b</td>
<td>64.3±16.5b</td>
<td>85.6±10.7a</td>
</tr>
<tr>
<td>Liver (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>181.4±45.1d</td>
<td>342.2±32.5a</td>
<td>291.1±21.4b</td>
<td>241.8±34.3c</td>
</tr>
<tr>
<td>TC</td>
<td>142.6±21.8c</td>
<td>305.2±38.2a</td>
<td>274.3±47.1a</td>
<td>211.4±31.9b</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD (n=6) and different letters indicate a significant difference (p<0.05) as determined by ANOVA followed by the Bonferroni test for multiple comparisons.

TG: triglyceride, TC: total cholesterol, HDL-C: high density lipoprotein-cholesterol.

1 WT control group; ob/ob control group; Pyc-10 mg/kg supplemented group; Pyc-30 mg/kg supplemented group.
Figure 1: Effects of pycnogenol (Pyc) supplementation on mRNA expression of lipases in ob/ob mice. WT, normal control; ob/ob control, positive control; Pyc-10, Pyc 10 mg/kg body weight/day; Pyc-30, Pyc 30 mg/kg body weight/day. Adipose tissue was prepared and used in real-time PCR, as described in “Materials and Methods.” Values were calculated as a percentage of lipase mRNA expression versus that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal control. Results are presented as mean±SD with at least three independent experiments, each performed in triplicate (n=3). Different letters indicate a significant difference (p<0.05) as determined by the Bonferroni test for multiple comparisons.

**Intracellular cAMP level and lipolysis-related protein expression in primary cultured adipocytes**

To understand the regulatory signaling mechanism of Pyc-mediated lipolysis, we performed an enzyme immunoassay and Western blotting in order to analyze mediators known to be involved in lipogenesis and lipolysis in primary cultured adipocytes. As shown in Fig. 2B, treatment with Pyc at 100 μg/mL clearly decreased the expression of PDE3B. Subsequently, intracellular cAMP levels (Fig. 2A) and PKA expression (Fig. 2B) were increased by treatment with Pyc at both 50 and 100 μg/mL. In addition, treatment with Pyc at 100 μg/mL suppressed the expression of FAS, which indicated a reduction of de novo lipogenesis.

**Measurement of glycerol release in primary mouse adipocytes**

H89, as a PKA specific inhibitor, was used to confirm the effect of Pyc on PKA-dependent lipolysis. Treatment of primary cultured adipocytes with Pyc significantly increased glycerol release in a dose-dependent manner (Fig. 3). But addition of H89 to Pyc-treated adipocytes significantly inhibited glycerol levels, suggesting that H89 blocked Pyc-induced glycerol release in primary cultured adipocytes. These results could imply that Pyc...
is able to increase lipolysis via the PKA pathway.

Expression of lipolysis-related genes in primary mouse adipocytes

The involvement of perilipin A and HSL was determined to clarify the mechanism of Pyc-mediated lipolysis. Pyc treatment significantly down-regulated perilipin A in a dose-dependent manner (Fig. 4A). In contrast, treatment with Pyc significantly increased HSL in both 50 and 100 μg/mL (Fig. 4B). However, addition of H89 to Pyc-treated adipocytes significantly increased the cellular levels of perilipin A and decreased the cellular levels of HSL, suggesting that perilipin A and HSL were involved in the Pyc-induced PKA pathway, resulting in lipolysis.

DISCUSSION

Drugs for obesity treatment, such as orlistat and sibutramine, have common side effects that include headache, dry mouth, insomnia, diarrhea, bloating, and dyspepsia (24). Thus, recent studies have reported on the anti-obesity effects of natural compounds containing flavonoids or anthocyanin in both in vitro and in vivo models (25, 26). Pyc was well known as an antioxidant containing a standardized extract of procyanins and flavonoids that were active on chronic diseases. According to Lee et al. (22), treatment with Pyc at 100 μg/mL inhibits lipid accumulation by controlling adipogenic gene expression and antioxidant enzyme responses in 3T3-L1 adipocytes. But the lipolytic mechanism of Pyc on anti-obesity has not been fully elucidated. Therefore, this study employed an obese animal model to estimate the anti-obesity effect of Pyc by measuring body weight gain, fat mass and lipid profiles. In addition, lipolytic or lipogenic factors such as glycerol levels, perilipin A, fatty acid synthetase (FAS) and HSL were measured in primary-cultured adipocytes to understand the cAMP-related PKA dependent mechanism.

In the present study, body weight gain, liver weight and fat tissue mass were significantly reduced by Pyc supplementation, especially at 30 mg/kg. FER was significantly reduced by Pyc supplementation even though Pyc supplementation did not affect food intake. Thus, the results suggest that Pyc supplementation is associated with anti-obesity effect via suppression of fat accumulation in adipose tissue.

Obesity is a risk factor of coronary heart disease, in
levels were significantly increased by Pyc supplementation. In addition, HDL-cholesterol increased in the ob/ob control group compared to the WT control group, whereas they were clearly decreased by Pyc supplementation. Devaraj et al. reported similar results, in that Pyc supplementation (150 mg/d) for 6 wk significantly reduced low density lipoprotein (LDL)-cholesterol and increased HDL-cholesterol concentrations in the plasma of 25 healthy subjects. Taken together, these results suggest that Pyc might play a beneficial role in obesity-related diseases by improving lipid profiles.

On the other hand, stored TGs in adipose tissue are hydrolyzed to fatty acids and glycerol by lipases such as HSL, ATGL, and monoacylglycerol lipase (MAGL) (11, 29, 30). HSL and ATGL are expressed predominantly in adipose tissue. HSL is a rate-limiting enzyme for fat catabolism, namely the hydrolysis of TG and diacylglycerol (DG) (16). ATGL is specifically initiated in TG hydrolysis, resulting in production of DG and fatty acids (16, 27). In the present study, Pyc supplementation significantly increased HSL and ATGL mRNA expression that could help lipolysis in adipose tissue. In addition, LPL is a key enzyme involved in TG accumulation, which hydrolyzes TG-rich lipoproteins, mainly chylomicrons and very low-density lipoproteins (31, 32). LPL levels are high in the adipose tissue of obese humans and rodents (33). Consistent with these findings, LPL mRNA expression in adipose tissue of ob/ob mice was higher than in non-obese control mice, whereas Pyc supplementation at 30 mg/kg tended to decrease LPL mRNA expression. These data suggest that reduction of fat mass by Pyc supplementation could be associated with up-regulation of HSL and ATGL and down-regulation of LPL in adipose tissue.

Some fatty acids are synthesized from non-lipid substrates in the liver and adipocytes through de novo lipogenesis (8). Fatty acid synthetase (FAS) is an essential enzyme for fatty acid synthesis in adipose tissue. FAS regulates de novo lipogenesis from acetyl-CoA, malonyl-CoA and NADPH, and is expressed at high levels in adipose tissue, liver and lung (34). In contrast, lipolysis is critically dependent on intracellular levels of cAMP, which is regulated by the enzyme phosphodiesterase3 (PDE3) (35). The PDE3 gene family consists of two members, PDE3A and PDE3B, which break down cAMP to 5’AMP. PDE3 is expressed in insulin-sensitive cells, including hepatocytes, adipocytes and pancreatic β cells, and plays a key role in anti-glycogenolysis, anti-lipolysis and insulin secretion (35, 36). Under physiological conditions, adipocyte lipolysis is primarily stimulated by elevating the cellular cAMP content and by activating cAMP-dependent PKA (37). Thus, reduction of PDE3B leads to decreased hydrolysis of cAMP and increased cAMP-dependent PKA. Subsequently, the phosphorylations of HSL and perilipin by PKA efficiently increase lipolysis (12, 13, 38). HSL translocation from cytosolic locations to lipid droplets occurs rapidly, and catalyzes the hydrolysis of TG stored in adipocytes (12, 13). At a basal state, perilipin, which is located on the lipid droplet surface of fat cells, protects the TG core from attack by lipases (13, 39). However, phosphorylated perilipin may modify the lipid droplet surface in order to facilitate interaction of HSL with the core TG within the droplet (13, 40). Several studies have demonstrated that reduced levels or absence of perilipin results in increased basal lipolysis (39, 40). This study used a PKA inhibitor, H9, in primary-cultured mice adipocytes to investigate whether Pyc-mediated lipolysis involves PKA-dependent phosphorylation of perilipin A and HSL. Mochizuki and Hasegawa reported that Pyc significantly increases glycerol release and cAMP content in 3T3-L1 adipocytes (20). Moreover, procyandin, a major compound of Pyc, has a lipolytic effect by activating of HSL in 3T3-L1 adipocytes (21). Consistent with these findings, in this study, treatment of primary cultured adipocytes with Pyc at 50 and 100 µg/mL significantly increased glycerol release, decreased perilipin A and increased HSL levels. But H89 treatment clearly blocked the cellular levels of perilipin A and HSL, suggesting that Pyc promotes lipolysis through a PKA-dependent pathway in primary cultured adipocytes.

In conclusion, Pyc supplementation reduced the fat mass, resulting in a reduction of body weight and amelioration of serum and hepatic lipid profiles. In mechanistic aspects, Pyc promotes lipolysis of adipocytes through activation of cAMP-dependent PKA, resulting in induction of HSL and reduction of perilipin A. In addition, Pyc has been shown to reduce lipid accumulation by down-regulating FAS expression in de novo lipogenesis. Although there is no direct evidence that a concentration at 100 µg/mL in primary adipocytes is equal to a dose at 30 mg/kg in animal studies due to different experiment systems, Pyc might be, at least in part, a useful natural ingredient for controlling obesity.

Acknowledgments

This work was supported by a grant from Kyung Hee University in 2007 (KHU-20071384).

REFERENCES


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