Antiobesity action of ε-polylysine, a potent inhibitor of pancreatic lipase

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Abstract In vitro, ε-polylysine (EPL) strongly inhibited the hydrolysis of trioleylglycerol emulsified with phosphatidylcholine (PC) and taurocholate by either pancreatic lipase or carboxylester lipase. The EPL concentration required for 50% inhibition of pancreatic lipase, 0.12 μM, was eight times lower than the concentration of orlistat required for the same effect. The 50% inhibition concentration by EPL was affected by emulsifier species: it was increased ~150 times, 70 times, and 230 times on gum arabic, phosphatidylserine, and phosphatidic acid emulsion, respectively, compared with PC emulsion. The 50% inhibition concentration by orlistat was little changed by emulsifier species. Gel-filtration experiments suggested that EPL did not bind strongly to pancreatic lipase, whereas orlistat did. To test the effect of EPL on obesity, mice were fed a high-fat diet containing 0.1, 0.2, or 0.4% EPL. EPL prevented the high-fat diet-induced increase in body weight and weight of the liver and visceral adipose tissues (epididymal and retroperitoneal). EPL also decreased plasma triacylglycerol and liver and visceral adipose tissues (epididymal and retroperitoneal) and liver triacylglycerol content after they had been increased by the high-fat diet. The fecal weights of mice were increased by the high-fat diet containing EPL compared with the high-fat diet alone. Fecal lipid was also increased by the diet containing EPL.14 These data clearly show that EPL has an antiobesity function in mice fed a high-fat diet that acts by inhibiting intestinal absorption of dietary fat.—Tsujita, T., H. Takaichi, T. Takaku, S. Aoyama, and J. Hiraki. Antiobesity action of ε-polylysine, a potent inhibitor of pancreatic lipase. J. Lipid Res. 2006. 47: 1852–1858.

Supplementary key words orlistat • high-fat diet • obesity

Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerols to 2-monacylglycerol and fatty acids. Typical substrates for this enzyme are long-chain triacylglycerols, which are separated from the aqueous medium by the surface phase. Thus, for catalysis to occur, lipase must be adsorbed on the substrate lipid surface (oil-water interface), and the nature of the surface of the substrate is an important factor for lipase activity. Enzymes are sometimes activated or denatured by surface adsorption. As a consequence of these properties, amphoteric substances would be expected to influence the surface properties of substrate lipid and might affect the lipase reaction rate. There have been many reports of lipase inhibitors derived from natural materials, for example, proteins (1, 2), phytic acid (3), and saponins (4). Most of these inhibitors are amphoteric substances. It is well known that bile salts and synthetic detergents behave as inhibitors of lipolysis (5, 6). Amphiphilic proteins, such as BSA and β-lactoglobulin (7), have been shown to inhibit lipase activity toward its triacylglycerol substrate. Basic materials also influence the lipase reaction rate. Basic carbohydrates, such as chitin-chitosan, inhibited the hydrolysis of trioleylglycerol (TO) (8). Previously, we demonstrated that a basic protein, protamine and polylysine, strongly inhibited the hydrolysis of TO emulsified with phosphatidylcholine (PC) (9, 10). Polylysine has been suggested as a food antiseptic because it binds to some proteins, nucleic acids, viruses, or bacteria through electrostatic or hydrophobic interaction and inhibits their functions. Another form of polylysine, ε-polylysine (EPL), is synthesized by linking the α-carboxyl groups of lysine with its ε-amino groups. As a consequence of this linkage, it is not hydrolyzed by proteases, such as trypsin, but retains its basic character. Therefore, EPL is a candidate agent to inhibit intestinal lipid absorption while resisting proteolysis.

Only a few substances interact directly with lipases themselves, one example being orlistat (tetrahydrolipstatin), a derivative of the naturally occurring lipase inhibitor orlistat produced from Streptomyces toxytricini, which strongly inhibits pancreatic lipase (11–13). The mechanism of lipase inhibition by orlistat is through a covalent bond to the active site serine of the lipase (14, 15). In this study, we compared the inhibition of lipase activity by EPL.

**Abbreviations:** EPL, ε-polylysine; PA, phosphatidic acid; PC, phosphatidylcholine; PL, phosphatidylethanolamine; PS, phosphatidylserine; TES, N,N-tris(hydroxymethyl)-methyl-2-aminooethanesulfonic acid; TO, trioleylglycerol.

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and orlistat. We also examined the effect of EPL on obesity in mice induced by a high-fat diet.

MATERIALS AND METHODS

Reagents

The enzyme substrates and reagents used were obtained as follows: TO, taurocholate, and colipase were from Sigma (St. Louis, MO). PC (from soybean) was from Nippon Shoji (Tokyo, Japan). Phosphatidylethanolamine (PE; from egg), phosphatidylserine (PS; from bovine brain), and phosphatidic acid (PA; from egg PC) were from Serdary Research Laboratories (London, Canada). EPL (average molecular weight, 4,090) was from Chisso Co. (Tokyo, Japan). Orlistat (tetrahydrolipstatin) was extracted from Xenical (Roche, Ltd.) and was recrystallized from n-hexane. The chemical structure was determined using $^1$H and $^{13}$C nuclear magnetic resonance spectra [JNMGSX400 (400 MHz); JEOL, Ltd., Tokyo, Japan] and electron-impact mass spectrometric data (GCMS-QP5050A; Shimadzu Co., Kyoto, Japan).

Pancreatic lipase was purified from rat pancreas by the procedure of Gidez (16) with some modifications. Activity during purification was monitored using the PC-TO emulsion described below (Enzyme activity assays). The purified enzyme (3,200 U/mg protein, pH 6.8) gave a single band on SDS-PAGE, from which its molecular weight was estimated to be 49,000. Carboxylester lipase was purified from porcine pancreas by the procedure of Rudd, Mizuno, and Brockman (17) with some modifications (5). The purified enzyme preparations were found to have specific activities of 700–800 $\mu$mol $p$-nitrophenol released/mg protein/min with $p$-nitrophenyl butyrate as the substrate.

Enzyme activity assays

Pancreatic lipase activity was determined by measuring the rate of release of oleic acid from TO. A suspension of 90 $\mu$mol of TO, 12.6 $\mu$mol of soybean PC, and 9.45 $\mu$mol of taurocholate in 9 ml of 100 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.0, containing 0.1 M NaCl was sonicated for 5 min (TO-PC emulsion). The assay system was composed of the following components in a total volume of 200 $\mu$l: 50 $\mu$l of enzyme (0.998 pmol), 50 $\mu$l of inhibitor solution, 0.25 $\mu$g of colipase, 0.5 $\mu$mol of TO, 0.03 $\mu$mol of taurocholate (except where noted), 0.07 $\mu$mol of phospholipid, 10 $\mu$mol of TES, and 20 $\mu$mol of NaCl. Incubation was carried out at pH 7.0 and 37°C for 30 min. The amount of oleic acid produced was determined by the method of Zapf et al. (18) with slight modification (19). Carboxylester lipase activity was determined using the same assay without colipase.

In some experiments, pancreatic lipase activity against TO was determined using the assay described above but with the 12.6 $\mu$mol of soybean PC replaced by other phospholipids or gum arabic (45 mg).

Sephacryl S-200 gel filtration

Purified pancreatic lipase was incubated with TO-PC emulsion containing EPL or orlistat. The incubation system was composed of the following components in a total volume of 3 ml: 0.5 ml of enzyme (7.66 nmol), 0.5 ml of TO-PC emulsion, 3 $\mu$g of colipase, 2.0 ml of TES buffer (0.1 M, pH 7.0) containing 0.1 M NaCl, and inhibitor (733 nmol of EPL or 6.05 $\mu$mol of orlistat). After 10 min of incubation at 37°C, 3 ml of diethyl ether was placed onto the incubation mixture, which was then shaken for 10 s and centrifuged at 1,200 $g$ for 5 min at 4°C. The upper ether phase was aspirated, and the ether extraction was repeated two more times. A 2 $\mu$l aliquot of the supernatant was applied to a Sephacryl S-200 HR column (2.6 × 60 cm; GE Healthcare Bio-Sciences KK, Tokyo, Japan) equilibrated with 0.05 M phosphate buffer (pH 7.0) containing 0.1 M NaCl. Fractions of 5 ml were collected, and aliquots of the fractions were taken to measure lipase activity.

Antiobesity activity of EPL

The antiobesity potential of EPL was tested in a rodent model of dietary obesity. Male C57BL/6 mice (8 weeks old) were obtained from CLEA Japan (Osaka, Japan) and housed in a temperature- and humidity-controlled room set to a 12 h/12 h light/dark cycle. After the animals were given a standard laboratory diet (Oriental Yeast Co., Ltd., Osaka, Japan) and water ad libitum for 1 week, they were divided into five groups matched for body weight (each n = 10). One group, the control diet group, was fed a low-fat diet [6% milk butter, 20% casein, 56% corn

Fig. 1. Effect of increasing concentrations of $\varepsilon$-polysine (EPL; open circles) or orlistat (closed circles) on the rate of reaction of rat pancreatic lipase on trioleoylglycerol (TO) emulsified with phosphatidylcholine (PC; A) or gum arabic (B). The results are expressed as means ± SEM of four experiments.
TABLE 1. Effect of emulsifiers on 50% inhibition concentration of EPL and orlistat

<table>
<thead>
<tr>
<th>Emulsifier</th>
<th>EPL</th>
<th>Orlistat</th>
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<tbody>
<tr>
<td>Pancreatic lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>0.122 ± 0.003</td>
<td>0.991 ± 0.156</td>
</tr>
<tr>
<td>PE</td>
<td>0.063 ± 0.004</td>
<td>0.800 ± 0.105</td>
</tr>
<tr>
<td>PA</td>
<td>28.31 ± 2.38</td>
<td>1.193 ± 0.239</td>
</tr>
<tr>
<td>PS</td>
<td>8.72 ± 0.073</td>
<td>0.712 ± 0.086</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>17.95 ± 4.86</td>
<td>2.08 ± 0.266</td>
</tr>
<tr>
<td>Carboxylester lipase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>0.160 ± 0.033</td>
<td>37.42 ± 7.20</td>
</tr>
<tr>
<td>Gum arabic</td>
<td>&gt;60</td>
<td>54.74 ± 7.25</td>
</tr>
</tbody>
</table>

EPL, ε-polylysine; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine. Trioleoylglycerol was emulsified with phospholipids or gum arabic, as described in Materials and Methods, and trioleoylglycerol-hydrolyzing activity was measured at various concentrations of orlistat and EPL. The results are expressed as means ± SEM of four experiments.

RESULTS

The hydrolytic activity of rat pancreatic lipase toward TO emulsified with soybean PC and taurocholate was determined in the presence of increasing concentrations of EPL and orlistat (Fig. 1A). EPL strongly inhibited TO hydrolysis; the molecular concentration of 50% inhibition by EPL (0.122 μM) was approximately eight times lower than that by orlistat (0.991 μM). However, the weight

![Fig. 2. Inhibition of pancreatic lipase at various concentrations by increasing concentrations of EPL. TO emulsified with PC was used as the substrate. A: The lipase concentrations were 0.055 (open squares), 0.111 (closed circles), and 0.222 (open circles) μg/ml. B: The experimental values in A were replotted as percentages of the remaining lipase activity.](image-url)
concentration of 50% inhibition by EPL (0.50 μg/ml) was almost the same as that by orlistat (0.49 μg/ml), because the molecular weight of EPL was approximately eight times higher than that of orlistat. When TO was emulsified with gum arabic instead of soybean PC, the 50% inhibition concentration by EPL was increased ~150 times compared with TO-PC emulsion (Fig. 1B). However, the 50% inhibition concentration by orlistat was increased only two times. When TO was emulsified with either PS or PA, the 50% inhibition concentration by orlistat was not much changed from that of TO-PC emulsion, but the 50% inhibition concentration by EPL was increased; 70 times for PS and 230 times for PA (Table 1). EPL also strongly inhibited the hydrolytic activity of pancreatic carboxylester lipase toward TO emulsified with soybean PC and taurocholate. The molecular concentration of 50% inhibition by EPL was ~230 times lower than that of orlistat. When TO was emulsified with gum arabic instead of soybean PC, the 50% inhibition concentration by EPL for carboxylester lipase was increased >370 times compared with PC emulsion. However, the 50% inhibition concentration by orlistat was increased only 1.4 times (Table 1).

The inhibitory action of EPL was determined using three different concentrations of pancreatic lipase (Fig. 2A). When normalized to the activity observed in the absence of inhibitor, the extent of lipase inactivation by each EPL

![Fig. 5. Sephacryl S-200 gel filtration of rat pancreatic lipase. Rat pancreatic lipase was incubated with TO-PC emulsion (A) containing EPL (B) or orlistat (C) and applied to a Sephacryl S-200 column. Aliquots of the fractions were taken to measure pancreatic lipase activity.](image)

![Fig. 4. A: Effect of EPL on body weight in mice fed a high-fat diet for 60 days. Each point represents the mean ± SEM of 10 mice. * P < 0.05, and ** P < 0.01 versus values in the high-fat diet group. B: Effect of EPL on energy intake. Each symbol represents the following: low-fat diet (closed circles), high-fat diet (open circles), high-fat diet plus 0.1% EPL (open squares), high-fat diet plus 0.2% EPL (closed squares), and high-fat diet plus 0.4% EPL (open triangles).](image)
concentration tested was independent of the enzyme concentration (Fig. 2B). Pancreatic lipase was incubated with TO-PC emulsion containing EPL or orlistat and applied to a Sephacryl S-200 column (Fig. 3). Lipase activity was eluted in a single peak, and the molecular weight was estimated to be 45,000. The total lipase activity of the peak eluted in a single peak, and the molecular weight was estimated to be 45,000. The total lipase activity of the peak fraction was not changed by incubation with EPL: ~97% compared with pancreatic lipase alone. However, the total lipase activity was reduced by incubation with orlistat: ~17% compared with pancreatic lipase alone.

Mice fed the high-fat diet containing 45% milk butter for 60 days had a significantly higher body weight than mice fed the low-fat diet (Fig. 4A). In mice fed the high-fat diet plus 0.4% EPL, the body weight increase was reduced significantly. The high-fat diet containing 0.1% or 0.2% EPL also suppressed an increase in body weight induced by the high-fat diet alone. The energy intake per mouse during the whole experimental period differed between the high-fat diet group and the high-fat diet group, but it did not differ much between the high-fat diet group and the high-fat diet plus EPL (0.1, 0.2, or 0.4%) group (Fig. 4B). The adipose tissue (epididymal and retroperitoneal) weight of mice fed the high-fat diet was significantly higher than in mice fed the low-fat diet (Table 2). EPL significantly suppressed the increase in adipose tissue weight induced by the high-fat diet alone. The high-fat diet containing 0.4% EPL also significantly suppressed the liver weight increase induced by the high-fat diet.

As shown in Tables 3 and 4, the high-fat diet induced hyperlipidemia, with increases of plasma triacylglycerol, cholesterol, phospholipids, and free fatty acids, and induced fatty liver, with accumulation of triacylglycerol and total cholesterol in the liver. Plasma triacylglycerol was concentration-dependently reduced by the high-fat diet containing EPL compared with the high-fat diet alone. Plasma total cholesterol was also reduced by the diet containing EPL, but plasma HDL cholesterol was not reduced by the diet containing EPL. Therefore, plasma non-HDL cholesterol was reduced in mice by feeding the diet containing EPL compared with the high-fat diet alone (Table 3). Plasma free fatty acid and glucose were not changed significantly by feeding the diet containing EPL (data not shown). Feeding the high-fat diet containing 0.4% EPL significantly reduced triacylglycerol and phospholipid accumulation in liver compared with the high-fat diet alone (Table 4). The accumulation of cholesterol and free fatty acid in the liver was not significantly changed by feeding the diet containing EPL.

As shown in Fig. 5, the feces of mice were EPL concentration-dependently increased in weight by the high-fat diet containing EPL compared with the high-fat diet alone. Fecal lipid was also increased by the diet containing EPL compared with the high-fat diet alone.

### DISCUSSION

EPL is a strong inhibitor of gastrointestinal lipase reactions, and sometimes its inhibition is stronger than that of orlistat (Fig. 1). The inhibition mechanism of EPL differs from that of orlistat. The inhibition by EPL was specific to substrate emulsifier, but the inhibition by orlistat was not specific to substrate emulsifier (Table 1). Orlistat has been reported as a selective and potent inhibitor of lipase (13, 14). The inhibition of orlistat is irreversible. The functional group of orlistat is the reactive β-lactone ring, leading to an ester with the serine hydroxyl group of the catalytic triad of pancreatic lipase, and a stoichiometric enzyme inhibitor complex of an acyl-enzyme type (a long-lived covalent intermediate) is formed (21, 22). Therefore, we can name orlistat an “enzyme inhibitor” or “lipase inhibitor,” because it interacts directly with enzyme (lipase) and inhibits lipase action. Alternatively, the inhibition of EPL has the characteristics of a reversible

### TABLE 3. Effect of EPL on plasma lipids in mice fed a high-fat diet for 60 days

<table>
<thead>
<tr>
<th>Diet</th>
<th>Triacylglycerol (mg/dl)</th>
<th>Phospholipid (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Non-HDL Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat diet</td>
<td>69.95 ± 4.17a</td>
<td>318.1 ± 8.73a</td>
<td>117.0 ± 2.81a</td>
<td>43.04 ± 1.66a</td>
<td>74.00 ± 2.98a</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>129.2 ± 8.71a</td>
<td>428.0 ± 8.46a</td>
<td>187.8 ± 8.78a</td>
<td>67.08 ± 2.72a</td>
<td>120.7 ± 8.80a</td>
</tr>
<tr>
<td>High-fat diet + 0.1% EPL</td>
<td>118.8 ± 4.83b</td>
<td>422.4 ± 14.48b</td>
<td>167.7 ± 4.12b</td>
<td>72.82 ± 3.14b</td>
<td>94.83 ± 3.07b</td>
</tr>
<tr>
<td>High-fat diet + 0.2% EPL</td>
<td>106.3 ± 4.56c</td>
<td>383.8 ± 14.39c</td>
<td>170.7 ± 8.47c</td>
<td>64.66 ± 5.43b</td>
<td>102.1 ± 4.06b</td>
</tr>
<tr>
<td>High-fat diet + 0.4% EPL</td>
<td>93.10 ± 3.85d</td>
<td>362.9 ± 9.09d</td>
<td>165.0 ± 5.56d</td>
<td>64.28 ± 2.63d</td>
<td>98.68 ± 4.01d</td>
</tr>
</tbody>
</table>

Results are means ± SEM. Values not sharing a letter are significantly different (P < 0.05).

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reaction. The extent of inhibition of the lipase reaction by EPL was independent of lipase concentration (Fig. 2), and EPL did not bind strongly to lipase (Fig. 3) but may have interacted with the TO-PC emulsion (10). Inhibition by EPL was dependent on how the substrate was presented to the lipases: it occurred strongly with TO-PC and TO-PE emulsions but not with TO-PS, TO-PA, or TO-gum arabic emulsions (Table 1). The distinction between the emulsifiers is that PC and PE are zwitterionic, whereas the others are anionic. Common to the emulsion systems in these assays was taurocholate, an anionic bile salt. EPL might form a surface-active complex with taurocholate, and it binds to TO-PC emulsion particles, thereby retarding lipase adsorption to the emulsion particles and retarding lipase reaction.

The lipase reaction occurs at the substrate surface (lipid-water interface) and is dependent upon surface adsorption of the enzyme. It is well known that amphiphilic substances acting as emulsifiers influence the lipase reaction rate. Hydrophobic proteins, such as BSA and β-lactoglobulin, which are also amphiphilic, have been shown to inhibit lipase activity toward its substrate by competing for the surface (7). However, the EPL concentration required for complete inhibition of the pancreatic lipase reaction was 1,000 times lower than that of the hydrophobic proteins required for the same effect (10). Other basic proteins, such as protamine and histone, also strongly inhibit the hydrolysis of TO-PC emulsion by pancreatic lipase (9). The effective concentration of these basic proteins is similar to that of EPL. The inhibitory activity of EPL was not changed by incubation with peptide digestive enzymes, such as trypsin, whereas the inhibitory activity of protamine or histone was decreased by incubation with trypsin (data not shown). Trypsin cannot attack the isopeptide linkage of EPL (α-carboxyl-ε-amino linkage), whereas other basic proteins are degraded by trypsin and their inhibitory activities are decreased (23). Therefore, EPL might be an effective inhibitor for the lipase reaction in the digestive tract. Indeed, EPL prevented the high-fat diet-induced increases in the body weight of mice (Fig. 4). However, we have not yet shown the effectiveness of EPL in humans. The emulsification environment in human gut lumen would be much less constant and predictable, because a human diet would be variable. Further experiments are needed to confirm the effectiveness of EPL on human obesity.

Obesity is a major worldwide public health problem. It predisposes one to lifestyle-related diseases, including hy-

**TABLE 4.** Effect of EPL on liver lipids in mice fed a high-fat diet for 60 days

<table>
<thead>
<tr>
<th>Diet</th>
<th>Triacylglycerol</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/liver</td>
<td>µEq/liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat diet</td>
<td>20.20 ± 1.53</td>
<td>4.32 ± 0.22</td>
<td>27.74 ± 0.97</td>
<td>5.72 ± 0.26</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>66.10 ± 8.86</td>
<td>5.38 ± 0.34</td>
<td>34.45 ± 1.13</td>
<td>11.49 ± 0.93</td>
</tr>
<tr>
<td>High-fat diet + 0.1% EPL</td>
<td>77.09 ± 8.23</td>
<td>4.87 ± 0.19</td>
<td>31.72 ± 0.94</td>
<td>14.52 ± 1.30</td>
</tr>
<tr>
<td>High-fat diet + 0.2% EPL</td>
<td>56.62 ± 7.70</td>
<td>5.06 ± 0.18</td>
<td>30.95 ± 1.09</td>
<td>13.25 ± 1.29</td>
</tr>
<tr>
<td>High-fat diet + 0.4% EPL</td>
<td>36.41 ± 2.91</td>
<td>5.48 ± 0.22</td>
<td>28.46 ± 0.73</td>
<td>10.54 ± 0.50</td>
</tr>
</tbody>
</table>

Results are means ± SEM. Values not sharing a letter are significantly different ($P < 0.05$).

**Fig. 5.** Effect of EPL on fecal weight (A) and fecal lipid weight (B) in mice fed a high-fat diet. Fecal samples were obtained from each group at 24 h and on the 18th and 36th days. Results are means ± SEM. Values not sharing a letter are significantly different ($P < 0.05$). HF, high-fat diet; LF, low-fat diet.
perlipemia, atherosclerosis, diabetes, and hypertension. Therefore, weight reduction is important in the treatment of overweight patients. Dietary fat plays the central role in obesity: to bring about and maintain weight loss, the amount of fat available to be metabolized must be decreased. Excessive consumption of dietary fat probably plays a major contributory role in the development of obesity (24–26). Orlistat, which belongs to a class of antiobesity agents (27, 28), has since been developed for the long-term management of obesity. Its mechanism of action is well understood: it strongly inhibits gastrointestinal lipase reactions on TO-PC emulsion. Because the major bile phospholipid is PC, accounting for >90% of the total, EPL might be an effective inhibitor in vivo: oral administration of EPL to rats reduced the peak plasma triacylglycerol concentration (10, 23). Therefore, EPL might suppress dietary fat absorption by inhibiting fat digestion. In this study, we tested the effect of EPL on obesity using mice fed a high-fat diet containing 0.1, 0.2, or 0.4% EPL. EPL prevented the high-fat diet-induced increases in body weight and the weight of liver and visceral adipose tissues (Fig. 4, Table 2). EPL also decreased plasma triacylglycerol and plasma cholesterol concentrations and liver triacylglycerol content after they had been increased by a high-fat diet (Tables 3, 4). These results suggest that EPL has an antiobesity function: it prevents the hydrolysis of dietary fat in the small intestine and reduces the subsequent intestinal absorption of dietary fat.

In conclusion, this investigation demonstrated that EPL is an antiobesity agent. EPL resembles orlistat in that it is a strong inhibitor of gastrointestinal lipase reactions. However, the inhibition mechanism of EPL differs from that of orlistat: EPL is a reversible reaction inhibitor, whereas orlistat is an irreversible enzyme inhibitor. EPL also behaves as an in vivo inhibitor while resisting proteolysis and could be considered a potential new tool for reducing intestinal lipolysis.

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