

# Inhibitory Effects of Grape Seed Extract on Lipases

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**OBJECTIVE:** The aim of the present study was to assess the effects of grape seed extract (GSE) on the fat-metabolizing enzymes pancreatic lipase, lipoprotein lipase, and hormone-sensitive lipase in vitro and evaluate its potential application as a treatment for obesity.

**METHODS:** Crushed grape seeds were extracted in ethanol, and the extract was assayed for the measurement of inhibitory effects on pancreatic lipase and lipoprotein lipase activities and on lipolysis of 3T3-L1 adipocytes.

**RESULTS:** The GSE rich in bioactive phytochemicals showed inhibitory activity on the fat-metabolizing enzymes pancreatic lipase and lipoprotein lipase, thus suggesting that GSE might be useful as a treatment to limit dietary fat absorption and the accumulation of fat in adipose tissue. The observed reduction in intracellular lipolytic activity of cultured 3T3-L1 adipocytes may reduce the levels of circulating free fatty acids that have been linked to insulin resistance in obese patients.

**CONCLUSION:** The GSE rich in compounds that inhibit lipases may provide a safe, natural, and cost-effective weight control treatment. *Nutrition* 2003;19:876–879. ©Elsevier Inc. 2003

**KEY WORDS:** *Vitis vinifera*, natural products, obesity, plant extracts

## INTRODUCTION

Obesity is one of the main public health problems in developed countries. It is considered to be a risk factor associated with the genesis or development of major chronic diseases, including cardiovascular disease, diabetes, and cancer.<sup>1,2</sup> In the United States, obesity is increasing at an alarming rate. Overweight and obesity are the most common nutrition disorders in the United States, affecting the majority of adults in the country.<sup>3</sup>

Characterization of obesity-associated gene products has shown new biochemical pathways and molecular targets for pharmacologic intervention that will likely lead to new treatments.<sup>4,5</sup> Ideally, these treatments will be viewed as adjuncts to behavioral and lifestyle changes aimed at maintenance of weight loss and improved health.<sup>6,7</sup> “Non-traditional” or “alternative” treatments using nutrition supplements are extremely popular, especially with respect to obesity and body composition. Although such treatments are widely used, none has been convincingly demonstrated to be safe and effective.<sup>8</sup> Under the guidelines of the US Food and Drug Administration, botanical drugs can be developed faster and cheaper than conventional single-entity pharmaceuticals. Many botanicals may provide safe, natural, and cost-effective alternatives to synthetic drugs.<sup>9,10</sup>

It has been reported that increased intake of foods with high-energy and dietary fat content promotes body fat storage, and that diets that consistently contain high levels of fat lead to increased calorie intake and therefore body weight and adiposity in humans and animals.<sup>11,12</sup> Western diets are high in fat and tend to promote obesity, and the pharmacologic inhibition of the digestion and absorption of dietary fat has been used as a strategy to treat obesity.<sup>3,13</sup>

Pancreatic lipase (PL) is the most important enzyme for the digestion of dietary triacylglycerols.<sup>14</sup> Orlistat, a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, is a potent inhibitor of gastric, pancreatic, and carboxyl ester lipase and has proved to be effective for the treatment of human obesity.<sup>13</sup> The existence of lipase inhibitors in various plant species has been investigated and reported in different species including *Cassia mimosoides*,<sup>15,16</sup> *Camelia sinensis*,<sup>17</sup> and *Salacia reticulata*.<sup>18</sup>

Lipoprotein lipase (LPL) is an enzyme responsible for the hydrolysis of triacylglycerols from plasma lipoproteins, mainly chylomicrons and very low-density lipoproteins, and its activity is known to be influenced by nutritional and hormonal status and by environmental conditions. Further, LPL is a factor that contributes to the development of obesity.<sup>19,20</sup> In adipose tissue, hormone-sensitive lipase (HSL) functions as the rate-limiting enzyme catalyzing the lipolysis of triacylglycerol and diacylglycerol after the phosphorylation of the enzyme by protein kinase A.<sup>21</sup>

A variety of health-promoting proanthocyanidins, also commonly called condensed tannins, are present in grape solid parts (seeds and skins).<sup>22–24</sup> A great deal of research effort is being devoted to testing the putative beneficial effects of grape polyphenols<sup>23,24</sup> extracted from grape seeds and widely used as nutrition supplements. The results from several studies have indicated a lack of toxicity and support the use of proanthocyanidin-rich extract from grape seeds for various foods.<sup>25,26</sup>

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Ingestion of natural antioxidants, such as grape seed extract (GSE) rich in proanthocyanidins, has been demonstrated to improve insulin sensitivity and/or ameliorate free radical formation and reduce the signs and symptoms of chronic age-related disorders including syndrome X.<sup>27,28</sup>

The present study tested the hypothesis that GSE can inhibit the enzymatic activity of PL and LPL in vitro and lipolytic activity in cultured adipocytes. The results of this study show promise for the potential use of GSE as a lipase inhibitor in the treatment of obesity.

## MATERIALS AND METHODS

### Sourcing of the Grape Seed Extract

Crushed grape seeds (50 g) obtained from Bramble Berry (Bellingham WA, USA) were extracted in 500 mL of 95% ethanol with mechanical agitation for 24 h. The organic solvent was then evaporated, and the crude extract was partitioned between H<sub>2</sub>O and hexane. The aqueous solution was freeze dried to yield approximately 1.3 g of extract (GSE).

In vitro assays for the measurement of inhibitory effects of GSE on fat-metabolizing enzymes were tested as follows.

**PANCREATIC LIPASE (EC 3.1.1.3) ACTIVITY.** Lipase-PS reagents were obtained from Sigma Diagnostics (procedure 805; Sigma-Aldrich, St. Louis, MO, USA). Human PL (Lipase-PS standard, 230 U/L) was obtained from Sigma Diagnostics (Sigma-Aldrich). Aliquots (30  $\mu$ L) of lipase standard, blank (water as reference), and GSE samples were added to 500  $\mu$ L of reconstituted substrate solution and mixed gently and incubated for 5 min at 37°C. Activator reagent (300  $\mu$ L) was added and mixed by gentle inversion, and the samples were incubated again for 3 min at 37°C. The recorded rate of increase in absorbance at 550 nm due to the formation of quinone di-imine dye was used to determine the PL activity in the samples prepared, as detailed elsewhere.<sup>29</sup>

**LIPOPROTEIN LIPASE (EC 3.1.1.34) ACTIVITY.** LPL was measured according to the method of Nilsson-Ehle and Schotz.<sup>30</sup> A pool of LPL was made by incubating human adipose tissue fragments with 10 U/mL of heparin (500 mg/5 mL) for 45 min at 24°C. Aliquots of this heparin eluate were preincubated with different concentrations of GSE for 30 min at 4°C. After addition of <sup>3</sup>H-triolein substrate<sup>30</sup> containing albumin and human serum as a source of apolipoprotein CII, samples were incubated for 60 min at 37°C. Released <sup>3</sup>H-oleic acid was extracted and measured.

**HORMONE-SENSITIVE LIPASE (EC 3.1.1.-) ACTIVITY.** Lipolytic activity in cultured mouse 3T3-L1 adipocytes was used as a measure of HSL activity. 3T3-L1 cells were cultured as described previously<sup>31</sup> in Dulbecco's Minimum Essential Medium (4500 mg of glucose/L) supplemented with 10% fetal bovine serum, 2 mM of glutamine, 100 U/mL of penicillin, 100  $\mu$ g/mL of streptomycin, 110  $\mu$ g/mL of sodium pyruvate, and 8  $\mu$ g/mL of biotin in a 5% CO<sub>2</sub> atmosphere at 37°C. Cells were maintained in dishes from Corning/Costar (Corning, NY, USA). The differentiation of 3T3-L1 cells into adipocytes was initiated by the addition of 10  $\mu$ M of dexamethasone, 0.5 mM of isobutyl-methylxanthine, and 10  $\mu$ g/mL of insulin to the culture medium of confluent cells for 3 d, followed by the cultivation of cells without supplements for an additional 3 d or more. We added GSE as indicated in Figure 1, and after 18 h of incubation the stimulation of lipolysis was accomplished by incubating differentiated 3T3-L1 adipocytes in culture medium supplemented with 10  $\mu$ M of isoproterenol and 2% fatty acid-free bovine serum albumin; when samples were

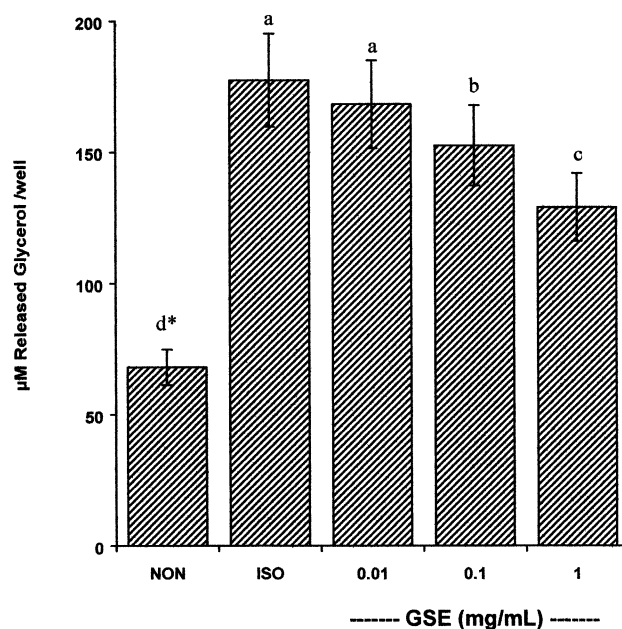


FIG. 1. Inhibitory effect of GSE on hormone-sensitive lipase activity in cultured murine 3T3-L1 adipocytes. Each column represents mean  $\pm$  standard deviation,  $n = 4$ . \*Means followed by a are not significantly different, whereas b is significantly different from a at  $P < 0.05$ , and c is significantly different from a and b at  $P < 0.05$  according to Duncan's multiple range test. GSE, grape seed extract; ISO, stimulated lipolysis (without GSE); NON, non-stimulated or basal lipolysis.

assayed for glycerol content to measure the extent of lipolysis, Dulbecco's Minimum Essential Medium supplemented with 5% fatty acid-free bovine serum albumin was used as a diluent for the assay. Lipolysis was determined by measuring glycerol levels released from the GSE-treated murine 3T3-L1 adipocytes with a fluorometric enzymatic assay.<sup>32</sup>

### Statistical Analysis

All data were subjected to analysis of variance. The data (means  $\pm$  standard deviation) shown are mean values, and the significance of differences was compared by using Duncan's multiple range test at the  $P < 0.05$  probability level. The letters (a, b, c) used in Figure 1 and the tables indicate the significance of the differences according to Duncan's multiple range test.

## RESULTS

Seeds of various plants are rich in fat. Further, these seeds contain lipase inhibitors among other bioactive phytochemicals.<sup>33</sup> We tested GSE for inhibitory action against PL in vitro and confirmed that the extracts contain lipase inhibitors that act in a dose-dependent manner (Table I). The application of the extract at a concentration of 1 mg/mL resulted in the inhibition of 80% of enzyme activity during 5 min of incubation.

GSE mildly reduced LPL activity in vitro (Table II), with 30% inhibition of enzyme activity compared with the control at a concentration of 1 mg/mL of GSE.

Eighteen hours of incubation of 3T3-L1 adipocytes with medium supplemented with the GSE increased the isoproterenol-stimulated glycerol release during a subsequent 1-h incubation (Fig. 1). There were 28% and 14% reductions of glycerol release at 1 and 0.1 mg/mL of GSE, respectively. Further, the inhibition of

TABLE I.

INHIBITORY EFFECTS OF GSE ON HUMAN PANCREATIC LIPASE ACTIVITY MEASURED WITH THE LIPASE-PS KIT FROM SIGMA DIAGNOSTICS		
GSE (mg/mL)	Lipase activity* (U/L)	Inhibitory effect† (%)
0	230 ± 2.8 <sup>a</sup>	
0.01	222 ± 1.2 <sup>a</sup>	3
0.1	179 ± 2.2 <sup>b</sup>	22
1	47 ± 5 <sup>c</sup>	80

\* Results represent the mean ± standard deviation,  $n = 4$ . Means followed by a are not significantly different, whereas b is significantly different from a at  $P < 0.05$ , and c is significantly different from a and b at  $P < 0.05$  according to Duncan's multiple range test.

† The inhibitory effect was defined as the lowering of relative activity (%) compared with the activity of the control (0 mg/mL of GSE = 100% activity).

GSE, grape seed extract

stimulated lipolysis by GSE demonstrated that the active components of GSE were taken up by the cells.

## DISCUSSION

Our findings indicated that GSE inhibits a number of lipases, including PL and LPL. Further, GSE decreased isoproterenol-stimulated lipolysis in 3T3-L1 adipocytes, presumably by decreasing HSL activity. Reducing the absorption of fat may be an effective adjunct to dieting in obese patients, as seen in the clinical use of Orlistat. Orlistat is a prescription medication available as a PL inhibitor that is not absorbed and, hence, works only within the intestine. Orlistat reduces the absorption of dietary fat by about 30% in adults.<sup>13</sup> Long-term clinical trials have shown that Orlistat results in about 5% greater weight loss and better maintenance of the lost weight over a 1-y period and also improves the lipid profile.<sup>13</sup> However, LPL hydrolyzes the triacylglycerols of very low-density lipoproteins and chylomicrons, thus releasing free fatty acids for uptake into adipocytes<sup>19,20</sup>; thus, the inhibition of LPL may slow the deposition of fat into adipose tissue. Because GSE inhibited PL and LPL, it might affect fat absorption and the uptake of fatty acids in the periphery, if enough of the active components can be absorbed and enter the circulation. However, inhibition of LPL in muscle may tend to slow clearance of circu-

lating triacylglycerol. Thus, in vivo studies are needed to further address the potential effects of GSE extract.

HSL is controlled by several hormonal pathways and hydrolyzes the fat stored in adipocytes, resulting in the release of free fatty acids into the blood.<sup>21</sup> This lipolysis may play a central role in the development of insulin resistance and the metabolic syndrome (syndrome X). Thus, the inhibition of HSL has the potential to reduce levels of circulating free fatty acids linked to insulin resistance in obese patients. This is a highly desirable clinical outcome that may provide treatment and prevention.<sup>3,34</sup> Our studies showed that the active components within GSE can cross the plasma membrane of cultured 3T3-L1 adipocytes and decrease the activity of HSL, so GSE may be useful therapeutically, if enough of the active components can be ingested. However, to date, it is not clear that a pharmacologically induced reduction of the release of free fatty acids from adipocytes would be beneficial in the reduction of obesity or would prove detrimental by increasing net fat deposition.

The effect of the GSE on lipases may be caused by a synergistic action of several compounds within the extract, rather than by a single compound.<sup>22</sup> These flavonoid procyanidins and their antioxidative metabolites<sup>25,26</sup> have the potential to be obesity-preventive agents. More studies and in vivo experiments are required to ascertain whether GSE, rich in lipase-inhibitory compounds, may provide a natural weight-control treatment, improve plasma lipid profiles, and thus decrease risk for heart disease, and to further define the physiologic extent of these effects.

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TABLE II.

INHIBITION OF LPL ACTIVITY BY GSE		
GSE (mg/mL)	LPL (U/mL)* ( $\mu$ M glycerol released h/mL)	Inhibition† (%)
0	41.7 ± 0.1 <sup>a</sup>	
0.1	41.0 ± 0.8 <sup>a</sup>	2
1	29.3 ± 0.5 <sup>b</sup>	30

\* Results represent the mean ± standard deviation,  $n = 4$ . Means followed by a are not significantly different; b is significantly different from a at  $P < 0.05$  according to Duncan's multiple range test.

† The inhibitory effect was defined as the lowering of relative activity (%) against the activity of control (0 mg/mL of GSE = 100% activity).

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