

Inhibition of Pancreatic Lipase by Grape Seed and Tea Extracts and Their Primary Catechins *in vitro*

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Abstract

Inhibiting pancreatic lipase activity is an effective means of reducing fat absorption, which may have a role in combating obesity by limiting energy intake. The use of pharmaceutical lipase inhibitors has been limited due to the prevalence of undesirable side effects. Polyphenols represent an alternative strategy to inhibit digestive enzymes using safe, gentle, and cost-effective extracts derived from plant sources. Thus, this study evaluated alternative options by testing various polyphenol-rich plant extracts (grape seed, green tea, TeaVigo®, and white tea) for their ability to inhibit pancreatic lipase activity. Using an *in vitro* fluorescence assay, the relative potency of extracts tested was determined in comparison to the pharmaceutical orlistat. In addition, the most abundant catechins found in these extracts, epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), were also tested for the ability to inhibit pancreatic lipase. Results showed the most effective plant-based lipase inhibitors were the concentrated green tea product, TeaVigo® and green tea. Results with the individual catechin trials showed EGCG and EGC were the most potent lipase inhibitors in the plant extracts.

Introduction

Obesity is characterized by excessive body fat resulting from an imbalance between energy intake and expenditure, and is a strong risk factor for diabetes, hypertension, hyperlipidemia, and arteriosclerosis. One suggested method for decreasing energy intake is to prevent lipid absorption in the small intestine, which may be achieved by inhibiting pancreatic lipase. The pharmaceutical orlistat is a commonly prescribed pancreatic lipase inhibitor shown to be effective in treating obesity and hyperlipidemia. However, many gastrointestinal side effects have been associated with orlistat (marketed as Alli or Xenical) making it an undesirable option for some people.

Grapes and tea leaves are rich sources of polyphenols that are reported as having many beneficial biological activities, including anti-obesity, antioxidant, anti-cancer, anti-mutagenic, and hypocholesterolemic activity. Extracts from both tea and grape seed have been reported as having inhibitory effects on carbohydrate digestion, but their relative efficacy of limiting fat digestion by inhibiting lipase activity has not been well studied. The use of plant extracts like tea and grape seed potentially represent a cheaper, safer route of limiting fat absorption while avoiding the undesirable side effects of orlistat. Since these extracts are an abundant source of catechins, especially EGCG, semi-purified green tea extracts may demonstrate greater lipase inhibitory potential. The inhibition of pancreatic lipase by grape seed, green and white tea extracts, TeaVigo® (a concentrated green tea product), and their primary catechins were examined *in vitro*, and compared to orlistat.

Materials and Methods

Chemicals. Plant extracts: grape seed, green tea, white tea, and TeaVigo® were supplied by USANA Health Sciences, Inc. (Salt Lake City, UT, USA) with chemical analysis performed by HPLC. All other chemicals were obtained from Sigma (St. Louis, MO, USA).

Lipase Assay. The pancreatic lipase assay was adapted from Nakai *et al.* with the adjustments of 22.5 μ M (micromolar) 4-methylumbelliferyl oleate (4-MU oleate) in buffer and 1.25 μ g/mL lipase in water. Lipase activity was monitored by the digestion of 4-MU oleate, resulting in an increase in fluorescence over time. Fluorescence was analyzed via spectrophotometry (ex/em:320/460 nm). Positive and negative controls were included with each trial. Results are presented as the mean \pm standard error from at least three independent trials.

Composition of Extracts Tested

	Grape Seed	Green Tea	White Tea	Tea Vigo
Epigallocatechin Gallate (EGCG)	0.0%	53.5%	33.6%	97.7%
Epicatechin Gallate (ECG)	1.2%	13.1%	14.5%	2.2%
Epigallocatechin (EGC)	0.0%	18.3%	30.4%	0.0%
Epicatechin	4.7%	6.9%	8.8%	0.0%
Other Catechins	6.7%	4.8%	5.3%	0.1%
Procyanidins	87.4%	0.0%	0.0%	0.0%

Analysis of catechins represented as percent of total catechins found. Other non-catechin components are present in extracts including ash (minerals) and non-soluble material.

Lipase Inhibition Measured by 4-MU Fluorescence

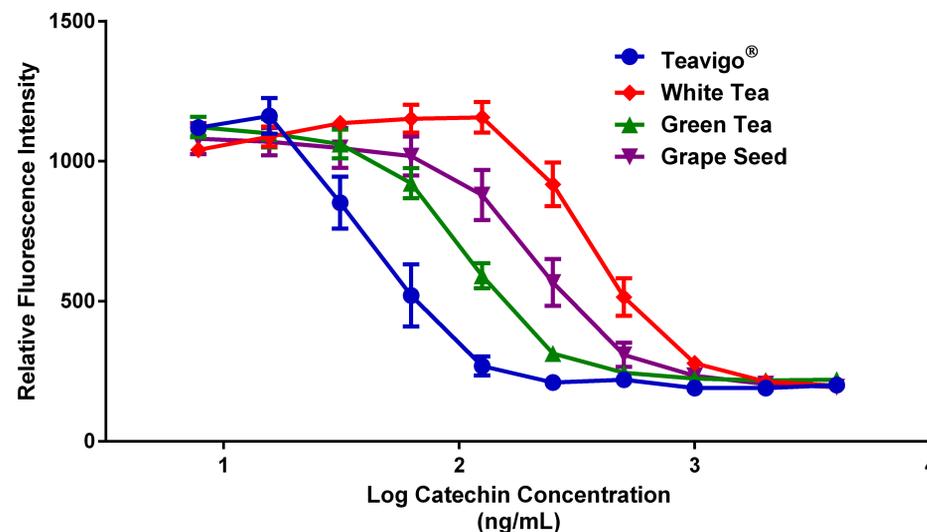


Figure 1. Inhibition of pancreatic lipase activity by tea and grape seed extracts. Pancreatic lipase activity was indicated by change in relative fluorescence generated by 4-MU oleate cleavage. Lipase inhibition was observed in the presence of each extract, with Tea Vigo®, and green tea extracts being the most potent inhibitors. Grape seed and white tea extracts were less effective, and only provided meaningful inhibition at high concentrations.

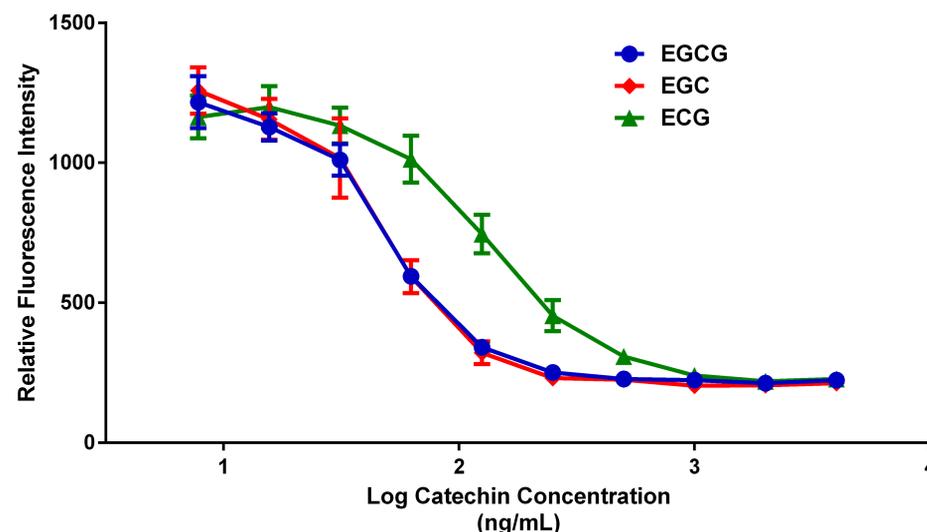


Figure 2. Inhibition of pancreatic lipase activity by epicatechin derivatives. Results from Figure 1 and extract composition suggested that EGCG, EGC, or ECG were likely candidates for pancreatic lipase inhibition. Pancreatic lipase activity was indicated by change in relative fluorescence, as previously described. Lipase inhibition was most effective with EGC and EGCG, though inhibition was also achieved with high doses of ECG.

Pancreatic Lipase Inhibition IC₅₀

Extract	# of Trials	IC ₅₀ (ng/mL)	Catechin	# of Trials	IC ₅₀ (ng/mL)
Grape Seed	4	216 \pm 22 ^{a*}	ECG	3	134 \pm 8 ^d
Green Tea	5	109 \pm 6 ^b	EGC	3	52 \pm 4 ^e
TeaVigo®	4	50 \pm 7 ^b	EGCG	3	55 \pm 2 ^e
White Tea	4	398 \pm 33 ^c	Orlistat (Control)	3	0.42 \pm 0.02 ^f

*Extracts with differently numbered superscripts represent significant differences ($p < 0.05$) in IC₅₀ value as measured by ANOVA. Extracts and individual compounds (ECG, EGC, EGCG, Orlistat) were compared in separate analyses.

Results

As briefly described in Materials and Methods, pancreatic lipase inhibition was determined by a fluorescence-based assay with 4-MU oleate as substrate. 4-MU fluoresces when the oleate side chain is cleaved by lipase, therefore a decreased fluorescence over time is associated with lipase inhibition when compared to 4-MU oleate and lipase only as a negative control. Positive controls were simultaneously conducted with orlistat to ensure enzyme properties remained unchanged between trials.

Results showed that all extracts tested were able to inhibit lipase activity (Figure 1). The concentrated green tea product TeaVigo® resulted in the strongest lipase inhibition, followed by green tea. Grape seed and white tea were moderately less effective. As a measure of the relative potency of the extracts tested, the IC₅₀ values were calculated from the enzyme activity data. Statistical analysis by ANOVA showed that TeaVigo® and green tea extracts were both significantly ($p < 0.05$) more effective than grape seed and white tea. Furthermore, white tea was significantly ($p < 0.05$) less effective than grape seed. The apparent differences in IC₅₀ for green tea and TeaVigo® did not achieve statistical significance.

Grape seed and tea extracts are both rich sources of catechins. A partial characterization of the extracts showed that ECG, EGC, and EGCG are the primary catechin constituents of the two most effective extracts, green tea and TeaVigo®. As observed with the whole extracts, each of these individual compounds inhibited pancreatic lipase activity (Figure 2). Analyses of these data and IC₅₀ values demonstrates that while orlistat was the most effective pancreatic lipase inhibitor, the epicatechins EGCG and EGC were nearly equivalent in potential for lipase inhibition, and were significantly ($p < 0.05$) more effective than ECG.

Summary and Conclusions

These results indicate TeaVigo® was the most effective lipase inhibitor of the natural compounds tested. EGCG and EGC were the two most prominent catechins in green tea and TeaVigo®, and were as effective as TeaVigo®. Since TeaVigo® is a concentrated green tea product, these results suggest yet another beneficial role of green tea. Green tea has been shown to have additional health benefits, including glycemic control and improvement of blood lipid profiles. These findings suggest the potential of natural products to inhibit pancreatic lipase activity; however, they are far less effective than the pharmaceutical orlistat. Despite this lowered effectiveness, these natural extracts may still be a promising alternative for those wishing to avoid pharmaceuticals and their side effects. Tea products are inexpensive, convenient, and safe to use, making them a practical option for those wishing to avoid the cost and potential side effects of pharmaceuticals. *In vivo* studies would be needed to determine the effectiveness of these compounds in human subjects, and to establish the practicality of their use as a weight loss or blood lipid lowering alternative.

Acknowledgements

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