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ORIGINAL RESEARCH

ProAlgaZyme and its subfractions increase plasma HDL cholesterol via upregulation of *ApoA1*, *ABCA1*, and *SRB1*, and inhibition of *CETP* in hypercholesterolemic hamsters

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Background: Plasma HDL cholesterol levels are inversely related to cardiovascular disease, which is the leading cause of death worldwide. This study investigated the effect of an algae infusion, ProAlgaZyme (PAZ), and its subfractions (P1, P2, P3, P4) on plasma HDL in a hamster model.

Methods: Sixty male golden Syrian hamsters (8 weeks old) were randomized into controls (W) or PAZ (P), P1, P2, P3, and P4 (n = 10 per group). An infusion of either 5% (P1, P2, P3) or 20% (P, P4) concentration (v/v) was administered via the drinking water for 4 weeks, while the hamsters were being fed a high-fat diet (30% of calories from fat). Serum lipids were assayed and liver samples subjected to reverse transcription polymerase chain reaction to determine the relative transcription levels of genes involved in HDL/reverse cholesterol transport metabolism, ie, *ApoA1*, *ABCA1*, *CETP*, and *SRB1*.

Results: Non-HDL cholesterol was significantly reduced in the P ($P < 0.05$), P3 and P4 ($P < 0.001$) groups as compared with the W group, while HDL cholesterol showed a significant increase in the P, P3, and P4 groups ($P < 0.001$). Moreover, the total cholesterol/HDL ratio was significantly improved in the P, P1, and P2 ($P < 0.05$), and P3 and P4 ($P < 0.001$) groups. The shift in cholesterol towards the higher density fractions was validated by density gradient ultracentrifugation. Real-time quantitative polymerase chain reaction showed a significant increase in hepatic *ApoA1* (P, P4) and *ABCA1* (P3, P4) expression, consistent with an increase in HDL production, biogenesis, and maturation. A two-fold increase in *SRB1* expression indicates that P4 further augments the reverse cholesterol transport mechanism. Reduction of *CETP* expression (P4) is consistent with a decrease in the transfer of cholesteryl ester to LDL, further increasing the amount of cholesterol held as HDL particles.

Conclusion: ProAlgaZyme and its subfractions significantly improved the plasma cholesterol profile by lowering non-HDL and increasing HDL, possibly via the reverse cholesterol transport mechanism.

Keywords: ProAlgaZyme, *ApoA1*, *ABCA1*, *SRB1*, *CETP*, reverse cholesterol transport

Introduction

Despite significant progress in medical therapeutics, cardiovascular disease (CVD) remains the leading cause of mortality in the US and other industrialized nations.[1] The prevalence of CVD is expected to increase in the next decade, mainly due to an increase in sedentary lifestyle and aging of the population, which will increase the incidence of stroke, acute myocardial infarction, atherosclerosis, and other CVD-related

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disorders. In 2008, the approximate total cost of treatment for CVD was nearly US$500 billion, making CVD a top expense in terms of both human lives and economics.[1] Moreover, it is predicted by the American Heart Association that, by 2030, 40.5% of the US population will be diagnosed with some form of CVD, and the total direct medical costs of CVD are projected to triple from $273 billion to $818 billion annually, while the indirect costs will increase by 61%.[2] Therefore, it is imperative that effective measures be taken in the prevention, early detection, and management of risk factors for CVD.

Current treatment strategies for reducing the risk of CVD include a focus on increasing low levels of high-density lipoprotein (HDL) cholesterol, because it is well established that reducing low-density lipoprotein (LDL) cholesterol alone is insufficient in preventing CVD events.[3,4] HDL and its major protein constituent, apolipoprotein (Apo) A1, are known to protect against CVD, possibly via the reverse cholesterol mechanism, which protects peripheral cells from accumulation of excess cholesterol.[5,6] Moreover, HDL cholesterol is thought to have antioxidative, anti-inflammatory, anticoagulatory, platelet antiaggregatory, and profibrolytic effects,[7] which can alleviate the effects of increased levels of LDL cholesterol.

Recently, functional foods, such as algal infusions and seaweed extracts, have attracted much attention as a therapy to improve the LDL/HDL cholesterol ratio.[8–10] Several authors have reported that seaweeds, isolated algal polysaccharides, and their water-soluble fractions, have hypocholesterolemic effects in experimental animals.[11,12] ProAlgaZyme (PAZ) algae infusion is the fermentation product of a blend of freshwater organisms, including green algae.[13] It contains less than 100 ppm of total dissolved solids, consisting of a mixture of approximately 90% salts (free of heavy metals at a detection limit of <0.1 ppm), while the remaining 10% is a proprietary mixture of organic constituents. In a preliminary study, Oben et al evaluated the effects of PAZ on body weight, body mass index, blood lipids, fasting blood glucose levels, and markers of inflammation in individuals with the metabolic syndrome. The results showed a significant beneficial outcome on the various parameters analyzed, including the lipid profile, suggesting that PAZ could be an effective method for prevention of CVD.[13] Changes in plasma lipids were not apparent until 8 weeks into the study, but were exceptionally large, with some subjects experiencing an almost two-fold increase in HDL cholesterol. However, no measures of triglycerides or LDL cholesterol were reported at 10 weeks.

The aim of the present study was to document the potential effect of PAZ on HDL cholesterol and to characterize further the underlying mechanism. In addition, we analyzed different subfractions of the filtrate for potential effect on lipids. Because an earlier study[13] showed that PAZ significantly increased the concentration of HDL cholesterol, hepatic mRNA expression was also analyzed for potential effects on genes involved in HDL/reverse cholesterol transport. Male golden Syrian hamsters were used because they have been shown to be a good model for human lipid metabolism.[14,15] Administration of a high-fat diet with 30% calories from fat and 99% coconut oil was used to induce a rapid hypercholesterolemic state in the hamsters.

Materials and methods

Hamsters and diet

The animal study protocol was approved by the Institutional Animal and Care Use Committee at Wayne State University, Detroit. Sixty 8-week-old male golden Syrian hamsters (*Mesocricetus auratus*), LVG strain (Charles River Laboratories, Wilmington, MA), weighing approximately 80 g, were acclimatized and given water and laboratory rodent diet 5001 (Lab Diet, Richmond, IN) ad libitum for one week prior to initiation of the experimental treatment. The animals were housed individually in a temperature-controlled room (25°C) and maintained on a 12-hour light/dark cycle. The hamsters were randomized into six groups of ten animals each according to mean body weight and fed a high-fat diet containing 30% calories from fat (Dyets Inc, Bethlehem, PA, Table 1).

Table 1 Composition of the experimental custom purified diet with Coconut oil and Soybean oil

Ingredient	Kcal/gm	Grams/kg
Casein	3.58	110
Lactalbumin	3.9	110
L-Arginine	4	2.5
L-Tryptophan	4	0.3
Cornstarch	3.6	370.2
Dyetrose	3.8	175
Coconut oil	9	138.6
Soybean oil	9	1.4
TBHQ	0	0.028
Cellulose	0	44
Cholesterol	0	1
Mineral mix #260001*	0	35
Vitamin mix #360001**	3.84	10
Choline bitartrate	0	2
Total	**44.72**	**1000**

Notes: *Hamster salt mixed prepared by Dyets, Inc; **Hamster Vitamin Mix prepared by Dyets, Inc.
Abbreviation: TBHQ, Tertiary Butylhydroquinone.

Each group received either water (W), PAZ (P), or one of the subfractions of PAZ (P1, P2, P3, P4) at either 5% (P1, P2, P3) or 20% (P, P4) concentration (v/v) as their drinking fluid for four weeks. This dose was established based on the previously reported dose of 4 oz of PAZ per day in humans.[13] Modifications were made to account for body weight and amount of fluid intake per day in hamsters versus humans.[13] ProAlgaZyme was fractionated by sequential affinity gel chromatography (Oxford Biomedical Research, Rochester Hills, MI.) Complete PAZ was passed through four chromatography columns (2.7 cm × 23 cm; approximately 90 mL of resin at full capacity) at a flow rate of approximately 6 mL per minute using a peristaltic pump. The ProAlgaZyme material was passed in series through these four chromatography columns. Column 1 containing a weak anion exchange resin (diethylaminoethyl cellulose) captured proteins and on elution resulted in the P1 fraction. Column 2 containing a strong anion exchange resin (BioRad AG 1-X8) was designed to capture molecules containing carboxyl groups and other negatively charged functionalities, as well as negatively charged ions, and was designated the P2 fraction. Column 3, designated the P3 fraction, was a strong cation exchange column (Dowex Monosphere 88) intended to capture molecules containing amino groups and other positively charged functionalities, as well as positively charged ions. Column 4 (silica gel 90 C18 reversed phase) was a C18 derivatized column that binds nonpolar organic molecules. The elute from this column was not used in this study. Instead, the flow through (labeled as P4), which contains relatively few molecules, including polar but uncharged organic molecules, as well as molecules of low polarity that were not captured by columns 1, 2, 3, or 4, was assessed for its effect on the hamster lipid profile. Body weight, food, and water intake were recorded weekly. The animals were maintained in accordance with the guidelines of the Institutional Animal and Care Use Committee at Wayne State University.

Plasma and tissue collection

Hamsters were fasted for 8 hours and anesthetized under CO_2/O_2 (50%:50%) gas (Metro Welding, Detroit, MI) prior to sacrifice. Blood was collected by cardiac puncture with syringes previously rinsed in potassium ethylenediamine tetraacetic acid solution (15% w/v) and kept on ice. Plasma was separated after centrifugation at 3500 rpm for 15 minutes at 4°C. Liver and adipose tissue were collected and immediately flash-frozen in liquid nitrogen for subsequent analysis.

Plasma lipid analysis

Plasma total cholesterol (TC) and triglyceride concentrations were determined enzymatically, while HDL cholesterol was measured in the supernatant following precipitation with Mg^{2+}/dextran sulfate according to the manufacturer's protocol (Pointe Scientific, Canton, MI). The concentration of non-HDL cholesterol was calculated as the difference between the measured total cholesterol and HDL cholesterol, and includes the sum of very low-density lipoprotein cholesterol, intermediate-density lipoprotein cholesterol, and LDL cholesterol. For separation of HDL cholesterol, a 500 µL plasma sample was mixed with 50 µL of reagent, and after 5 minutes at room temperature was centrifuged at 2000 × g for 5 minutes. For determination of the HDL cholesterol concentration, a 96-well assay microplate was used to mix 200 µL of reagent with 10 µL of supernatant, followed by incubation for 10 minutes at 37°C. The plate was read at a wavelength of 490 nm using KC4 software (EL × 800 microplate absorbance reader, Bio-Tek, Winooski, VT).

The lipoproteins were isolated by density gradient ultracentrifugation, essentially as stated by Chapman et al.[16] For each group, lipoprotein isolations (n = 2) were carried out using plasma pooled from 4–5 hamsters in 16 × 93 mm ultracentrifuge tubes. Lipoproteins were isolated using a Beckman SW-41 rotor (Beckman Coulter, Brea, CA) at 35,000 rpm and 15°C for 46 hours.[17] Following ultracentrifugation, 26 × 500 µL fractions per tube were collected by sequentially pipetting from the top. The total cholesterol concentration in each fraction was measured using enzymatic reagents as described above (Pointe Scientific).

Real-time reverse transcription polymerase chain reaction

Total RNA from the liver was extracted using the miRNeasy Mini Kit (Qiagen, Valencia, CA) and reverse transcription of RNA into cDNA was performed using the high-capacity mRNA to cDNA Master Mix kit (Applied Biosystems, Carlsbad, CA) as per the manufacturer's protocol. Total RNA was obtained by adding 40 µL of RNAase-free H_2O and centrifugation at 1000 × g for one minute. mRNA was further subjected to reverse transcription using the high-capacity RNA to cDNA Master Mix kit. The reaction was carried in a total of 20 µL of mixture (4 µL of Complete Master Mix, 8 µL of total RNA, and 8 µL of nuclease-free H_2O) in a Mastercycler (Eppendorf, Hauppauge, NY). The program was set for 5 minutes at 25°C, 30 minutes at 42°C, 5 minutes at 85°C, and one hour at 4°C. A 2 µL sample of the cDNA obtained was used for each real-time reverse transcription

polymerase chain reaction using SYBR Green Master Mix (Applied Biosystems) and an MX3005P instrument (Strategene, Santa Clara, CA) to determine the relative transcription levels of specific genes (*ABCA1*, *ApoA1*, *CETP*, and *SRB1*) involved in HDL/reverse cholesterol transport metabolism. The cycle conditions were 10 minutes at 95°C followed by 40 cycles of incubation at 95°C for 15 seconds each, then 60°C for one minute. The sequences for the primers used in this study are defined in Table 2.[18–20] No accumulation of nonspecific products or primer dimers was observed using nontemplate control wells as a result of prior optimization of the concentration used. The data were analyzed according to the comparative threshold cycle (C_t) method and normalized by glyceraldehyde-3-phosphate dehydrogenase expression in each sample. Glyceraldehyde-3-phosphate dehydrogenase was used as an invariant control because levels of this transcript were not altered by the experimental diets. Levels of mRNA expression were reported as fold differences when compared with hamsters fed the high-fat diet and water.

Statistical analysis

All data are expressed as the mean ± standard error. Differences between the control and treatment groups were determined using one-way analysis of variance tests (IBM SPSS Inc, Chicago, IL). The data were analyzed to determine the effect of the algae extract relative to distilled water while the animals were fed the high-fat diet. Pearson correlation coefficients were calculated to investigate relationships between plasma total cholesterol, HDL cholesterol, triglyceride concentrations, and expression of hepatic genes. Statistical significance was defined as $P < 0.05$.

Table 2 Real time RT-PCR primers

Gene	Primers	Reference
CETP	F:5′-AAGGGTGTCGTGGTCAGTTCT-3′ R:5′-ACTGATGATCTCGGGGTTGAT-3′	18
ApoA1	F:5′-ACC-GTT-CAG-GAT-GAA-AAC-TGT-AG-3′ R:5′-GTG-ACT-CAG-GAG-TTC-TGG-GAT-AAC-3′	19
SRB1	F:5′-AAG-CCT-GCA-GGT-CTG-TGA-AGC-3′ R:5′-AGA-AAC-CTT-CAT-TGG-CTC-CCT-A-3′	19
ABCA1	F:5′-ATA-GCA-GGC-TCC-AAC-CCT-GAC-3′ R:5′-GGT-ACT-GAA-GCA-TGT-TTC-GAT-GTT-3′	20

Abbreviations: *CETP*, Cholesteryl ester transfer protein; *ApoA1*, Apolipoprotein A-1; *SRB1*, Scavenger receptor class B member 1; *ABCA1*, ATP-binding cassette transporter A1.

Results

Metabolic effects of high-fat diet and PAZ supplementation

All hamsters survived for the entire duration of the study. Animals in the six experimental groups consumed similar amounts of food and water. The final body weight and weight gain, as well as the food efficiency ratio, were not significantly different between the treatment groups and controls. Liver weights were also not significantly different between the groups (Table 3).

Plasma total cholesterol was not significantly different in the treatment groups when compared with the controls. However, the total cholesterol/HDL cholesterol ratio was significantly lower in all experimental animals when compared with controls ($P < 0.05$ in the P, P1, and P2 groups; $P < 0.001$ in the P3 and P4 groups, Table 4). Consumption of PAZ or its fractions lowered non-HDL cholesterol concentrations in the P group ($P < 0.05$) and in the P3 and P4 ($P < 0.001$) groups, as compared with the controls. In addition, the P3 group had significantly lower non-HDL cholesterol levels as compared with P1 ($P < 0.05$) and P2 ($P < 0.001$) treatment groups (Table 4). Moreover, the concentration of plasma HDL cholesterol was significantly increased in the P, P3, and P4 groups ($P < 0.001$) when compared with controls, and this was confirmed by ultracentrifugation (Figure 1). Lipoprotein fractionation indicated that a higher proportion of cholesterol was carried in the HDL fraction in the P group. Triglyceride concentrations were not significantly different between the treatment groups and controls. However, the triglyceride concentrations in groups P1, P2, and P4 were significantly ($P < 0.05$) lower than in the p group (Table 4).

Effect of PAZ and its subfractions on gene expression

In an attempt to elucidate the mechanism by which PAZ and its subfractions alter the lipoprotein profile by increasing HDL, the activities of *ABCA1*, *ApoA1*, *SRB1*, and *CETP* were assessed. Hamsters fed the PAZ and P4 fraction had the highest fold increase (about five times) in *ApoA1* expression ($P < 0.01$), while the P3 group showed a smaller increase (two-fold, Figure 2A). *ABCA1* sterol transporter expression showed a moderate increase in the P3 and P4 groups (1.7-fold and 1.8-fold, respectively), which was statistically significant ($P < 0.01$, Figure 2B). In addition, *SRB1* activity levels were also modestly but significantly higher in the P3 and P4 groups (approximately two-fold, $P < 0.01$, Figure 2C). The differences in the other treatment groups (P1 and P2) were

Table 3 Anthropometrics of male hamsters fed high fat diet and PAZ or its fractions for 4 weeks

	W	P	P1	P2	P3	P4
Anthropometric data						
Body weight, g	119 ± 3	117 ± 3	117 ± 3	118 ± 3	122 ± 2	119 ± 3
Body weight gain, g/4 wk	32.7 ± 2.1	29.7 ± 2.0	30.3 ± 2.6	33.1 ± 3.0	29.9 ± 3.1	31.1 ± 1.0
Food intake, g/d	7.1 ± 0.1	6.8 ± 0.1	6.7 ± 0.02	6.8 ± 0.2	7.0 ± 0.2	6.6 ± 0.2
Food efficiency ratio, g gain/g feed	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
Water intake, mL/d	8.3 ± 0.2	8.2 ± 0.6	8.3 ± 0.7	7.7 ± 0.5	7.7 ± 0.4	7.2 ± 2.1
Liver weight, g	5.4 ± 0.2	5.8 ± 0.3	5.5 ± 0.2	5.4 ± 0.3	5.5 ± 0.3	5.4 ± 0.2

Notes: Values are mean ± SE, n = 10/group. Statistical program ANOVA with Tukey's procedure was used, SPSS software.

not significant. The data suggest that the increase in HDL cholesterol concentrations in the P, P3, and P4 groups was in part attributable to the increase in production of nascent HDL cholesterol particles and/or clearance via *SRB1* receptors.

Hepatic *CETP* expression was characterized by a two-fold decrease only in the P4 group as compared with those receiving water alone (Figure 2D). Inhibition of *CETP* is consistent with the decrease in plasma non-HDL lipoprotein along with the increase in HDL cholesterol.

Plasma lipoprotein concentrations levels and hepatic gene expression

Correlations between plasma lipid concentrations and hepatic gene expression levels were sought to identify potential relationships between molecular processes and circulating lipid concentrations (Table 5). Total cholesterol, non-HDL cholesterol, and total cholesterol/HDL ratios were negatively correlated with hepatic expression of *ABCA1* and *SRB1*. There was a significant positive correlation between plasma HDL cholesterol concentrations and mRNA levels of *ApoA1* ($P < 0.01$). In addition, positive correlations between HDL cholesterol concentrations and mRNA levels of *SRB1* were observed.

Discussion

The present study was designed to examine the effect of the algal fermentation product, PAZ, and its subfractions, on plasma lipoproteins in a diet-induced hypercholesterolemic hamster model. More specifically, the objective was to determine the mechanism of action whereby certain components of PAZ can influence the pathway of cholesterol metabolism. Hamsters were used in this study because their lipoprotein metabolism is comparable with that of humans in terms of having similar components and metabolism of both lipoproteins and bile acids.[21,22]

Different types of algal cellular biomass and algal extracts have been studied for their ability to lower circulating cholesterol concentrations in hamsters consuming hypercholesterolemic diets[8] and in humans,[23] and all have shown a reduction in total plasma cholesterol, non-HDL cholesterol, and/or triglyceride concentrations.

When PAZ was administered to humans for 10 weeks to evaluate its effect on cardiovascular risk factors associated with the metabolic syndrome, there was a decrease in plasma total cholesterol, non-HDL cholesterol, and triglyceride concentrations. Further, HDL cholesterol levels were significantly increased, and PAZ was well tolerated with no adverse effects noted.[13] However, one drawback of the human study was the extreme variation in lipid profile between the treatment group versus the control group, as well as a lack of information on underlying potential mechanisms. The main objective of the current study was to verify the hypolipidemic effects of PAZ and to evaluate if an increase in HDL cholesterol could be attributed to hepatic mRNA

Table 4 Plasma lipid concentrations in male hamsters fed high fat diet and PAZ or its fractions for 4 weeks

	W	P	P1	P2	P3	P4
Plasma lipids						
Triglyceride mmol/L	2.28 ± 0.15	2.78 ± 0.21	2.00 ± 0.12***	2.02 ± 0.21***	2.3 ± 0.18	1.93 ± 0.15***
Total cholesterol mmol/L	6.13 ± 0.18	6.31 ± 0.16	5.97 ± 0.21	6.31 ± 0.03	5.64 ± 0.13	5.92 ± 0.23
HDL cholesterol mmol/L	2.69 ± 0.1	3.57 ± 0.21**	3.13 ± 0.16	3.34 ± 0.23	3.49 ± 0.18**	3.54 ± 0.16**
Non HDL cholesterol mmol/L	3.44 ± 0.1	2.72 ± 0.18*	2.84 ± 0.18	2.97 ± 0.16	2.15 ± 0.1**	2.38 ± 0.1**
TC/HDL cholesterol	2.28 ± 0.05	1.77 ± 0.13*	1.91 ± 0.09*	1.89 ± 0.9*	1.62 ± 0.06**	1.67 ± 0.03**

Notes: Values are mean ± SE, n = 10. *$P < 0.05$; **$P < 0.001$ when compared with W group; ***$P < 0.05$ when compared with p group. Statistical analysis ANOVA with Tukey's procedure was used, SPSS software.

Abbreviations: W, water group (control); P, proAlgazyme group; P1, 2, 3, 4, fraction 1, 2, 3, or 4 of proalgazyme; HDL, high density lipoprotein; TC, total cholesterol.

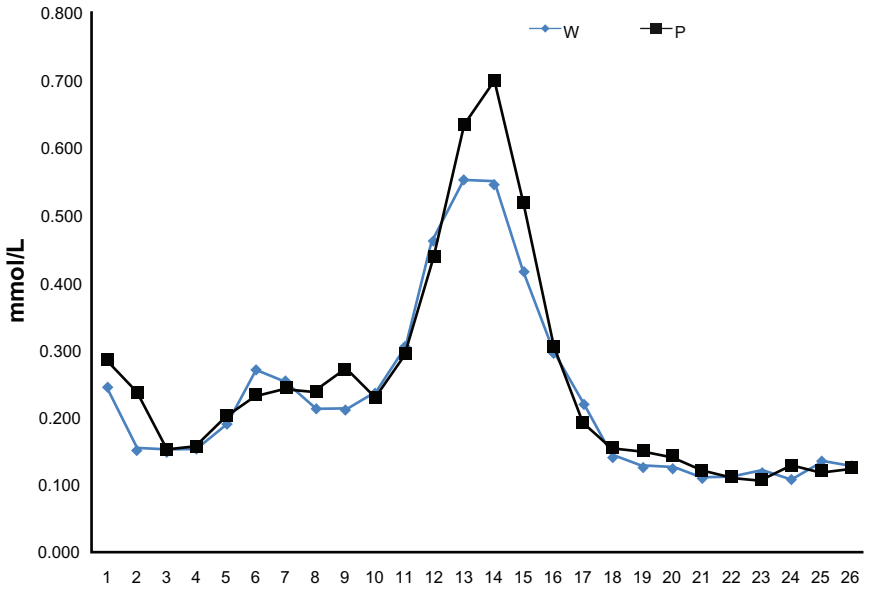

Figure 1 Effect of PAZ on lipoprotein particle distribution showing a shift from lower to higher density particles in P group vs W control group.
Notes: Total cholesterol concentrations were measured in all eluted fractions (0.5mL/fraction) using standard enzymatic reagents. Data represents the mean of pooled plasma for W and P groups.

expression of key genes involved in HDL/reverse cholesterol transport metabolism.

The results of our study corroborate previous findings concerning plasma lipoprotein concentrations upon administration of dietary PAZ, whereby non-HDL cholesterol was reduced by 38%, 31%, and 21% in P3, P4, and P groups, respectively, while total cholesterol/HDL ratio was improved by 30% in P3 and P4 groups, and by 21% in a P group. In addition, the HDL cholesterol concentration was significantly increased by over 30%

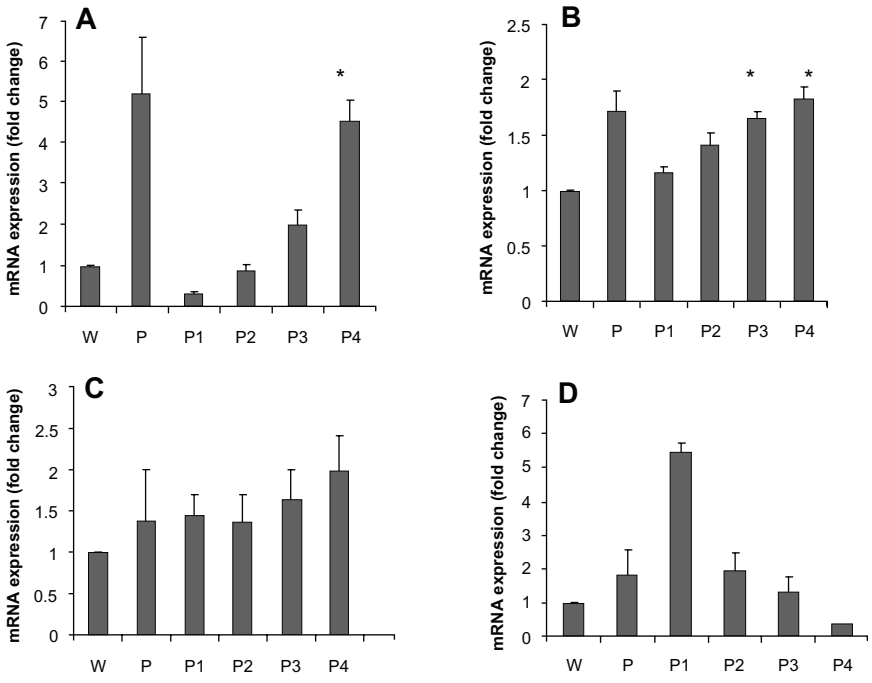

Figure 2 Relative levels of expression of genes that encode key proteins involved in the regulation of cholesterol and HDL metabolism in animals fed a high fat diet plus complete PAZ (P) or one of the fractions as compared with the control group on high fat diet and water (W).
Notes: Values are expressed as mean ± SE; n = 5 animals per group (n = 10 for P group). Each mRNA was normalized with GAPDH and is expressed as a fold change. *$P < 0.01$.
Abbreviations: *ApoA1*, Apolipoprotein A-1 (**A**); *ABCA1*, ATP-binding cassette transporter A1 (**B**); *SRB1*, Scavenger receptor class B member 1 (**C**); *CETP*, Cholesteryl ester transfer protein (**D**).

Table 5 Correlation between plasma cholesterol concentrations and expression of hepatic genes in male hamsters fed a high fat diet and PAZ or its subfractions

	Total cholesterol	HDL cholesterol	TC/HDL ratio	Non-HDL cholesterol
Genes				
ApoA1	0.153	0.399*	–0.342*	–0.243
ABCA1	–0.068	0.252	–0.33*	–0.33*
SRB1	–0.190	0.252	–0.420*	–0.459*
CETP	0.040	–0.055	0.056	0.099

Note: Values are Pearson correlation, n = 10. *$P < 0.01$, SPSS software.

in the P, P3, and P4 groups. The increase in plasma HDL cholesterol concentrations was validated by analysis of lipoproteins following ultracentrifugation. A shift in the lipoprotein distribution consistent with a reduction in non-HDL cholesterol and an increase in HDL cholesterol was noted. To evaluate potential underlying mechanisms for this effect, genes involved in HDL metabolism (*ApoA1*, *ABCA1*, *SRB1*, and *CETP*) were evaluated in liver samples.

ApoA1 is involved in the production of nascent HDL particles, while *ABCA1* transports lipids from peripheral tissues to nascent HDL to form larger HDL particles. The mature HDL particle is removed via the *SRB1* receptor in the liver into bile, clearance from plasma occurs, and the *ApoA1* molecule is recycled. *CETP* facilitates the transport of cholesteryl esters and triglycerides between lipoprotein particles, and partial inhibition of this enzyme is beneficial for lowering cholesterol concentrations in plasma.

Using a real-time reverse transcription polymerase chain reaction technique, we examined the ability of ProAlgaZyme to regulate these genes transcriptionally. Administration of PAZ and its subfractions did indeed alter mRNA levels of *ABCA1*, *ApoA1*, *SRB1*, and *CETP*. Transcription of *ApoA1*, *ABCA1*, and *SRB1* genes was upregulated, whereas transcription of the gene encoding *CETP* was downregulated after 4 weeks of dietary intervention with PAZ. To our knowledge, this is the first report investigating the effect of this algal infusion on HDL gene transcription and metabolism. Therefore, high non-HDL cholesterol and total cholesterol, and low HDL cholesterol, can be treated by upregulating *ABCA1*, *ApoA1*, and *SRB1* expression and downregulating *CETP* expression.

In conclusion, our results suggest that PAZ administration results in a favorable lipoprotein cholesterol distribution profile in hamsters, primarily via its effects on multiple targets in the reverse cholesterol transport pathway. Coupled with previous data, the potential for PAZ to raise HDL warrants further investigation.

Acknowledgments

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Disclosure

AG and SVG have rights to a application covering the biological activity of PAZ. NS, AG, MW, and XJ report no conflicts of interest in this work.

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