Effect of BSN272 on Hyperlipidemia and Atherosclerosis in LDLr-/- Mice

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Effect of BSN272 on Hyperlipidemia and Atherosclerosis in LDLr-/- Mice

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Abstract

This study was designed to compare the effects of D-tagatose with BSN272 on serum lipids and prevention of atherosclerosis in LDLr-/- mice. BSN272 is a combination drug of D-tagatose and polydatin (trans piceid). LDLr-/- mice were divided into four groups and were all fed a standard chow. Mice were dosed by gavage and received water (group 1), a glucose/fructose mixture (group 2), or glucose/fructose mixture with D-tagatose (group 3) or with BSN272 (group 4) for a period of 9 weeks. Food intake, body weight, serum cholesterol, triglyceride and lipoprotein concentrations, and aortic atherosclerosis were measured. Cholesterol and triglyceride levels in the BSN272 treated group were consistently lower than in the water and glu/fruc groups throughout the course of the experiment. BSN272 reduced atherosclerotic lesions by 57% in LDLr-/- mice and significantly reduced VLDL and LDL cholesterol by 35 and 17%, respectively. From these results we conclude that the BSN272 combination is the most effective of the treatments for lowering cholesterol and triglycerides and for inhibiting the development of atherosclerosis.

Introduction

The two objectives of this study were to (1) compare the effect of D-tagatose with the effect of a combination of D-tagatose and polydatin on serum triglycerides and cholesterol, and (2) compare the effect of these two treatments on the development of atherosclerosis in LDLr-/- mice.

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In western countries, cardiovascular disease is the leading cause of mortality. While there are multiple factors that increase the risk of developing cardiovascular disease, there is evidence supporting a strong link between abnormal blood lipids (dyslipidemia) and increased risk for cardiovascular disease. Dyslipidemia is typically characterized by elevated levels of triglycerides and low-density lipoprotein (LDL) cholesterol and by low levels of high-density lipoprotein (HDL) cholesterol. Atherosclerotic lesions form from lipoproteins, macrophages, and lymphocytes in arterial blood vessels. Blood cholesterol moves into damaged vessel endothelium layers. These modified lipids, changed by oxidation, cause macrophages and lymphocytes to enter the area to remove the LDLs. Over time, these components develop into plaque. Macrophages will display the lipoproteins on their cell surface and form foam cells initiating the plaque formation.

BSN272 is a combination drug therapy composed of a carbohydrate, D-tagatose, and polydatin, a glucoside derivative of resveratrol. D-tagatose, a naturally occurring epimer of fructose, was originally developed as a low-calorie sweetener (1.5 kcal/g compared to 4 kcal/g for sucrose) but was found to have an antihyperglycemic effect in animal and in human studies and showed promise as a treatment for type 2 diabetes and obesity. Clinical studies have shown D-tagatose to be a potential anti-diabetic drug through its beneficial effects on postprandial hyperglycemia and hyperinsulinemia. In addition to treating diabetes, D-tagatose may also be an effective treatment for obesity and for reducing cardiovascular risks by increasing high-density lipoprotein (HDL) levels. After over 10 years of animal and human studies, D-tagatose was classified as being "generally recognized as safe (GRAS)" by the FDA and has been used since in food and beverage products.

Police et al. found that the equivalent substitution of D-tagatose for sucrose as a dietary carbohydrate did not result in the same extent of obesity, hyperglycemia, hyperlipidemia, and atherosclerosis in LDLr-/- mice. Mice fed standard lab chow and mice fed D-tagatose chow exhibited similar energy intake, body weights and blood glucose and insulin concentrations, while sucrose-chow fed mice exhibited increased energy intake and became obese and hyperglycemic. Sucrose-fed mice had increased serum cholesterol, triglyceride concentrations and atherosclerosis compared to mice fed D-tagatose or a standard diet.

Polydatin is a natural substance that is a glucoside form of resveratrol. Evidence suggests that resveratrol, a naturally occurring polyphenol commonly found in a variety of plants and foods, most notably grapes, can produce a variety of beneficial effects, including the promotion of weight loss and anti-inflammatory and neuroprotective properties. Recently, it was found that the concentration of polydatin in grapes is as much as seven times that of resveratrol and is probably the most abundant form of resveratrol in nature. Polydatin as a number of advantageous properties that increase its bioavailability compared to resveratrol, including a greater resistance to enzymatic
oxidation. Polydatin enters cells by an active transport mechanism using glucose carriers, unlike resveratrol which penetrates the cell passively. A number of studies suggest that polydatin has biological properties similar to those of resveratrol. Current evidence suggests polydatin may inhibit platelet accumulation, improve microcirculation, decrease lipid peroxidation, and reduced neutrophil-endothelial aggregation.

These proactive factors may limit the growth of plaque in arteries.

There is considerable interest in the use of trans-resveratrol and its derivatives, including polydatin, for the treatment of many human diseases. Extracts derived from Polygonum cuspidatum have long been a part of traditional Chinese herbal medicine, being used to treat pain, fever, coughs, inflammation and a variety of other ailments. Polydatin, a glucoside derivative of resveratrol, is the major component of these extracts. In addition to Polygonum, polydatin has been found in wines and grapes, cocoa, peanuts and peanut butter, pistachios and almonds. As a derivative of resveratrol, polydatin is believed to have many of the same beneficial effects, but also has some properties that may make it more effective from a pharmacological standpoint than resveratrol. Polydatin is structurally the same as resveratrol except that it has a glucoside group attached to the C-3 position in place of a hydroxyl group. This substitution makes polydatin more water soluble and in some ways more resistant to enzymatic breakdown than resveratrol. It is also actively taken up by cells via glucose carriers in the cell membrane instead of being passively transported like resveratrol. These properties suggest that polydatin would have greater bioavailability than resveratrol.

Claims for health benefits of polydatin abound. Studies almost too numerous to count have presented evidence that polydatin has many positive effects including anti-inflammatory, hepatoprotective, anti-cancer, neuroprotective, and cardioprotective activities. Pharmacological studies and clinical practice have demonstrated that polydatin also has protective effects against shock, ischemia/reperfusion injury, congestive heart failure, endometriosis, and prevention of fatty liver disease and insulin resistance, and that it can regulate glucose and lipid metabolism. Polydatin has found its way into clinical trials for the treatment of hemorrhagic shock and irritable bowel syndrome.

The way in which polydatin is able to have all of these activities is still being studied, but multiple mechanisms of action are evident, including; an antioxidant, free radical-elimination mechanism, activation of protein kinase C, suppression of NF-kappaB, inhibition of the activation of renin-angiotensin-aldosterone system and decreasing the excretion of endothelin 1, TNF-alpha, and angiotensin II, reduction of lipid peroxidation levels, up regulation of the expression of hippocampal brain-derived neurotrophic factor, enhanced insulin sensitivity in the liver as shown by improved insulin receptor substrate 2 expression levels and Akt phosphorylation, decreasing the content of malondialdehyde, promoting the activities of total superoxide dismutase (T-SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in plasma, increasing the content of glutathione (GSH) in myocardial tissue, restoring decreased deacetylase sirtuin-1 activity and protein expression in liver tissue following severe shock and activation of sirtuin, and suppressing oxidative stress-induced lysosomal instability and mitochondrial injury by increasing the protein expression of SOD2.

The treatment of dyslipidemia using polydatin has been suggested by a number of studies using animal models. Polydatin given orally at 100 mg/kg lowered low-density lipoprotein (LDL) cholesterol by approximately 18% and serum triglycerides by 40% in rats consuming a standard chow containing a mixture of corn oil, 10% cholesterol, and 1% cholic acid. Lower doses of trans-polydatin (50 mg/kg body weight) were ineffective at preventing hyperlipidemia, however they were able to prevent the accumulation of cholesterol and triglycerides in the liver. In a study using Syrian golden hamsters, polydatin was found to decrease total cholesterol levels and total triglyceride levels by 47% and 63%, respectively, in hamsters on a high fat, high cholesterol diet. In a study using rabbits, polydatin decreased the serum levels of total cholesterol, triglycerides and LDL. The ratio of total cholesterol to HDL was also reduced. In our laboratory, the combination of polydatin and D-tagatose has been shown to reduce cholesterol, triglycerides, and the extent of atherosclerosis in apoE mice. The apoE mouse model is generally resistant to obesity, shows increased VLDL and LDL, and decreased HDL, and not particularly subject to developing insulin resistance.

In the present study, we have examined the effect of BSN272, a combination of D-tagatose and polydatin, on blood lipids and atherosclerosis in LDLR mice. In contrast to the apoE mouse model, in the LDLR mouse model obesity, increased LDL, and insulin resistance are induced by a high fat diet. Also, VLDL does not increase in the LDLR mouse as it does in the apoE mouse. These differences might affect the development of atherosclerosis in this model, and lead to different treatment effects.
to differences between the apoE and LDLr⁻/⁻ mouse results.

Methods

Mice

Male 6-7 weeks old C57BL6-LDLr knockout (LDLr⁻/⁻) mice (JAX Strain Name: B6.129S7-Ldlr<sup>tm1Her</sup>/J) were used for this study. Mice were acclimated for 2 weeks prior to start of the study and were individually housed in solid bottom cages and kept on a standard light cycle: 12 hours light, 12 hours dark at 72 ± 8ºF.

Treatment

This study was carried out at Covance Laboratories (Madison, WI). Animals were randomized by body weight into four groups (Illustration 1). Mice in group 1 (Control group, n=10) were dosed with water, while mice in group 2 (n=10) were dosed with 50% glucose + 50% fructose (see Table 1). The remaining mice were placed into groups 3 and 4 and randomly selected doses for animals 21-30 (group 3) and 31-40 (group 4) were forced to be uncorrelated by principal axis transformation. This orthogonalization of doses allowed the contribution to the reduction of lipids by polydatin and each sugar to be measured independently while still being in the presence of the other molecules.

For treatment groups 3 and 4, D-tagatose was added to ground feed (meal) each day. Groups 1 & 2 had ground TD.2014 (Teklad, Harlan Laboratories, Madison, WI) with no D-tagatose added. The dose in the feed of D-tagatose for groups 3 and 4 was increased by ~7.1% daily during the D-tagatose lead-in phase until the final maximum dose was reached. Individual animal feed bags were provided for each day of the lead-in phase of the study for all animals. Remaining feed was disposed of daily and the cages cleaned of the remaining crumbled feed. Duration of lead-in phase was 14 days. During the lead-in period all mice were handled daily to acclimate the animals to dosing by scuffing the animal to simulate gavage dosing. The study design is summarized in Illustration 1.

On Day 15 all animals were placed on TD.2014 for the remainder of the study. Mice were dosed by gavage based upon most recent body weight, twice per week for 9 weeks. Illustration 2 shows the components in the solutions given to the mice by gavage. Each animal in groups 3 and 4 had a different formulation as shown in Illustration 3 “Dosing/Formulation Table for Each Animal”. Inside each dose group, the doses ranged from 0 to 0.853 g/kg/dose for the sugars and 0 – 0.150 g/kg/dose for polydatin. Dose volume was 10 mL/kg. Animals were weighed and food consumption was measured weekly. Blood samples were taken from the tail vein. Animals were not fasted prior to taking blood samples. On day 78 animals were anesthetized with isoflurane, bled by cardiac puncture, and the tissues removed.

Serum Lipids

Blood samples were obtained through tail cuts every two weeks throughout the experiment, and triglycerides, cholesterol and free fatty acids were measured at Covance Laboratories using a Roche Hitachi 917 or Cobas 6000 analyzer using a photometric:enzymatic method. Animals were not fasted prior to bleeding, but were bled approximately 1 hour post dosing. On the final day of the study, mice were anesthetized with isoflurane and bled by cardiac puncture. Body weights and food consumption were measured weekly.

At the end of the study mice were sacrificed via cardiac puncture. Aortic arches were harvested nine weeks after treatments began and the extent of atherosclerotic lesions was determined by false color imaging. Lesion area was measured as a fraction of the aortic arch area. The percent of atherosclerotic lesions in the BSN272 treated group was determined by dividing the mean atherosclerotic lesion in the Glu/Fruc/BSN272 group by the mean atherosclerotic lesion in the Glu/Fruc group.

The amount of VLDLs, LDLs, and HDLs were determined by collecting serum nine weeks after the treatments began, resolving the lipoprotein complexes by FLPC, and quantifying the amount of cholesterol in each FPLC fraction using an enzymatic cholesterol assay. Samples for analysis were chosen from 5 mice in each of the glucose + fructose and glucose + fructose + BSN272 groups. The samples selected had total cholesterol values closest to the mean.

Results and Discussion

Food Intake

There was no significant difference in amount of food eaten between mice in the different groups once treatments were started. There was a difference in amount of food eaten during the two week D-tagatose lead-in (Illustration 4). Mice being fed the D-tagatose during the lead-in phase ate less (1.9 ± 0.10 g for Groups 3-13 and 2.1 ± 0.11 g for Groups 13-22) than mice in Group 1 (3.2 ± 0.052 g) or 2 (3.3 ± 0.04 g). This was somewhat expected as studies in humans have found D-tagatose produced a feeling of satiety<sup>92-95</sup>.
Once mice were place on the standard chow and gavage treatments began, food consumption was the same for all three groups (Illustration 5).

**Body Weights**

Mice in the D-tagatose and BSN272 groups weighed slightly less at the start of the study before any treatment began than mice in the water or Glu/Fruc groups. This slight difference was maintained throughout the course of the experiment. Even though mice in the groups receiving the D-tagatose during the 2 week lead-in phase ate less (see Illustration 4), their weight gain during the lead-in phase was no different than mice not receiving the D-tagatose that ate more. There were no significant differences in body weights of the mice in the four groups during the course of the study (Illustration 6). The rate of weight gain was similar for all mice during the course of the study.

**Tagatose and BSN272 reduce serum lipids in LDLr<sup>-/-</sup> mice**

**Cholesterol**

Day 78, end of study result. Glucose/Fructose raised total serum cholesterol in LDLr<sup>-/-</sup> mice compared to control mice. Treatment with D-tagatose or BSN272 prevented the increase due to the glucose/fructose (Illustration 7). End point mean cholesterol was 322 ± 18 mg/dl for the control group, 378 ± 18 mg/dl for the Glucose/Fructose group, 309 ± 27 mg/dl for Glucose/Fructose/Tagatose group, and 305 ± 16 mg/dl for the Glucose/Fructose/BSN272 group (Illustration 7).

**Triglycerides**

Day 78, end of study result. Glucose/Fructose did not change serum triglyceride levels compared to control mice (118 ± 14 mg/dl for Glucose/Fructose mice compared to 114 ± 13 mg/dl for control mice). However, treatment with D-tagatose or BSN272 reduced serum triglyceride levels (81 ± 6 mg/dl and 71 ± 4 mg/dl, respectively), with the BSN272 having the lowest study end point triglyceride level (Illustration 8).

**Free fatty acids**

Free fatty acid levels in all groups on Day 14 look approximately the same as their respective levels on Day 78 when the study ended (Illustration 9). In the middle of the study (days 36 and 50) values for all groups go up by about 50%. On day 14 the D-tagatose and BSN272 groups have significantly lower fatty acid levels than the glucose/fructose or water control groups, which could be due to the D-tagatose given during the lead-in phase. Fatty acids drop considerably for all four groups from Day 50 to 64, and then go back up in the group on water and drop in the BSN272 group. The drop in all groups makes it difficult to determine if any change is treatment related. There is no statistically significant difference between the D-tagatose and BSN272 groups at any time point.

**BSN272 reduces VLDL and LDL in LDLr<sup>-/-</sup> mice**

The amount of VLDLs, LDls, and HDLs were determined by collecting serum nine weeks after the treatments began, resolving the lipoprotein complexes by FLPC, and quantifying the amount of cholesterol in each FPLC fraction using an enzymatic cholesterol assay. A 25% trimmed mean FPLC chromatogram was calculated using the total cholesterol values from the enzymatic cholesterol assay. BSN272 reduced VLDL and LDL by 35% and 17%, respectively (p<0.05). HDLs were not significantly altered by treatment (Illustration 10).

**BSN272 reduces atherosclerotic lesions in LDLr<sup>-/-</sup> mice**

Aortic arches were harvested 9 weeks after treatments began and the amount of atherosclerotic lesions was determined by false color imaging (Illust. 11 inset; red areas indicate locations of the lipid deposits). Lesion area was measured as a fraction of the aortic arch area. The percent of atherosclerotic lesions in the BSN272 treated group was determined by dividing the mean atherosclerotic lesions area in the Glu/Fruc/BSN272 group by the mean atherosclerotic lesion area in the Glu/Fruc group. BSN272 in the diet reduced atherosclerotic lesions to less than one-half of their original level.

**Factor Analysis Results for the LDLr<sup>-/-</sup> Mice**

The use of oral gavage doses orthogonalized by transformation to principal axes permits the efficacy of each molecule (D-tagatose and polydatin) to be calculated in the presence of the other. The BSN272 combination is the most effective of the treatments for lowering triglycerides.

- Both glucose and fructose raise triglycerides.
- D-tagatose lowers triglycerides by -3.1 mg/dl per g/kg/dose of the sugar in the combination, while the polydatin lowers triglycerides by -372 mg/dl per g/kg/dose of the drug in the combination.

The polydatin/tagatose combination (BSN272) is the most effective of the treatments for lowering cholesterol.

- Both glucose and fructose raise cholesterol.
- D-tagatose lowers total cholesterol by -3.9 mg/dl per g/kg/dose of the sugar in the combination, while the polydatin lowers total cholesterol by -629 mg/dl per g/kg/dose of the drug in the combination.
• Paradoxically, in some animal models, D-tagatose and polydatin can raise serum triglycerides. Polydatin administered alone in the Syrian Golden hamster on Western diet increases serum triglycerides. D-tagatose administered alone in the Syrian Golden hamster on Western diet increases serum triglycerides. Polydatin co-administered with D-tagatose (BSN272) in the Syrian Golden hamster on Western diet decreases serum triglycerides. Unlike the LDLr<sup>-/-</sup> mouse, the hamster has cholesteryl ester transfer protein (CETP), similar to humans. CETP transports cholesteryl esters and triglycerides between the lipoproteins. CETP can pick up triglycerides from very-low-density (VLDL) or low-density lipoproteins (LDL) and swap them for cholesteryl esters from high-density lipoproteins (HDL), and vice versa.

**BSN272 Results Summary**

- Serum triglycerides (TG) were reduced by almost one-half. However, there was also a reduction in TG in mouse on water treatment, making it difficult to conclude that TG reduction is treatment related in the LDLr<sup>-/-</sup> mouse.
- Reduction on VLDL cholesterol was the next largest, followed by the reduction in LDL cholesterol.
- Reduction in TG, VLDL and LDL may explain the reduction in atherosclerotic lesion area in the aortic arch.

**Conclusions**

This study was designed to compare the effects of D-tagatose alone and in BSN272 on the levels of cholesterol and triglycerides and on preventing atherosclerosis in LDLr<sup>-/-</sup> mice. LDLr<sup>-/-</sup> mice maintained on a high-fat diet provide a model of hypercholesterolemia with somewhat elevated plasma cholesterol<sup>37</sup>. In addition, Zadelaar et al.<sup>36</sup> found that the lipoprotein profile in LDLr<sup>-/-</sup> mice closely mimics that of humans, with the cholesterol mainly tied up in the LDL fraction. LDLr<sup>-/-</sup> mice were used to study the effects of BSN272 versus D-tagatose alone on lipid levels in these mice, and to compare the LDLr<sup>-/-</sup> model with results provided by the apoE<sup>-/-</sup> model used in other published studies<sup>39</sup>.

A diet that was supplemented with Glucose/Fructose and D-tagatose or BSN272 produced no significant change in the food intake or body weight of LDLr<sup>-/-</sup> mice. However, free fatty acids and lipids, including triglycerides and total cholesterol, significantly decreased in mice given BSN272. Serum triglycerides (TG) were cut almost in half. LDL and VLDL, but not HDL, levels were also decreased. Not surprisingly, aortic atherosclerotic lesions were reduced by 57%, as BSN272 reduced the amount of lipids moving through the blood.

BSN272 differs from Lovaza and other omega-3-acid ethyl esters in that it not only reduces triglycerides it also reduces LDLs. Triglyceride levels have been found to be particularly important to women as the Farmingham Study found women with triglyceride levels below 50 mg/dl have one the highest rates of coronary heart disease (CHD)<sup>100</sup>. Various studies have found that Omacor does significantly reduce...
mean triglyceride concentrations, including the Harris study (1997)\textsuperscript{106} which reported a 45% reduction in triglycerides, increased HDL cholesterol by 13% and LDL cholesterol by 31%, dosed at 3.4 g eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) and 18 mg vitamin E per day. Additionally, the Pownall (1999) study\textsuperscript{107} reported a 38.9% decrease from baseline fasting triglyceride levels and an increase in HDLs of 5.9% and an increase in LDLs of 16.7%. EPA and DHA inhibit acyl-COA, resulting in decreased triglyceride synthesis, while increasing fatty acid metabolism, and increasing lipase production which results in increased triglyceride binding\textsuperscript{108}.

Foam cell formation is one of the early steps in the process of atherosclerosis\textsuperscript{109}. Free metal ions can help to oxidize the LDL and convert it into a form that can be taken up by macrophages. BSN272 seems to prevent LDL oxidation and may prevent oxidation caused by macrophages at the atherosclerotic site.

Although glucose and fructose had modest effects on serum lipids and body weight over the course of the study, the levels of serum cholesterol and triglycerides in the groups receiving glucose, fructose, and D-tagatose, and glucose, fructose, D-tagatose, and BSN272 were consistently equal to or less than the levels in the groups receiving either water or glucose and fructose. Furthermore, the combination of D-tagatose and polydatin appeared to be more potent than D-tagatose alone in reducing serum cholesterol and triglycerides over the course of the experiment.

D-tagatose and polydatin also prevented the increase in serum cholesterol induced by glucose and fructose, and was capable of reducing atherosclerosis in LDLr-deficient mice.

Importantly, the sera collected on days 14, 22, 36, 50 and 64 were from fed (not fasted) mice, making it difficult to clearly define a role for D-tagatose and polydatin on the metabolism of endogenous lipids. However, considering that the levels of serum triglycerides and cholesterol in the mice treated with either glucose, fructose, and D-tagatose or glucose, fructose, D-tagatose, and BSN272 were always less than or equal to those in mice receiving either water or mixtures of glucose and fructose and that blood samples were collected one hour after gavaging, these results suggest that supplementing high carbohydrate meals with D-tagatose or combinations of D-tagatose and BSN272 may be effective at reducing postprandial carbohydrate-induced hyperlipidemia and lowering serum cholesterol and triglycerides over time.
Illustrations

Illustration 1

Illustration 1

Study Design

Animals were dosed with test articles 2x/day (~8 to 12hrs between doses)
Blood lipids were measured biweekly
n=10 per group

Illustration 2

Illustration 2

<table>
<thead>
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<th>Group</th>
<th>Test Article</th>
<th>Dose g/kg/dose</th>
<th>No. of animals</th>
<th>Animal Numbers</th>
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<td>Water</td>
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<td>10</td>
<td>1-10</td>
</tr>
<tr>
<td>2</td>
<td>Glucose (50%) + Fructose (50%)</td>
<td>See Illust. 3</td>
<td>10</td>
<td>11-20</td>
</tr>
<tr>
<td>3-12</td>
<td>Glucose + Fructose + D-tagatose</td>
<td>See Illust. 3</td>
<td>10</td>
<td>21-30</td>
</tr>
<tr>
<td>15-22</td>
<td>Glucose + Fructose + BSN272</td>
<td>See Illust. 3</td>
<td>10</td>
<td>31-40</td>
</tr>
</tbody>
</table>
Illustration 3

Illustration 4

Illustration 4. Food consumption during the 14 day D-tagatose lead-in period during which animals in Groups 3 and 4 were given increasing amounts of D-tagatose each day to acclimate them to the sugar. Lead in chow was powdered for the Glucose/Fructose/Tagatose and Glucose/Fructose/BSN272 groups to simplify mixing, leading to lower consumption.
Illustration 5

Food intake measured by weight of food eaten per day was the same for all four groups after the 14 day D-tagatose lead-in period.

Illustration 6

Body weights of mice during the course of the study.
Illustration 7

Illustration 7. Total Serum Cholesterol. The total serum cholesterol of fed mice treated with D-tagatose or BSN272 is always lower than for mice treated with glucose/fructose or even water.

Illustration 8

Illustration 8. Serum Triglycerides. The total serum triglycerides of mice treated with D-tagatose or BSN272 is always lower than for mice treated with glucose/fructose or even water.
Illustration 9

Illustration 9. Free fatty acids. There is no statistically significant difference between the D-tagatose and BSN272 respective groups at days 14 and 78.

Illustration 10

Illustration 10. A) Effects of combination of D-tagatose and BSN272 on atherogenesis in LDL- deficient mouse atherosclerotic lesion area was determined by false color imaging and the results were normalized to dividing the mean atherosclerotic lesion area in the Glucose+Fruuctose+BSN272 groups by the Glucose+Fruuctose group. (B) Trimmed mean VLDL, LDL, and HDL FPLC chromatograms at 3 Weeks. (C) Results calculated from FPLC chromatograms treated with BSN272 significantly reduces VLDL and LDL, while leaving HDL essentially unchanged.
Illustration 11

Illustration 11. The group treated with the BSN272 combination has the lowest atherosclerotic lesion area.