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Potential Beneficial Health Effects of a Grape Seed Procyanidin Extract (GSPE): Results From a Mouse Model

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This publication highlights the underlying molecular actions of a grape seed extract in a mouse model that lead to a significant reduction in serum cholesterol and triglycerides levels, two important risk factors associated with the development of cardiovascular disease. Although results from animal studies are not directly translatable to humans, the research provides new insight and compelling evidence suggesting that further investigations are warranted in order to determine whether grape seed extract, as a supplement, exerts similar effects in humans.

Introduction

This publication summarizes ongoing USDA-funded research in the Department of Agriculture, Nutrition and Veterinary Sciences, at the University of Nevada, Reno, aiming to further our understanding regarding the beneficial health effects associated with consumption of a grape seed procyanidin extract (GSPE). Laboratory-based research to date in mice suggests that GSPE may play an important role in mitigating a variety of human health problems; including, but not limited to, reducing serum cholesterol and triglyceride levels, two important risk factors associated with the development of cardiovascular disease. **Table 1** provides a collection of terms, abbreviations and definitions useful in understanding the results presented.

Table 1: Terms, abbreviations and definitions.

Term	Abbreviation	Definition
Bile Acid	BA	A primary component of bile that is made from cholesterol and secreted into the intestine to assist in dietary fat digestion and absorption
Catabolism		The breakdown in the body of complex large molecules into smaller ones
Complementary and Integrative Medicine		A group of diagnostic and therapeutic disciplines that are used together with conventional medicine
Cholesterol	CHOL	A lipid found in most human tissues, including blood, that is important in a variety of bodily functions
Dyslipidemia		A condition in which total or low-density lipoprotein (LDL) cholesterol levels are elevated, serum triglyceride levels are increased, and high-density lipoprotein (HDL) levels are reduced in the blood. These are important risk factors for coronary heart disease and stroke
Enterohepatic bile acid recirculation		Refers to the movement of bile acids and other substances from the liver into bile and then into the gall bladder, followed by release into the small intestine. Bile acids not used in digestion and absorption in the upper (proximal) portion of the small intestine are reabsorbed in the lower (distal) portion of the small intestine and transported back to the liver via the portal vein for subsequent reuse
Grape Seed Procyanidin Extract	GSPE	An extract made from the seeds of white grapes, *vitis vinifera*, that contains a high concentration of procyanidins
Homeostasis		The tendency of the body to maintain a condition of balance/stability or equilibrium within its internal environment
Hypercholesterolemia		Elevated cholesterol levels in the blood
Hypertriglyceridemia		Elevated triglyceride levels in the blood
In vivo		A biological process taking place within a living organism
Lipid		A class of compounds that includes triglycerides and cholesterol
Lipogenesis		The transformation of non fat foods (e.g. glucose) into fat and triglyceride
Procyanidins		Water-soluble plant pigments
Triglyceride	TG	A type of lipid commonly known as fat

Chronic human health issues are the driving force for lab-based research

Several chronic human health issues, including cardiovascular disease, obesity and the metabolic syndrome, are a constant burden to ever-increasing medical costs in the United States. Investigations designed to identify dietary components that may help to alleviate or prevent such disorders are an important area of active research. Increased knowledge regarding mechanisms by which dietary compounds function in the body may lead to additional dietary recommendations to help maintain overall health.

Cardiovascular disease

Cardiovascular disease is currently the leading cause of death in the U.S. and around the world (World Health Organization, 2014). It includes a number of conditions that affect the structure or function of the heart, such as coronary artery disease, also known as atherosclerosis, or disease of the muscle within the heart, termed cardiomyopathy. Atherosclerosis occurs when the arteries that supply vital oxygen and nutrients to the heart become hard, due to the build-up of fat (cholesterol). Elevated serum cholesterol, and mild-to-moderate increased serum triglyceride levels, are important risk factors associated with the development of cardiovascular disease (Berglund et al., 2012). It is estimated that 90 percent of cardiovascular disease incidences are preventable (McGill, McMahan, & Gidding, 2008) by eating a healthy diet, engaging in regular exercise, and avoiding excessive alcohol consumption and smoking.

Obesity

Obesity is a complex disorder involving an excessive amount of body fat. Obesity increases the risk of diseases and health problems, including heart disease, diabetes, high blood pressure and certain types of cancer. It is reported that 34.9 percent or 78.6 million adults in the United States are obese (Ogden, Carroll, Kit, & Flegal, 2014).

Metabolic syndrome

Metabolic syndrome is the name for a group of risk factors that increase the risk for heart disease and other health problems, including diabetes and stroke. The risk factors include:

- **Central (or abdominal) obesity (a large waistline):** Excess fat in the stomach area is a greater risk factor for heart disease compared to the presence of excess fat in other parts of the body, such as the hips.
- **High triglyceride levels in the blood:** High triglycerides may contribute to hardening of the arteries or thickening of the artery walls (atherosclerosis), which leads to an increased risk for stroke, heart attack or heart disease.
- **Low *High Density Lipoprotein* (HDL) cholesterol levels:** HDL is often referred to as *"good"* cholesterol since it helps to remove cholesterol from arteries. Therefore, *low* HDL levels increase the risk for heart disease.
- **High blood pressure:** Blood pressure is the force of blood pushing against artery walls as the heart pumps blood. If this pressure rises and stays high over time, it can damage the heart.
- **High fasting blood sugar levels:** High blood glucose levels can pose a significant risk over both the short and long term. Since every cell in the body requires glucose, long-term problems include the risk for developing eye disease, heart attacks, strokes and kidney disease.

An individual's risk for heart disease, diabetes and stroke increases with the presence of an increasing number of these particular metabolic syndrome risk factors. The risk of having metabolic syndrome is closely linked to being overweight and obese and a lack of physical activity. The terms *overweight* and *obesity* refer to a body weight greater than that considered to be healthy for a certain height. The usual measurement indicating whether an individual is overweight or obese is body mass index (BMI), a measure of body fat based on height and weight. The table below shows the standard weight categories associated with BMI ranges for adults (Centers for Disease Control):

Body Mass Index (BMI)	Weight Status
Less than 18.5	Underweight
18.5 - 24.9	Normal/Healthy
25.0 – 29.9	Overweight
Greater than 30.0	Obese

Triglycerides, cholesterol and bile acids

Triglycerides are a type of fat found in the blood, and triglyceride levels are an important indicator of heart health. When a meal is eaten, the body converts any unneeded calories into triglycerides, which are then stored in fat cells. Later, hormones help to release the triglycerides for use as energy between meals. If more calories are consumed, particularly from carbohydrates or fat, than are expended on a regular basis, they will be converted into triglycerides and this may lead to a condition called hypertriglyceridemia (high levels of triglycerides within the blood). Hypertriglyceridemia is a condition often associated with atherosclerosis (buildup of plaque in the arteries) and cardiovascular disease.

Cholesterol is a waxy substance that comes from two main sources, either your body or from the food you eat. Inside the body, the liver makes cholesterol and circulates it through the blood. Cholesterol also comes from animal sources, such as meat, poultry and full-fat dairy products.

Within the body, cholesterol levels must be tightly regulated and are kept relatively constant through the following bodily functions:

- Regulation of production within the liver;
- Regulation of the amount absorbed from the diet; and
- Regulation of the amount broken down into bile acids (Lu et al., 2000), the main way to eliminate cholesterol from the body.

Bile acids are important in digestion for the efficient absorption of dietary fats and fat-soluble vitamins, such as A, D, E and K (Lefebvre, Cariou, Lien, Kuipers, & Staels, 2009), and they also function as signaling molecules. Bile acids control their own uptake, transport and production in the liver, and are important for maintaining the appropriate levels of cholesterol and triglyceride in the body (Goodwin et al., 2000; Makishima et al., 1999; Watanabe et al., 2004). Consequently, altering bile acid-related pathways has become an attractive target for treating conditions such as hypercholesterolemia (high blood cholesterol levels) and hypertriglyceridemia (high blood triglyceride levels).

Although we need cholesterol as a building block, excess cholesterol levels can be problematic. It can form plaque between layers of the artery walls, which will cause the heart to work harder in order to circulate blood around the body. Plaque can break open and cause blood clots. If a clot blocks an artery supplying blood to the heart, it will cause a heart attack; and if a clot blocks an artery supplying blood to the brain, it will lead to a stroke.

Although triglycerides store unused calories and provide the body with an easily accessible energy source, and cholesterol is important to build cells and make certain hormones, neither can dissolve in the blood, and instead circulate throughout the body with the help of proteins that transport the lipids, which are called lipoproteins. Although we need cholesterol as a building block, excess cholesterol levels can be problematic. It can form plaque between layers of the artery walls, which will cause the heart to work harder in order to circulate blood around the body. Plaque can break open and cause blood clots. If a clot blocks an artery supplying blood to the heart, it will cause a heart attack; and if a clot blocks an artery supplying blood to the brain, it will lead to a stroke.

Regulation of triglyceride and cholesterol homeostasis and the role of bile acids

As mentioned above, bile acids are produced from cholesterol within the liver (Lefebvre et al., 2009). They are then released into the gall bladder where they are stored. After eating a meal, the bile is released and travels to the small intestine, where it aids in the absorption of dietary fats and fat soluble vitamins. Ninety-five percent of the bile acids not used in the process of digestion and absorption are transported back to the liver via the portal vein, in a process termed *enterohepatic recirculation*. The remaining five percent are excreted in the feces; and that five percent is then replaced by conversion of cholesterol into bile acids within the liver. Bile acids recirculate from the intestine to the liver about four to 12 cycles per day (Lefebvre et al., 2009; Lu et al., 2000), as shown in **Figure 1**.

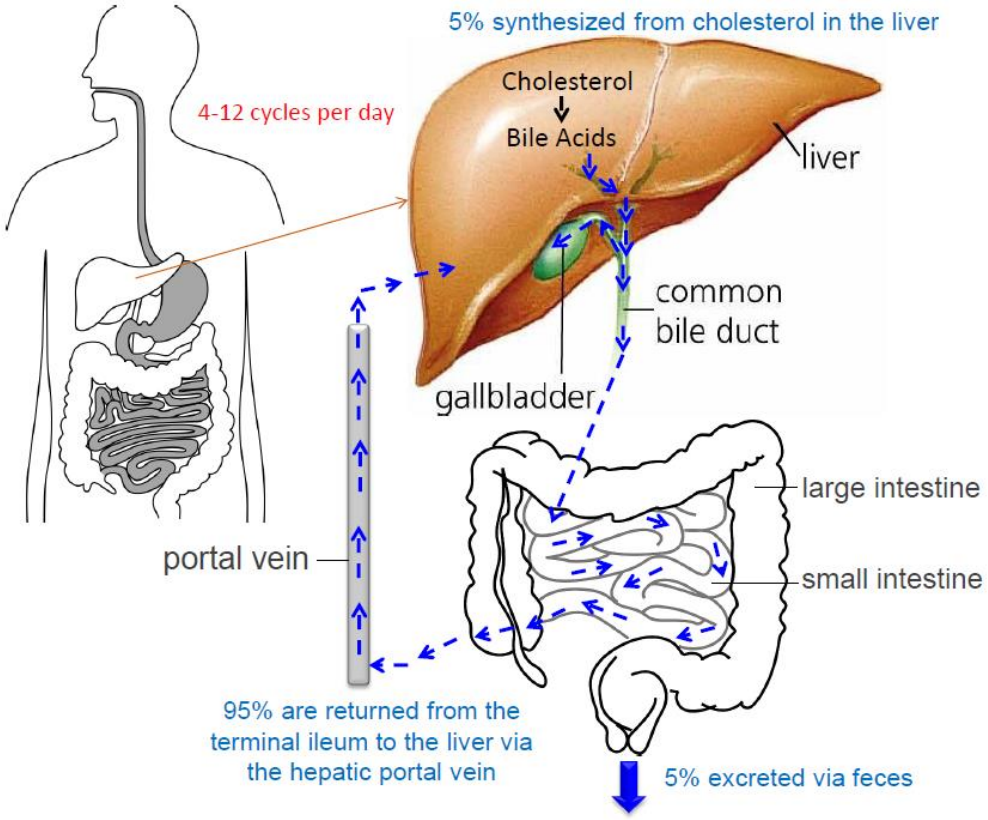

Figure 1: Enterohepatic recirculation of bile acids.

High cholesterol is one of the major controllable risk factors for cardiovascular disease, which includes coronary heart disease potentially leading to heart attack and stroke. Dietary and lifestyle modifications are often initial ways to treat dyslipidemia (characterized by high total or LDL cholesterol and triglycerides and low HDL levels) (Pejic & Lee, 2006), but patients may subsequently

be prescribed pharmaceutical drugs to treat these disorders. Complementary and integrative medicine therapies, including plant-derived extracts, are popular alternatives for a variety of conditions, including dyslipidemia. Identifying ways in which natural products and bioactive dietary components exert beneficial effects against cardiovascular disease risk factors, including triglycerides and cholesterol, is an important area of active research.

Dietary procyanidins protect against Cardiovascular disease-associated risk factors

Procyanidins are water-soluble plant pigments that are naturally present in vegetables, fruits and beverages such as tea and wine. Diets rich in fruits and vegetables tend to be high in flavonoids, and as such, exert cardioprotective effects in humans (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog et al., 1995). Dietary procyanidins, a class of flavonoids commonly found in grapes, apples and red wine, have been shown to ameliorate risk factors associated with MetS (Kastorini et al., 2011; Rasmussen, Frederiksen, Krogholm, & Poulsen, 2005). Research conducted in this laboratory focuses on identifying the ways in which a grape seed *procyanidin* extract (GSPE) functions. A rich source of procyanidins, GSPE is easily absorbed in the digestive tract and taken into the body. It has shown an ability to decrease serum triglyceride levels in mice (Del Bas et al., 2008; Del Bas et al., 2009; Heidker, Caiozzi, & Ricketts, 2016a, 2016b) and rats (Del Bas et al., 2005; Downing et al., 2015; Quesada et al., 2009), making it an attractive natural treatment for lowering triglyceride levels.

The main purpose of this lab-based research:

The research presented here aimed to further determine the underlying mechanisms used by GSPE to lower serum cholesterol and triglyceride levels in a mouse model (Heidker et al., 2016a). The research presented herein is a condensed version of the full scientific research report, which can be found at the Journal website:http://onlinelibrary.wiley.com/doi/10.1002/mnfr.201500795/full.

Methods

The experimental design consisted of laboratory mice (C57BL/6 strain: wild type, WT) or mice lacking the farnesoid x receptor ($Fxr^{-/-}$) that were fed with a standard rodent chow (a diet formulated to deliver constant and complete nutrition throughout the life cycle) for the first eight weeks of life. After eight weeks, the mice were administered with either water as a control (VEH) or 250 mg/kg body weight GSPE for 14 hours. After treatment overnight, intestine, liver and blood specimens were collected from the mice for subsequent analysis to determine gene and protein changes and the effects on serum parameters, including cholesterol and triglycerides.

Based on metabolic comparison between mice and humans, the dose of GSPE used in this study is equivalent to ~703 mg procyanidins per day in a 60-kg human (Clifton, 2004). The average procyanidin intake by adults in the United States is reported to be 95 mg per day (Wang, Chung, Song, & Chun, 2011). However, in comparison, the average intake in Finland ranges from 448 to 1278 mg per day (Ovaskainen et al., 2008), suggesting that a substantial increase in procyanidin intake can feasibly be achieved by consuming more procyanidin-rich fruits and vegetables.

Results

Effects on serum cholesterol and triglyceride levels

GSPE markedly reduced serum triglyceride levels by approximately 28 percent, compared to the water-treated mice (**Figure 2A**). Total serum cholesterol levels were also significantly reduced after

treatment with GSPE (**Figure 2B**). Both of these beneficial lipid-lowering effects were absent in mice lacking the farnesoid x receptor ($Fxr^{-/-}$), demonstrating the importance of Fxr in the actions of GSPE. Compared to the decrease observed in the presence of GSPE, fenofibrate, a commonly prescribed pharmaceutical drug used to treat dyslipidemia, lowers serum triglyceride levels by 36 percent (Birjmohun, Hutten, Kastelein, & Stroes, 2005; Moutzouri, Kei, Elisaf, & Milionis, 2010).

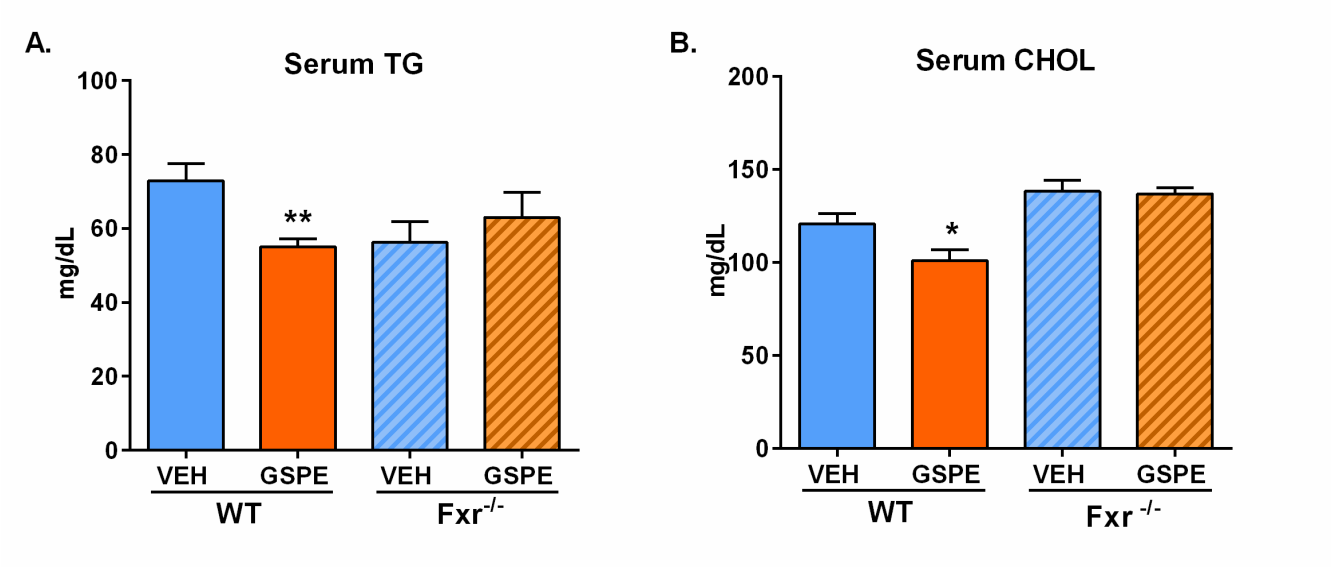

Figure 2: GSPE reduces serum triglyceride and cholesterol levels in wild type but not $Fxr^{-/-}$ mice *in vivo.* TG: Triglycerides. CHOL: Cholesterol. (n=6 per treatment, per group, in triplicate). Statistical significance: *p<0.05, and **p<0.01. Reproduced with permission from (Heidker et al., 2016a).

Decreased serum triglyceride and cholesterol levels correlated with decreased serum bile acid levels and increased fecal bile acid excretion

Further analysis revealed that the levels of bile acids in the serum were significantly reduced by treatment with GSPE in wild-type mice, which coincided with increased excretion of bile acids in the feces. These effects were absent in the $Fxr^{-/-}$ mice, indicating that this receptor is required for the effects observed following GSPE administration.

Gene expression

The expression of genes involved in bile acid reabsorption and transport in the intestine were decreased following GSPE treatment. The results show that GSPE reduces intestinal bile acid absorption, via altering gene expression, which then leads to an increase in the amount of bile acids excreted in the feces. The increased loss of bile acids in the feces leads to an increase in the conversion of cholesterol into bile acids in the liver, in order to maintain appropriate levels of bile acids within the body (Heidker et al., 2016a).

In addition, the expression of genes involved in making triglycerides in the liver was decreased, indicating that GSPE inhibited the production of triglycerides within the liver (Heidker et al., 2016a).

Key findings from this research study:

Laboratory results using a mouse model demonstrate that:

- GSPE exerts a triglyceride-lowering effect by inhibiting the reabsorption of bile acids in the intestine.
    - This is dependent on the presence of Fxr.
- Inhibiting re-uptake of bile acids in the intestine leads to:
    - A reduced number of bile acids being returned to the liver,
    - Reduced serum bile acid levels, and
    - Increased excretion of bile acids in the feces.
- The decrease in the amount of bile acids returning to the liver causes triglycerides to be broken down to provide building blocks to make more cholesterol.
- This newly made cholesterol is then used to make bile acids to replace those lost in the feces. (See **Figure 3.**)

The results from this laboratory research provide important insight into the actions of GSPE at the molecular level, and help to increase our understanding regarding the potential beneficial health effects of procyanidins found in grape seed extract in particular. Studies using mouse models help to identify underlying mechanisms by which GSPE lowers triglyceride levels, an important risk factor to both MetS and cardiovascular disease in humans.

Furthermore, understanding the mechanisms by which natural products work in order to mediate beneficial health effects will help in designing future human-based clinical trials aimed at identifying new complementary therapies for metabolic diseases and related health disorders. The results of clinical trials may help educate the American public regarding the inclusion of dietary components, such as procyanidins, beneficial to achieving optimal health.

Understanding how natural products may benefit health in mice helps in the design of human-based clinical trials aimed at identifying new complementary therapies for metabolic diseases and related health disorders. The results of clinical trials will educate health professionals and health educators about the benefits of natural procyanidins present in the diets that can aid in achievement of optimal health.

Future studies should include additional lab-based research to fully determine the mechanisms by which GSPE lowers serum triglyceride levels and exerts other beneficial health effects. In addition, studies using rodent models of disease will aid in the identification of the effectiveness of GFSPE as a potential treatment for human metabolic disorders. Ultimately, well-designed human clinical trials should be conducted in order to determine the efficacy of natural products such as GSPE for the treatment of risk factors associated with cardiovascular disease and MetS.

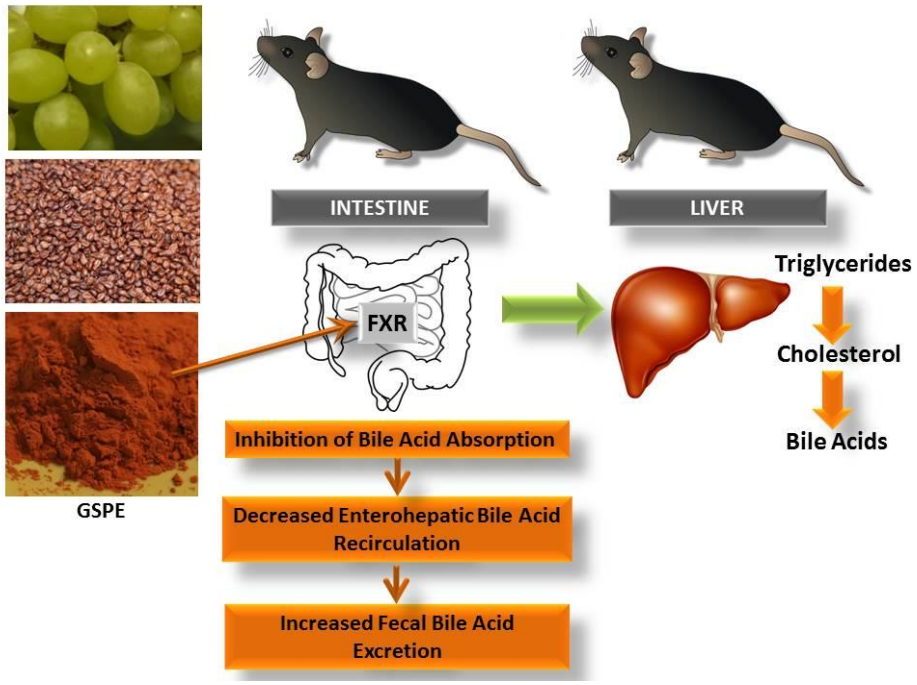

Figure 3: GSPE inhibits the absorption of bile acids in the intestine, leading to decreased serum cholesterol and triglyceride levels. Grape seed procyanidin extract (GSPE) decreases the reabsorption of bile acids in the intestine, leading to a reduced number of bile acids going back to the liver, reduced serum bile acid levels and increased excretion of bile acids in the feces. This collectively causes increased production of bile acids in the liver. The increased need for bile acid production leads to a decline in cholesterol stores within the liver, which initiates the breakdown of triglycerides to allow increased production of cholesterol. Overall, these effects contribute to the mechanism underlying the triglyceride- and cholesterol-lowering action of GSPE.

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