# THE EFFECT OF RICE BRAN OIL AND SAFFLOWER OIL ON SERUM LIPIDS IN THE RAT

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BY

LILIANA A. AGUILAR

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DENTON, TEXAS

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TEXAS WOMAN'S UNIVERSITY

DEPARTMENT OF NUTRITION AND FOOD SCIENCES
COLLEGE OF HEALTH SCIENCES
DENTON, TEXAS

December 15, 1994
Date

To the Associate Vice President for Research and Dean of Graduate Studies:

I am submitting herewith a thesis written by Liliana A. Aguilar entitled "The Effect of Rice Bran Oil and Safflower Oil on Serum Lipids in the Rat." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

John D. Radcliffe

John D. Radcliffe, Ph. D., R.D., Major Professor

We have read this thesis and recommend its acceptance:

Cindi M. Hsieh

Dorice M Narins

Accepted:

Dr. Dorice M Czajka Narins

Dr. Dorice M. Czajka-Narins, Chair

G. Ann Uhlir

Dr. G. Ann Uhlir, Dean

Leslie M Thompson

Associate Vice President for Research and Dean of Graduate Studies

ABSTRACT

The Effect of Rice Bran Oil and Safflower Oil
on Serum Lipids in the Rat.

Aguilar Liliana A.

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This study examined the effect of dietary rice bran oil and safflower oil on serum levels of cholesterol, phospholipids, and triglycerides in the male Sprague-Dawley rat. One group of 7 animals received a 10% rice bran oil diet, and a second group of 7 animals received a 10% safflower oil diet. Both groups of rats were fed the diets for a 21 day period. Type of oil had no effect on either food intake or growth. Animals fed safflower oil had non-significantly lower serum levels of all these lipid fractions measured, i.e., cholesterol, triglycerides and phospholipids. Serum cholesterol levels were 9% lower, serum phospholipids were 10.4% lower, and serum triglycerides were 37.5% lower. The results, for this particular strain of rat, indicate that dietary safflower oil shows a trend towards giving lower serum lipid levels than rice bran oil.

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# CHAPTER I

## Introduction

For many years, research has been done on cholesterol and cardiovascular disease in order to establish a link between blood cholesterol level and the development of coronary atherosclerosis. By the mid 1960's, researchers confirmed the transportation of blood cholesterol in lipoproteins (Steinberg,1987). These particles are considered to play a critical role in the development of atherosclerosis. Low density lipoprotein (LDL) cholesterol is the major carrier of cholesterol in the plasma (Steinberg, 1987). Research supports a direct relationship between the plasma LDL level and the development of atherosclerosis. Studies have shown that Americans who have high serum concentrations of cholesterol (over 220 mg/dL) are more likely than people with lower levels to develop cardiovascular heart disease (CVD). Populations, such as the Japanese, who have lower serum cholesterol levels than people in the United States, have a lower incidence of CVD. Increased serum cholesterol levels are associated with changes in dietary habits and decreased physical activity (Omae, 1990). These changes in dietary habits include an increase in the intake of dietary fat. For example, a study by Grundy (1987) demonstrated an association between the level of serum and dietary fat intake. There has been a great interest in dietary factors that lower serum cholesterol. These

include a diet low in saturated fats, a decrease in energy intake, and a decrease in the intake of dietary cholesterol and animal protein. In addition, it has been recently shown that plant-derived products (e.g., as psyllium, oat bran, and rice bran) can lower serum cholesterol. The active principle of the latter is thought to be the unsaponifable fraction of the oil contained in the rice bran. Bran is 12% to 25% oil by weight, depending on the quality of bran.

Rice bran oil (RBO) is desirable for human consumption. It has better keeping qualities than other refined oils, such as palm oil. These keeping qualities are due to its high content of tocopherol ("vitamin E"). Also, RBO has a high smoke point, making it acceptable as an oil for frying. RBO contains 80%-85% unsaturated fatty acids, principally as oleic acid (a monounsaturated fatty acid) and linoleic acid (a polyunsaturated fatty acid).

Further studies involving the use of RBO will help establish its cholesterol lowering action. This study compares dietary RBO to safflower oil (SFO) with respect to its effects on serum cholesterol, phospholipids and triglyceride because the effect of RBO on these parameters has not been compared to that of polyunsaturated oils, such as SFO, which are recommended by the American Heart Association as part of a diet designed to lower serum cholesterol.

## Statement of the Problem

The question to be answered by this study is as follows: What is the effect of rice bran oil, versus that of safflower oil, on serum levels of cholesterol, triglycerides (TG) and serum phospholipids?

## Null Hypotheses

In this study, the null hypotheses will be:

1. There will be no effect of diet (rice bran oil vs. safflower oil) on serum cholesterol levels in male Sprague-Dawley rats.
2. There will be no effect of diet on serum phospholipid levels in male Sprague-Dawley rats.
3. There will be no effect of diet on serum triglyceride levels in male Sprague-Dawley rats.

# CHAPTER II

## Review of the Literature

### Rice Bran Oil

The source of RBO is rice bran, which is the coating removed from brown rice during processing (Gupta, 1989). Rice bran contains 12% to 25% oil, depending on the quality and polishing of the bran. A better quality of rice bran is produced by parboiling (pre-cooking of rice within the husk) the rice paddy after milling, which gives a higher yield of oil (approximately 16%), and the oil contains less free fatty acids. The above process is desirable because otherwise the lipases, which are natural to the bran, will promote hydrolysis and rapid deterioration of the oil by splitting it into glycerol and free fatty acids. Table 1 gives a list of the fatty acids found in RBO and compares the composition of SFO and RBO. Gupta (1989) states that the refined grade of RBO used for cooking purposes has a good flavor and is rich in lipids and vitamin E. It also has a long shelf life,and this is attributed to the natural antioxidant (tocopherol) RBO contains. The refined grade of RBO contains 80%-85% unsaturated fatty acids and has a flashpoint of 250° Centigrade. The unsaponifiable components of RBO contain triterpene alcohols, such as oryzanol, tocotrienol (a form of vitamin E) and phytosterols, which are fat-like alcohol found in plants, such as compesterol, stigmasterol, and b-sitosterol. Oryzanol can be broken down further

Table 1

Major Fatty Acids of Rice Bran Oil and Safflower Oil Dietary Oils (%)

| Fatty-acid chain length | Lauric C<12 | Lauric C12 | Myristic C14 | Palmitic C16 | Stearic C18:0 | Oleic C18:1 | Linoleic C18:2 | Linolenic C18:3 |
|---|---|---|---|---|---|---|---|---|
| Rice bran oil | - | - | - | 17 | 2 | 42 | 36 | 2.0 |
| Safflower oil | - | - | - | 6 | 2 | 10 | 83 | - |

Note: Based on representative values from the literature oleic, linoleic and linolenic are the only unsaturated fatty acids listed above.

into cycloartenol (CA) and 24-methylene cycloartenol. These components are found in greater amounts in RBO than in safflower oil or olive oil. Rice bran oil contains 0.48% CA and 0.49% 24-methylene cycloartenol, whereas safflower contains 0.034% CA and 0.007% 24-methylene cycloartenol (Rukmini, 1991). Rice bran oil's qualities and its effect in lowering cholesterol have made it a desirable oil. In Japan, RBO is used in mayonnaise and snack foods, such as potato chips. In Taiwan, RBO is used for cooking oil and salads (Gupta, 1989).

Cholesterol

Cholesterol is a fat-like substance which is a major constituent of cell membranes and a precursor of steroid hormones, bile acids, and vitamin $D_3$. It is produced endogenously by the liver and obtained exogenously from the diet. Cholesterol is carried in the blood in particles containing both lipids and proteins, and these are called lipoproteins. There are five classes of lipoproteins: chylomicrons, very low density lipoproteins (VLDLs), intermediate density lipoproteins, LDLs and HDLs. Chylomicrons transport most of the dietary TG to the peripheral tissues and contain mostly TG (80-95%); VLDLs transport endogenous TG to adipose tissue and contain 50-80% TG and 10-40% total cholesterol. Intermediate lipoproteins are precursors of LDL; LDLs contain 45% total cholesterol, most of which is esterified cholesterol and apoprotein B-100 (apo B-100). Low density lipoprotein cholesterol delivers cholesterol to the liver

and peripheral tissues. Uptake of LDL cholesterol is facilitated by the LDL receptor's recognition of apo B-100. Low density lipoprotein cholesterol is the major atherogenic class of lipoproteins containing approximately 60-70% of total plasma cholesterol; HDL cholesterol seems to be anti-atherogenic because of its role in transporting cholesterol from the peripheral tissues to the liver (Expert Panel, National Cholesterol Education Program, 1993).

The National Research Council Diet and Health report as well as other major publications on disease prevention have confirmed that, along with obesity, excessive dietary intakes of saturated fatty acids and cholesterol are major factors responsible for high blood cholesterol levels (>220 mg/dL) that lead to coronary heart disease (CHD) (The Cholesterol Facts, 1990). The landmark study by the Lipid Research Clinic (1984) demonstrated that lowering the levels of plasma cholesterol decreased the risk of CHD. There is consistent evidence that supports and confirms the correlation between high total serum cholesterol and the development of CHD. Stehbens (1992) states that CHD usually represents atherosclerotic myocardial disruption, with 90% of the CHD cases being attributed to atherosclerosis. Approximately 5 million Americans are suffering from CHD, and as many women as men die from the disease. Coronary heart disease accounts for nearly 250,000 deaths annually in women.

In a study carried out by Wild, Conner, S., Sexton, and Conner, W. (1993), comparable dietary intakes of cholesterol and saturated fat were seen in France and Finland. Despite this, Finland had a higher CHD mortality rate in men aged 55 to 59. The researchers obtained the information from the annual vital statistics issued by the World Health Organization and the Food and Agriculture Organizations of the United Nations. In order to examine dietary variables associated with CHD other than from cholesterol and saturated fat, a Cholesterol Saturated Fat Index (CSI) was calculated. The index was computed by using partial correlation coefficients between CHD and diet variables.

In the study, the CSI values for Finland and France were similar, even though Finnish subjects consumed more milk and butterfat than what was consumed by French subjects. In France, four times more vegetables and vegetable oils containing unsaturated fatty acids were consumed. This study indicates that vegetables and vegetable oils containing monounsaturated and polyunsaturated fatty acids to be protective against CHD. Alcohol was not related to CHD after it was adjusted for by the CSI, but the researchers indicate it may be protective against CHD when the diet is high in cholesterol and high in saturated fat and low in plant foods. This seems to imply that in France the protective factor against CHD was not the consumption of wine but rather the higher dietary intake of vegetables and vegetable oils. The researchers

indicate that one of the factors in plants that may be protective against the CHD could be the antioxidants, such as α-tocopherol, which may prevent LDL cholesterol oxidation. Wild et al. (1993) state that oxidized LDL cholesterol has been shown to be atherogenic. Parthasarathy, Steinberg, and Witztum (1992) suggest that LDL is oxidized by smooth muscle cells and monocytes. This oxidation may be mediated by superoxide radicals. Furthermore, they identify many atherogenic properties of oxidized LDL. A few examples are as follows: oxidized LDL is rapidly taken up by macrophages, which then leads to cholesterol accumulation; oxidized LDL is cytotoxic and immunogenic; and oxidized LDL induce smooth muscle cell proliferation. The researchers also indicate that individuals differ in their susceptibility to the effects of oxidized LDL. They suggest that this may be due to the variation in the levels of antioxidants found in individuals. Antioxidants provide a defense against the damage of cellular membranes, protein and nucleic acids as a result of free radical and singlet oxygen formation during aerobic respiration. Tocopherols have been found to be potent antioxidants. Rice bran oil has a high content of α-tocopherol (322 mg/kg oil), having with a total tocopherol content of 400 mg/kg of oil or 0.04% of oil weight. Safflower oil has a total tocopherol content of 267 mg/kg, an α-tocopherol content of 223 mg/kg oil (White, 1988).

Research indicates that diets high in cereals, legumes, vegetables and vegetable oils have shown an association with low plasma cholesterol levels and a low incidence of CHD. A specific vegetable oil that is used extensively in India and Japan is RBO. Rice bran oil has been shown to significantly lower levels of total LDL and VLDL cholesterol more effectively than other vegetable oils. In a study carried out by Sharma and Rukmini (1986), the hypocholesterolemic affect of RBO was investigated in rats. Thirty-two rats were divided into four dietary groups of eight animals each. Groups were fed control (corn oil) and experimental (RBO) atherogenic diets containing cholesterol and cholic acid and two other groups (control and experimental) were fed cholesterol-free diets. The level of dietary fat for all diets was 10%. It was shown that rats fed the atherogenic diet 10% RBO for 8 weeks had significantly lower levels of total cholesterol (-37%) and higher levels of HDL cholesterol (+82%) than animals fed the atherogenic diet 10% GNO. The LDL cholesterol level was 52% lower for animals fed the atherogenic 10% RBO than for the ones fed the atherogenic 10% GNO; levels of TGs were decreased, but not significantly so. Rice bran oil and GNO are similar physiochemically, except for the difference in unsaponifiable content of the oils--(4.1%) in RBO and 0.3% in GNO. The researchers concluded that feeding RBO resulted in the enhanced excretion of bile acids and neutral sterols. It was suggested that the enhancement can be attributed to the large

amount of unsaponifiable matter in RBO. The unsaponifiable matter may affect the reabsorption of bile acids and disrupt micelle formation.

In a similar study on the hypocholesterolemic activity of RBO, total serum and liver lipids were analyzed to determine total and free cholesterol concentrations as well as TG and HDL cholesterol. In this study, Seetharamaih and Chandrasekhara (1989) found decreases in serum total cholesterol (-38%) and VLDL + LDL cholesterol (-39%) and an increase in HDL cholesterol (27%) in rats fed 10% RBO + 1% cholesterol + 0.15% bile salt compared to rats fed 10% GNO + 1% cholesterol + 0.15% bile salt. Serum lipids were further reduced by 20% with the addition of 0.5% oryzanol (component of RBO) to rats fed 10% RBO + 1% cholesterol + .15% bile salt. Liver lipids (cholesterol and TG) were 20% lower in rats fed RBO + 1% cholesterol + 0.15% bile salt + .5% oryzanol (component of RBO) than in those fed RBO + 1% cholesterol + 0.15% bile salt. Rice bran oil's cholesterol-lowering effect was attributed to the oryzanol, a component of the unsaponifiable fraction. In this study, it was also indicated that oryzanol is 20-30% of the unsaponifiable matter and 1.3 - 2.6% of the oil.

These two studies indicate that some part of RBO's unsaponifiable matter is responsible for the effects on lipoproteins and for the enhanced excretion of bile acids and neutral sterols. Since the fatty composition of RBO and GNO are similar, it was thus

concluded by the authors that the above effects can be attributed to the unsaponifiable matter of RBO and specifically to one component, i.e., oryzanol.

Nicolosi, Ausman, and Hegsted (1991) also suggest that a component of RBO's unsaponifiable matter can inhibit cholesterol synthesis and lower serum cholesterol levels in animal models. The specific component indicated was oryzanol. Their study demonstrated the hypolipidemic response of 9 cynomolgus monkeys that were first fed semi-purified stabilization diets to obtain baseline values for serum cholesterol levels and were then fed test diets. The test diets contained various levels of different kinds of oils as dietary fat. Rice bran oil, one of the test oils, was varied from 0 to 35% Kcals of the test diets. Safflower oil was also examined in this study for cholesterol lowering affects, but RBO resulted in having the greatest positive effect on lowering LDL cholesterol. When RBO was the only oil in a test diet, there was up to a 40% reduction in LDL cholesterol, but there was no affect on HDL cholesterol. In this study, RBO's unsaponifiable matter seemed to have masked the effects of the fatty acids in the diets as demonstrated by the results of the study.

Rice bran oil contains higher levels of saturated fatty acid (16%) and lower levels of polyunsaturated fatty acids (40%) than many other unsaturated vegetable oils. The cholesterol-lowering effects of RBO is equal or greater to that of other unsaturated

vegetable oils. This indicates that a nonlipid acid component of RBO has the cholesterol-lowering effect.

Other components in RBO's unsaponifiable matter are phytosterols. The phytosterols contained in vegetable oils have been found to have a cholesterol lowering effect. Rice bran oil contains higher amounts of phytosterols (campesterol, stigmasterol and ß-sitosterol) than other vegetable oils, such as SFO (Rukmini, 1991).

Rice bran oil also contains high amounts of tocotrienol (specially γ-tocotrienol),which is found at a level of approximately 75 mg/100g of oil as opposed to less than 4 mg/100g of oil in SFO. Tocotrienols are unsaponifiable components found in plants, and they have a biological activity as vitamin E compounds, thus they are also potent antioxidants (Nair, 1986). In a study by Quereshi, Burgers, Peterson, and Elson (1986), d-ß-tocotrienol was isolated from barley. The study demonstrated that this component suppresses hepatic 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase; this enzyme is rate limiting enzyme in the biosynthetic pathway of cholesterol. In their study, broiler chicks were fed a diet containing d-γ-tocotrienol from barley for a 3-week period. The results from part of the research indicated a lowering of HMG-CoA reductase activity and serum cholesterol by d-α-tocotrienol from barley for a 3-week period. A decrease in HDL cholesterol was noted, but this was not found to be significant. However, there was a significant fall in LDL cholesterol.

Germane to this study is the finding by researchers that RBO has significant cholesterol-lowering effects attributed to the unsaponifiable matter. Rice bran oil, as stated before, has a high content of unsaponifiable matter than other oils, such as SFO.

Rice Bran Oil and Safflower Oil

Even though further research is needed to determine the actual cholesterol lowering of RBO's unsaponifiable matter in humans, several studies have indicated possible mechanisms for this cholesterol lowering action. In a review study by Rukmini and Raghuram (1991), the following cholesterol-lowering mechanisms were indicated: oryzanol, a triterpene alcohol, may inhibit the absorption of cholesterol and increase the fecal excretion of bile acids; and the fractions of oryzanol, CA and 24-methylene cycloartenol, may inhibit cholesterol esterase activity.

In one study reviewed by Rukmini and Raghuram (1991), rats were fed an atherogenic diet containing either 10% RBO or 10% GNO with 1% cholesterol and 0.5% cholic acid. In this study, the phytosterols CA and 24-methylene cycloartenol, which were fed in amounts found in RBO, were given to hypercholesterolemic rats for 8 weeks, and thus resulted in a significant decrease in serum cholesterol and TGs. The researchers suggest that CA inhibits cholesterol esterase action, which results in the decreased hydrolysis of cholesterol esters and the decreased absorption of cholesterol. Due to the structural similarity of CA to cholesterol, CA may compete with

cholesterol binding sites in the liver. Consequently, this would result in cholesterol being excreted and metabolized to bile salts and pigments. In studies, where rats were not fed cholesterol, nearly 20% of intestinal cholesterol was synthesized by the mucosal lining, with other sterols being derived from skin grooming and bacterial action in the large intestine.

As mentioned previously, the study by Quereshi et al. (1986) demonstrated the ability of tocotrienol in RBO to inhibit HMG CoA reductase and to decrease the biosynthesis of cholesterol. As also stated previously, RBO's unsaponifiable matter has been shown to have a cholesterol-lowering effect. By contrast, SFO's cholesterol lowering mechanism has been attributed to the high linoleic acid content. Safflower oil is a vegetable oil having the highest content of linoleic of any known oil. It contains approximately 83% linoleic acid, as opposed to 36% in RBO. Safflower oil contains 78% polyunsaturated fatty acids and 12% monounsaturated fatty acids, as opposed to RBO, which has approximately 40% of fatty acids being polyunsaturated and 43% being monounsaturated fatty acids in RBO (White, 1988). Many studies have shown that polyunsaturated vegetable oils can lower serum cholesterol, especially LDL cholesterol. The American Heart Association recommends that up to 10% of total calories come from polyunsaturated fatty acids. One of the polyunsaturated oils recommended for cooking and salad dressings is SFO (American Heart Association, 1993). This

association also recommends that monounsaturated fatty acids provide between 10-15% of total calories. Two of the monounsaturated oils recommended are canola and olive oil.

Goldstein and Brown (1987) suggest that excessive consumption of dietary saturated fat and cholesterol delivered to the liver by the chylomicron remnant receptor may lead to an increased production of VLDLs by the liver, resulting in increased amounts of LDL. The VLDLs, when catabolized in the body, are precursors to LDLs. The increased amounts of LDL cause plasma LDL to increase due to the limited number availablity of LDL receptors. Human studies suggest that saturated fatty acids decrease LDL receptor levels. Furthermore, increased liver cholesterol (endogenous) may suppress the activity of the LDL receptor. The ultimate result is an increase in plasma LDL levels. Saturated fatty acids (such as lauric, myristic, or palmitic acid) and cholesterol are thought to produce increased levels of plasma VLDL and consequently increased levels of LDL cholesterol (Bonanome, 1988). Wahrburg, Martin, SandKamp, Schulte, and Aussman (1992) state that the major effect that saturated fatty acids have on LDL receptors is the suppression of their activity, resulting in the decreased catabolism of LDL and an increased conversion of VLDL remnants to LDL cholesterol. The excess cholesterol can cause atherosclerosis of arterial walls due to accumulation of macrophages.

A human study by Reaven et al. (1991) demonstrated that a diet rich in oleic acid (a monounsaturated fatty acid), as opposed to a diet rich in linoleic acid (a polyunsaturated fatty acid), decreased the susceptibility of LDL to oxidation. Oxidized LDL is atherogenic. In this study, monounsaturated fatty acids offered protection against LDL oxidation, whereas polyunsaturated fatty acids did not. It was also confirmed that a diet rich in either oleic and linoleic lowers serum cholesterol levels but that oleic acid did not decrease HDL cholesterol, as opposed to linoleic acid which did. Oleic acid is found in larger amounts in RBO (approximately 43%) than in SFO (approximately 14%).

Bonanome and Grundy (1988) demonstrated that stearic acid (a saturated fatty acid) lowers serum cholesterol levels in comparison to lauric acid, myristic acid, or palmitic acid. A possible mechanism for this action is that stearic acid is converted to oleic acid. In this human study, the oleic content of plasma, TG and cholesterol esters increased when a diet high in stearic acid was fed. Both RBO and SFO contain between 2.0 - 2.6% stearic acid, so that it is unlikely to contribute the effect of these oils on serum lipids. In a study by Mattson and Grundy (1985), diets high in polyunsaturated fatty acids were shown to lower serum concentrations of LDL and HDL cholesterol. Diets high in monounsaturated fatty acids lowered LDL cholesterol without

affecting HDL cholesterol. This may be the result of the LDL receptors' return to their normal activity (Grundy, 1988).

Wahrburg et al. (1992) suggest that polyunsaturated fatty acids increase the activity of LDL receptors, which may result in the increased catabolic rate of LDL and the decreased conversion of VLDL remnants to LDL. A probable mechanism is that PUFAs may suppress the activity of saturated fatty acids resulting in the return of LDL receptors to normal activity.

A study by Iwata et al. (1992) investigated the dietary effect of safflower phospholipid and soybean phospholipids on plasma and liver lipids in rats. Safflower phospholipid was shown to suppress the increase of plasma and liver cholesterol caused by possibly increasing the formation of HDL by the activation of lecithin-cholesterol acyltransferase. The mechanisms stated to be responsible were the inhibition of cholesterol absorption in the small intestine, the increased excretion of fecal neutral steroid, which was hypothesized to be the result of the inhibition of cholesterol absorption in the small intestine. Remla, Menon, and Kurup (1991) compared the effect of SFO and coconut oil on lipids in rats where myocardial infarction was induced by isoproterenol. Safflower oil demonstrated a protective effect against infarctions, indicated by the minimal necrosis of the heart in animals fed this oil compared to heart necrosis seen in rats fed the coconut oil.

Wardlaw, Snook, Lin, Puangco, and Kwon (1991) demonstrated no significant difference in diets enriched with SFO or canola oil in their ability to decrease LDL cholesterol or apoprotein B concentration in adult men. In this study, 16 men consumed 39 ±1% fat energy either from canola oil or safflower oil for 8 weeks. The men were stabilized for 3 weeks on a baseline, diet typical American.

It is apparent that further studies are needed to examine the effects of oils rich in polyunsaturated and monounsaturated fatty acids and serum levels of cholesterol and TGs in both human subjects and experimental animals. Rice bran oil should be investigated further to compare its effects on cholesterol with that of a polyunsaturated oil, e.g., SFO. There are no studies to compare RBO with SFO in human subjects; therefore, studies in animal models would be useful.

# CHAPTER III

## Methods and Procedures

Fourteen, 4-week-old, male Sprague-Dawley rats were randomly assigned to two experimental groups, with each having seven animals (Table 2). Group I received a 10% RBO diet; Group II received a 10% SFO diet. Both groups were fed the diets for a 21 day period. A detailed composition of the diets is given in Table 3.

Food and tap water were allowed ad libitum. All animals were housed individually in stainless steel suspended wire cages having mesh floors. The room was maintained at 22 ± 1° Centigrade with a relative humidity of 50-60% and a regular light/dark cycle of 12 hours. Food intakes and body weights of animals were recorded weekly between 10 a.m. to 11 a.m. throughout the experiment. At the conclusion of the 21 day period, rats were subjected to a 5-hour fast, anesthetized and were exsanguinated by cardiac puncture. Blood was collected and serum prepared. Sera were analyzed for total cholesterol, TGs and phospholipid levels. Cholesterol concentrations were measured by an enzymatic method using Stanbio Kit #1010; TGs were measured enzymatically by Stanbio Kit #2000 (Stanbio Laboratory, San Antonio, Texas). Serum phospholipid levels were determined by an enzymatic kit (Wako Chemical Co., Arlington, VA). Protocols for these lipid determinations are given in the Appendices (pages 46-55).

Care of animals was in conformance with the guidelines set forth by the Texas Woman's University Institutional Animal Care and Use Committee. The statistical analysis of the data consisted of the Students' t-test. The type of t-test was determined to be unpaired because samples were independent of each and because they were randomly selected. The hypothesis was nondirectional because there was no expected direction of outcome and therefore a two-tailed test was used. The Students' t-test was used to assess the significance of statistical differences; the level of significance was $P < 0.05$.

Table 2

Subject Distribution

| Group | Diet | N |
|---|---|---|
| 1 | Rice bran oil | 7 |
| 2 | Safflower oil | 7 |

Table 3

Diet Compositions

| | Rice bran oil diet | Safflower oil diet |
|---|---|---|
| | g/kg/diet | |
| Casein | 200 | 200 |
| Rice bran oil | 100 | - |
| Safflower oil | - | 100 |
| Mineral mix (AIN 76) | 35 | 35 |
| Vitamin mix (AIN 76A) | 10 | 10 |
| Choline bitartrate | 2 | 2 |
| Sucrose | 603 | 603 |
| Cellulose | 50 | 50 |
| Total | 1000 | 1000 |

# CHAPTER IV

## Results

The means, standard deviations, and the coefficients of variation for serum total cholesterol, phospholipids and triglycerides for the two experimental groups are shown in Table 4. As shown on Table 5, the type of oil fed to the rats had no effect on either food intake or growth. The mean value for serum total cholesterol in rats fed SFO was 81 mg/dL, with a coefficient of variation (CV) of 17%. In the groups fed 10% RBO, the mean serum total cholesterol level was 89 mg/dL, with a CV of 9.4%. The mean value for serum phospholipids in rats fed 10% SFO was 187 mg/dL, with a CV of 14%; in the RBO group, the mean value was 209 mg/dL, with a CV of 9%. The mean for serum TG in the RBO group was 136 mg/dL, with a CV of 5%, and in the SFO group the mean is 85 mg/dL with a CV of 5.4%. There were no statistically significant differences in the values for serum lipids between the group fed 10% SFO and those for the group fed 10% RBO; however, the animals in the group fed 10% SFO did have lower serum levels of all lipids than those in the group fed RBO.

The Student's t-test revealed no significant difference between the effect of 10% RBO and that of 10% SFO on the serum lipids; however the animals in the group fed 10% SFO did have lower serum levels of all lipids than those in the group fed RBO.

Table 4

Serum Lipids in Rats Fed Rice Bran Oil (RBO) or Safflower Oil (SFO), N=7 per Group.

| | Group I 10% RBO | | | Group II 10% SFO | | | |
|---|---|---|---|---|---|---|---|
| | $\bar{X}$[a] | SD[b] | CV[c] | $\bar{X}$ | SD | CV | P Value |
| Total cholesterol (mg/dL) | 89.0 | 8.4 | 9.4% | 81.3 | 14 | 17% | 0.22 (NS)[d] |
| Phospholipids (mg/dL) | 209 | 19 | 9.1% | 187 | 26 | 14% | 0.10 (NS) |
| Triglycerides (mg/dL) | 136 | 7.4 | 5.4% | 85.0 | 46 | 5.4% | 0.28 (NS) |

[a]$\bar{X}$ = Mean

[b]SD = Standard Deviation

[c]CV = Coefficient of Variation

[d]NS = Non Significant, P>0.05

Table 5

Initial, Final, and Change in Body Weight and Food Intake of Rats.

| | Group I 10% RBO | | | Group II 10% SFO | | |
|---|---|---|---|---|---|---|
| | $\bar{X}$ [a] | SD[b] | CV[c] | $\bar{X}$ | SD | CV |
| Initial (g) | 90.3 | 8.6 | 9.5% | 91.8 | 8.7 | 9.5% |
| Final (g) | 201 | 23 | 11% | 201 | 27 | 13% |
| Change in Body Wt. (g) | 111 | 18 | 17% | 109 | 20 | 18% |
| Food Intake (g/rat/d) | 14.6 | 1.5 | 10% | 14.7 | 1.6 | 11% |

[a] $\bar{X}$ = Mean

[b] SD = Standard Deviation

[c] CV = Coefficient of Variation

# CHAPTER V

## Discussion

This study was carried out with the objective of comparing safflower oil (12% monounsaturated fatty acids and 78% polyunsaturated fatty acids [principally as linoleic acid]) and RBO (43% monounsaturated fatty acids [principally as oleic acid] and 40% polyunsaturated fatty acids [principally as linoleic acid]) with regard to their effects on serum lipids.

Current dietary guidelines by the American Heart Association (AHA) for the prevention of CHD recommend that 30% or less calories come from dietary fat; of total energy intake, monounsaturated fatty acids provide up to 10%, polyunsaturated fatty acids should provide up to 10%, saturated fatty acids should provide less than 10% (American Heart Association, 1988). The National Cholesterol Education Program further indicates that following the AHA recommendations may decrease LDL cholesterol levels without affecting HDL cholesterol (The Expert Panel, National Cholesterol Education Program, 1993).

The present study found no significant differences between sample groups fed 10% safflower oil and those fed 10% RBO with regard to serum lipids (total cholesterol, phospholipids and triglycerides). Thus, these oils would both seem suitable as means to favorably alter intakes of fatty acids for the US population.

The present study's findings indicate a trend for SFO to decrease serum lipids. Lower serum lipids were not expected in rats fed 10% RBO, even though results of previous animal research have demonstrated RBO's hypolipidemic action as compared to other vegetable oils, such as GNO, as a lowering effect is only seen with an atherogenic diet. Researchers have attributed this action to the unsaponifiable fraction (CA, tocotrienols, phytosterol and tocopherols), which possibly results in increased cholesterol excretion, increased fecal excretion of bile salts, and an inhibition of HMG CoA reductase activity. These mechanisms were previously discussed in more detail in the literature review. Rice bran oil may be expected to be hypocholesterolemic in comparison to oils rich in saturated fatty acids, as research has confirmed oleic acid's role in decreasing LDL cholesterol without affecting HDL cholesterol (Reaven et al., 1991). Furthermore, high levels of HDL cholesterol have been proven to be protective against CHD. Safflower oil contains 83% linoleic acid and 10% oleic, but RBO contains 36% linoleic and 42% oleic. Linoleic acid has been shown to be effective in decreasing serum cholesterol, but it also decreases HDL cholesterol (Reaven, 1991). Thus, on the basis of fatty acid profile, SFO may be expected to give lower serum cholesterol levels than RBO.

The failure to detect a significant effect on serum lipid values may be the result of the small sample size and limited duration of

the study. When a sample size is too small, it is difficult to detect the desired effect, thus perhaps missing a significant effect.

In a study by Sharma and Rukmini (1986), rats were fed cholesterol-containing diets having either 10% RBO or 10% GNO for 8 weeks. Animals fed the 10% RBO diet had lower levels of total cholesterol, LDL-cholesterol, and VLDL cholesterol than ones fed GNO; serum TGs were lower than for those fed 10% GNO. Rats fed a cholesterol-free diet containing either 10% GNO or 10% RBO did not have significant differences in values for serum lipids. The HDL cholesterol levels in the animals fed a cholesterol-containing diet were higher when diets contained 10% RBO rather than 10% GNO. The animals fed a cholesterol-free diet supplemented with 10% RBO had similar HDL cholesterol levels to those fed 10% GNO. The cholesterol-free diet supplemented with 10% RBO is similar to the diet fed to animals in this current study. The two studies differ in that this current study's sample size is smaller, there were four groups in the latter, although the group sizes (n=7 versus n=8) similar. The duration time of the current study was 3 weeks versus 8 weeks in the study by Sharma and Rukmini (1986). Perhaps increasing the sample size, extending the duration of the study to eight weeks, and including dietary cholesterol may have shown favorable results for RBO's effect on serum lipids.

Some current studies support earlier research demonstrating that dietary intake of oils rich in polyunsaturated fatty acids (e.g.,

SFO) decrease serum cholesterol more than oils rich in monounsaturated fatty acids (e.g., canola oil). For example, a study by Heyden (1994) indicates that oils rich in linoleic acid decrease total cholesterol and LDL-cholesterol, whereas those rich in oleic acid have little or no effect. Heyden's study contradicts the findings in many recent studies that support oleic acid's effect in lowering serum cholesterol without affecting HDL cholesterol. A study by Shekelle and Stamler (1990) demonstrated that increasing polyunsaturated fatty acids from 3 to 5% of calories was linked with lowering the risk of CHD by decreasing LDL cholesterol, whereas increasing calories from 16 to 23% from monounsaturated fatty acids did not. Heyden (1994) also observed that a high oleic acid diet increased plasma total TGs, VLDL cholesterol, LDL cholesterol and LDL TGs. Furthermore, the protection against CHD by populations consuming a Mediterranean diet (fat calories are predominately from monounsaturated fatty acid, e.g., olive oil) may simply be due to consuming a diet where fat calories are predominately from either polyunsaturated fatty acids or monounsaturated fatty acids rather than saturated fatty acids. Heyden (1994) further states that many studies indicating the inverse relationship between the consumption of monounsaturated fatty acids and a decreased risk of CHD did so because saturated fatty acids were replaced by monounsaturated fatty acids.

Lichtenstein et al. (1994) used human subjects to investigate the effects of RBO on serum lipids. This study compared RBO to canola, corn, and olive oil as to their effect on plasma lipid and apolipoprotein concentrations in moderately hypercholesterolemic humans. The source of the RBO was not mentioned. There were 15 subjects, both male and female, who consumed diets with each of the test oils for 32 day periods. Mean caloric intakes ranged from 2000 to 4000 kcal and consisted of 17% calories from protein, 53% from carbohydrate and 30% from fat (20% came from the test oil). The findings indicated that diets enriched with RBO, canola or corn oil are comparable in decreasing plasma cholesterol and LDL cholesterol levels and that subjects fed these oils had levels of these two lipid fractions that were significantly lower than these for subjects fed olive oil by 6.3%. This is the only published human study comparing RBO to other vegetable oils with regard to its effect in lowering these plasma lipids. Unfortunately, there are no published human studies comparing RBO to SFO in its effect in lowering serum lipids. There was no significant difference in different test oils in the above study.

Adam, Wolfram, and Zollner (1983), using both in vitro and in vivo experiments, demonstrated the effects of dietary intakes of 20% of energy as linoleic acid versus 4% as linoleic acid on platelet aggregation and thromboxane formation. The two-week dietary intake of a diet having 20% linoleic acid decreased plasma

phospholipid levels, whereas a 4% linoleic diet had no effect. Plasma phospholipid is a main pool for the arachidonic acid, a prostaglandin precursor. The researchers indicate that the availability of arachidonic acid may be the rate-limiting factor for production of thromboxane $A_2$ (which contracts arteries and promotes platelet aggregation) and prostaglandin formation. Another indication by Adam et al. (1983) for the limited formation of prostaglandins when diets with high levels (20% of energy intake) are fed is that there is a decreased level of arachidonic acid in HDLs (involved in lipid transport from peripheral cell), possibly resulting in a decrease in the formation of tissue prostaglandins. The pathogenesis of atherosclerosis involves the development of plaque, which arises from injury to the endothelial lining caused by serum factors, e.g., increased serum cholesterol. Following endothelial injury, platelets adhere to endothelial cells or to the subendothelial connective tissue. The resultant platelet adhesion and aggregation can induce thrombosis as well as vasoconstriction and cellular proliferation. Inhibition of platelet aggregation and inflammatory responses may result from a decreased availability of arachidonic acid and thus a decreased production of thromboxane $A_2$. Subsequently, this may decrease the risk of atherosclerosis. In the studies by Adam et al. (1983), oleic acid was claimed to slightly decrease the mean arachidonic acid level in HDLs. Diets containing vegetable oils with a high content of linoleic acid, such as SFO,

which has 83% of its fatty acids in this form, may contribute to a decrease in plasma phospholipids and result in decreased prostaglandin formation (Adam, 1983). In the current study, animals fed the diet with 10% SFO had plasma phospholipid levels that were lower (10.4%) than ones fed the diet with 10% RBO. This may be due to the fact that SFO contains 83% linoleic acid versus 36% in RBO.

A professional paper by Trejus (1991) studied the effect of atherogenic diets (1% cholesterol and 0.5% cholic acid) containing either 10% RBO or 10% SFO on serum cholesterol and TG levels in the rat (see Figures 1 and 2). Here again, there were no statistically significant differences in serum cholesterol levels, but rats fed a diet with SFO had lower serum levels of cholesterol and TGs than ones fed RBO, and values for these two classes of lipid were 8.6% and 44.6% lower, respectively, than ones fed RBO. Serum cholesterol levels were higher in the former study because an atherogenic supplement was given. Serum TG levels were decreased. Atherogenic supplements decrease serum TGs in the rat, but the reason is unknown. In the current study, serum levels of cholesterol, phospholipids and TGs were 9%, 10.4% and 37.5% lower for animals fed SFO than for the ones fed RBO (see Figure 2). Another reason why these findings may have resulted is that RBO has a higher content of palmitic and (17%) than SFO (6%). McNamara (1994) states that only three saturated fatty acids increase plasma cholesterol levels, i.e., myristic, lauric, and palmitic acid. As

**Figure 1. Comparison of Rice Bran Oil (RBO) and Safflower Oil (SFO) on Total Cholesterol (Mean and SD) for Rats Fed Standard Purified Diets (Present Study) and Purified Diets having an Atherogenic Supplement (Study by Trejus).**

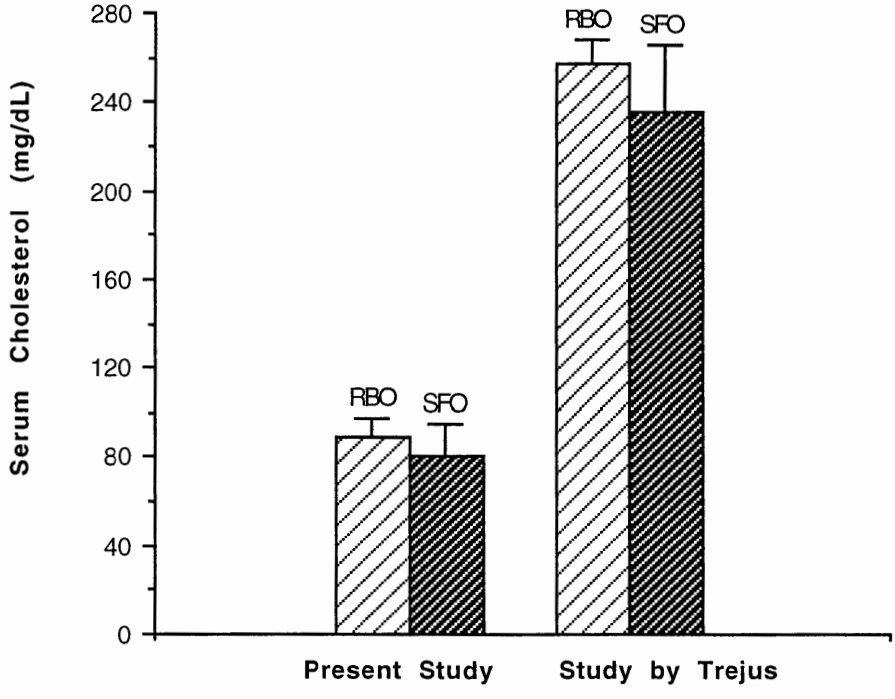
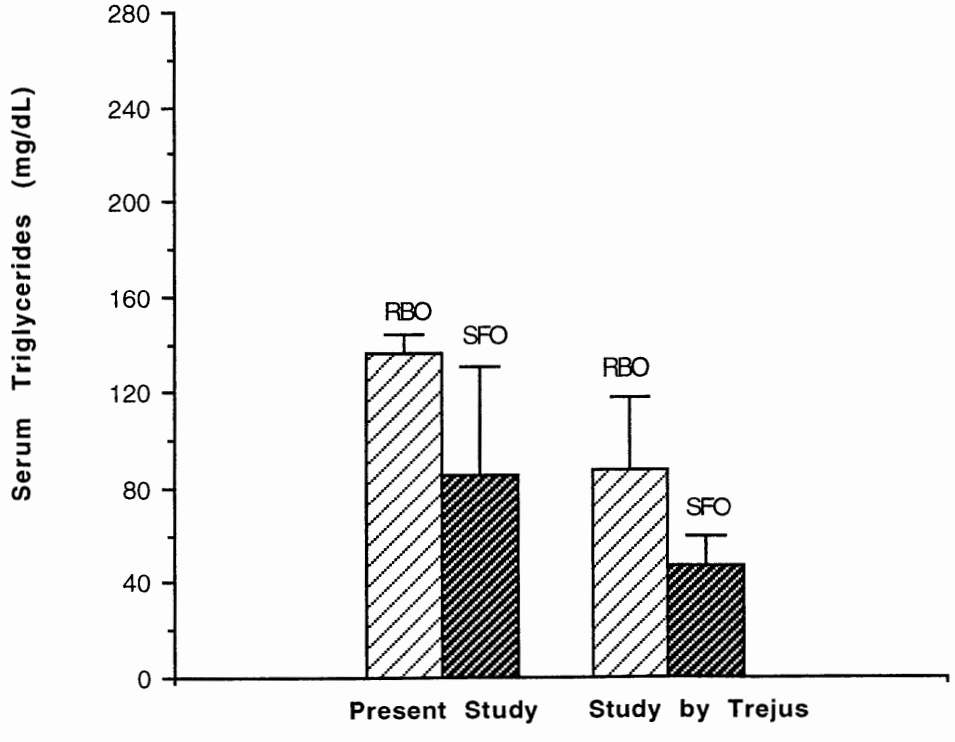


**Figure 2. Comparison of Rice Bran Oil (RBO) and Safflower (SFO) on Serum Triglycerides (Mean and SD) for Rats Fed Standard Purified Diets (Present Study) and Purified Diets having an Atherogenic Supplement (Study by Trejus).**



mentioned before, SFO contains 83% linoleic acid, which previous research has shown to decrease serum phopholipid levels (Adam, 1983); however, the effect of saturated fatty acids on serum phospholipids has received little attention. High levels of serum cholesterol, serum phospholipid and TGs have been claimed to be a risk for the development of CHD.

In conclusion, this present three-week animal study comparing SFO and RBO did not demonstrate any significant differences between RBO and SFO on serum lipids, but animals fed SFO had lower serum levels of all lipid fractions studied. An extensive review of the literature indicates that studies comparing safflower oil and RBO as to their effect on serum lipids in either humans or rodents have not been published. Contradictory information on whether polyunsaturated fatty acids or monounsaturated fatty acids differ with respect to their effects on total cholesterol and in the development of heart disease makes recommendations about the incorporation of one over the other into diets difficult. Further dietary studies (both human and animal) and mechanistic studies may make recommendations easier n the future.

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# Appendices

# Appendix A

# Laboratory Protocol

## Triglycerides

Introduction:

Measurement of serum triglyceride levels is a valuable screening tool for hyperlipidemia. Determination of serum triglyceride concentrations is important in the following: acute pancreatitis, nephrosis, diabetes mellitus and disorders of lipid metabolism.

Objective:

Serum triglyceride levels are determined by laboratory procedures.

Principle of the Method:

1. Glycerol and fatty acid are formed by the action of pancreatic lipase on triglycerides.
2. Glycerol is phosphorylated to produce glycerol-3-phosphate (G-3-P) and adenosine-5'-diphosphate (ADP). This is catalyzed by glycerol kinase (GK).
3. G-3-P is oxidized by glycerylphosphate oxidase (GPO) and produces dihydroxyacetone phosphate and hydrogen peroxide.
4. Peroxide reacts with 4-aminoantipyrine and 4-chlorophenol to form quinoneimine. Refer to Figure A-1 for reaction scheme.

Glycerol + ATP $\xrightarrow{GK}$ G-3-P=ADP

G-3-P + $O_2$ + $\xrightarrow{GPO}$ DAP + $H_2O_2$

$2H_2O_2$ + 4 aminoantipyrine + $\xrightarrow{POD}$ Quinoneimine + HCL + $4H_2O$

4-Chlorophenol

GK = Glycerol Kinase

GPO = Glycerol phosphate

POD = Peroxidase

Figure A-1. Reaction scheme for the enzymatic determination of triglyceride

## Serum Triglyceride

### Reagents

1. Enzymatic Triglyceride Reagent GPO

   Contains 4-aminoantipyrine, 4-chloro-phenol, ATP magnesium-ions, lipases, glycerol-kinase, glycerol-3-phosphate oxidase, peroxidase, PIPES buffer solution.

2. Enzymatic Triglyceride Standard GPO

   Contains glycerol (20.8 mg/dL) plus preservative and stabilizer.

### Procedure

Set up sample tubes and tubes for a blank and two standards. Add 30 µL samples of serum to test tubes; 30 µL of triglyceride standard having a concentration of 200 mg/dL; add 30 µL of saline to the blank; add 500 µL of triglyceride reagent to all tubes. Allow tubes to stand at room temperature for 10 minutes. Pipette samples, standards and blank into cuvettes. Read absorbance at 500 nm on the spectrophotometer.

### Calculations

Serum triglyceride mg/dL = AU/AS x 200

AU=absorbance of unknown

AS=absorbance of standard

200=concentration of the standard

## Serum Total Cholesterol

Introduction:

The presence of large amounts of cholesterol in the blood is a major risk factor in coronary heart disease. Epidemiological data from different countries have shown a strong positive correlation between coronary heart disease and serum cholesterol. The normal values for serum cholesterol are 150 to 250 mg/dl (adults).

Objective:

Lipid profile to determine total serum cholesterol and triglyceride.

Principle of the Method

1. Cholesterol esters are hydrolyzed by cholesterol esterase (CE) to form free cholesterol and fatty acids.
2. The free cholesterol and the preformed cholesterol are then oxidized by cholesterol oxidase (COx) to cholest-4-en-3 one and hydrogen peroxide.
3. Hydrogen peroxide, 4-aminoantipyrine and phenol are oxidized in the presence of peroxidase (POD).
4. The result is a quinoneimine chromogen with maximum absorption at 500nm.
5. The intensity of the red color indicates the total cholesterol concentration. Refer to Figure A-2 for reaction scheme.

Cholesterol Esters $\xrightarrow{CE}$ Cholesterol + Fatty Acids

Cholesterol + $O_2$ $\xrightarrow{COx}$ Cholest-4-en-3-one + $H_2O_2$

$H_2O_2$ + 4 - Aminoantipyrine + Phenol

$\xrightarrow{POD}$ $H_2O$ + O - Quinoneimine dye

CE = cholesterol esterase
COx = cholesterol oxidase
POD = peroxidase

Figure A-2. Reaction scheme for the enzymatic determination of total cholesterol.

## Serum Total Cholesterol

Reagents

1. Enzymatic Cholesterol Reagent

   Contains - 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase, buffers and stabilizers.

2. Enzymatic Cholesterol Standard

   Contains - cholesterol in ethylene glycol monomethyl ether.

Procedure

Set up sample tubes and tubes for a blank and two standards. Add 30 μL sample of serum to test tubes; 30 μL of cholesterol standard having a concentration of 200 mg/dL; add 30 μL of saline to the blank; add 500 μL of cholesterol reagent to all tubes. Vortex all tubes. Allow tubes to stand at room temperature for 45 minutes. Pipette samples, standards and blank into cuvettes. Read absorbance at 500 nm on the spectrophotometer.

Calculations

Serum Total Cholesterol (mg/dL) = AU/AS x 200

AU=absorbance of unknown

AS=absorbance of standard

200=concentration of the standard (mg/dL)

Expected values for the rat are 60-150 mg/dL.

## Serum Phospholipids

### Introduction

Measurement of serum phospholipid levels is a valuable screening tool for the diagnosis of liver diseases, for example, obstructive jaundice, in cardiovascular disease it can be used to derive glycerides by subtracting from total lipids cholesterol and phospholipids. It is also a useful tool in indicating fetal lung development.

### Objective:

Serum phospholipids are determined by laboratory procedure.

### Principle of the method

1. Phospholipids in the form of phosphatidyl choline are hydrolyzed by phospholipase D.
2. Liberated choline is oxidized by choline oxidase to betaine.
3. Hydrogen peroxide is produced and couples 4-aminoantipyrine and phenol in the presence of peroxidase.
4. A quinoneimine chromogen is produced and absorbance at 500 nm is measured. Refer to Figure A-3 for reaction scheme.

### Reagents

1. Enzymatic Phospholipid Reagent

   Contain: Phospholipase D and Choline Oxidase
2. Enzymatic Phospholipid Standard

Contains: Phosphatidyl choline dissolved in aqueous solution containing 5 g Triton X-100 per 1.

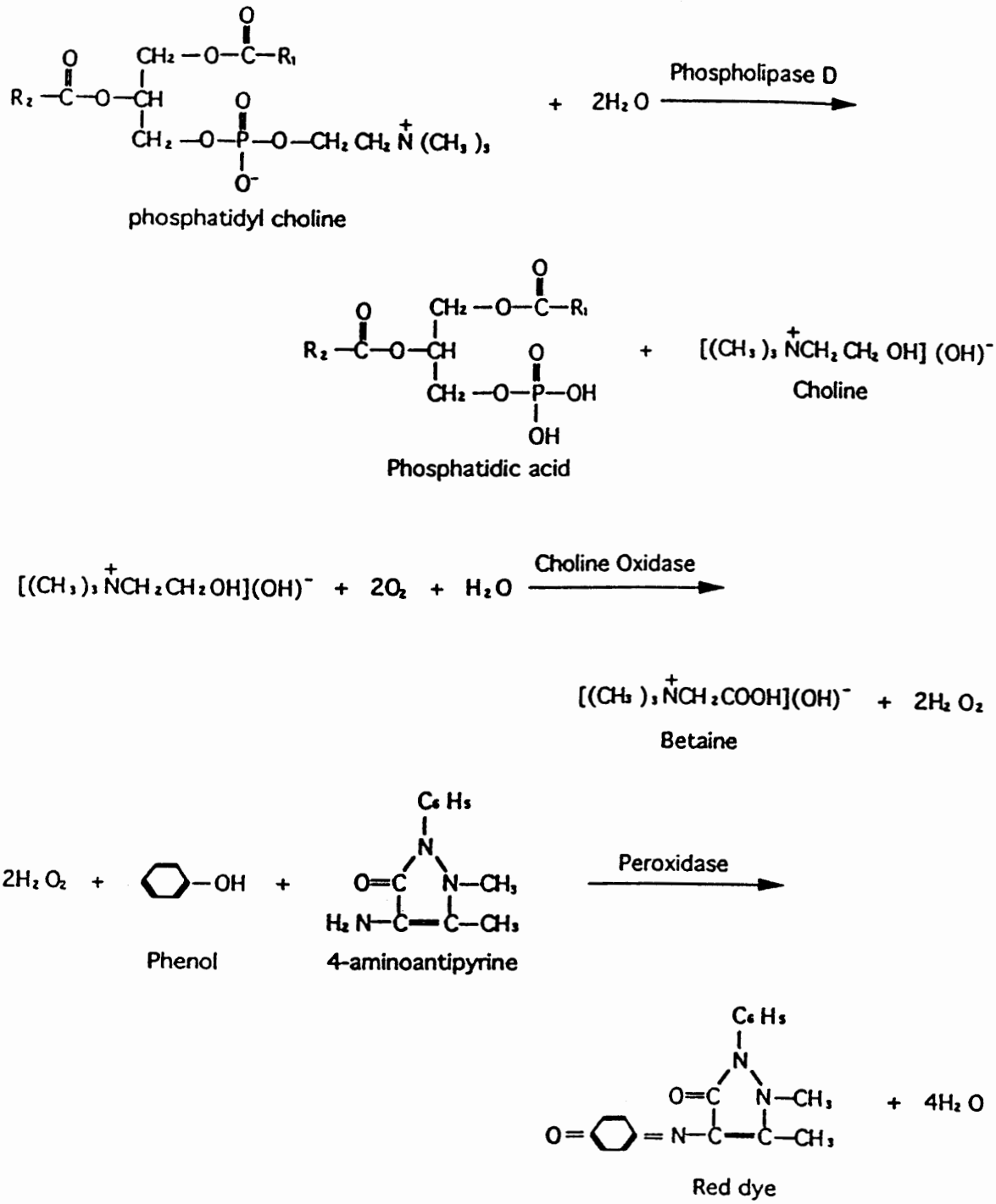


Figure A-3. Reaction scheme for the enzymatic determination of phosphatidyl choline

Procedure

Set up sample tubes and tubes for a blank and two standard tubes. Add 30 μL samples of serum to test tubes; use 30 μL of phospholipid standard having a concentration of 300 mg/dL; add 30 μL of saline to the blank; add 500 μL of phospholipid reagent to all tubes. Allow tubes to stand at room temperature for 10 minutes. Pipette samples, standards, and blank into cuvettes. Read absorbance at 500 nm on the spectrophotometer. Pipette samples into tubes. Allow to let stand for 45 minutes before second absorbance reading at 500 nm.

Calculations

Serum phospholipids mg/dL = $\Delta AU/\Delta AS$ x 300 (mg/dL).

$\Delta AU$ = Absorbance of Unknown.

$\Delta AS$ = Absorbance of Standard.

300 (mg/dL) is value of the standard solution.

Table A-1

Food Intake G/Rat/Day

| Safflower oil | Rice bran oil |
|---|---|
| 15.5 | 16.3 |
| 12.0 | 13.6 |
| 12.6 | 11.4 |
| 16.4 | 15.3 |
| 14.8 | 15.5 |
| 15.6 | 15.1 |
| 16.0 | 14.8 |
| $\bar{X}$ = 14.7 | $\bar{X}$ = 14.6 |
| SD = 1.6 | SD = 1.5 |
| SEM = 0.65 | SEM = 0.62 |

Table A-2

Initial, Final, and Change in Body Weight of Rats Fed 10% RBO and10% SFO

| Rat | Initial Weight (g) | Final Weight (g) | Δ Body Weight (g) |
|---|---|---|---|
| 10% RBO | | | |
| 1 | 87 | 207 | 60 |
| 2 | 92 | 185 | 93 |
| 3 | 75 | 156 | 81 |
| 4 | 94 | 202 | 108 |
| 5 | 90 | 218 | 128 |
| 6 | 104 | 220 | 116 |
| 7 | 90 | 218 | 128 |
| 10% SFO | | | |
| 8 | 87 | 208 | 121 |
| 9 | 83 | 164 | 81 |
| 10 | 80 | 167 | 87 |
| 11 | 97 | 200 | 103 |
| 12 | 98 | 222 | 118 |
| 13 | 104 | 222 | 118 |
| 14 | 94 | 214 | 120 |

Table A-3

Serum Lipids in rats Fed Rice Bran Oil (RBO) or Safflower Oil (SFO)

| Rat | Cholesterol (mg/dL) | Phospholipids (mg/dL) | Triglycerides (mg/dL) |
|---|---|---|---|
| RBO | | | |
| 1 | 90 | 196 | 208 |
| 2 | 89 | 187 | 103 |
| 3 | 86 | 208 | 92.5 |
| 4 | 76 | 191 | 86.8 |
| 5 | 90 | 217 | 98.5 |
| 6 | 88 | 224 | 92.6 |
| 7 | 105 | 238 | 273 |
| SFO | | | |
| 8 | 87 | 171 | 186 |
| 9 | 79 | 159 | 58.8 |
| 10 | 75 | 162 | 73.3 |
| 11 | 97 | 178 | 79.1 |
| 12 | 99 | 229 | 80.9 |
| 13 | 61 | 201 | 46.5 |
| 14 | 71 | 208 | 72.3 |

# Appendix B

## Outline of Poster Session

Poster Board Session Outline:

Size of Poster Board Display: 6' long 4' wide.

Project Title and Researcher's Names:

Title:

Comparison of the Effects of Safflower Oil and Rice Bran Oil on Serum Lipids in the Rat.

Researchers:

*A. Hsueh, L. Aguilar, J. Radcliffe, D. Czajka-Narins.

Title size: 4' by 1' and letter size 2 inches.

Researchers' name - 1 inch. Under title of project.

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*Name of first author will be name of presenter. Paper to be submitted for presentation at Annual Meeting of American Oil Chemists' Society in San Antonio, TX, May 1994.

## Abstract

This study examined the effect of dietary rice bran oil and safflower oil on serum levels of cholesterol, phospholipids, and triglycerides in the rat. One group of 7 animals received a 10% rice bran oil diet, and a second group of 7 animals received a 10% safflower oil diet. Both groups of Sprague-Dawley rats were fed the diets for a 21 day period. Animals fed safflower oil experienced non-significantly lower levels of serum cholesterol (9%), serum phospholipids (10.4%) and serum triglycerides (37.5%) than ones fed rice bran oil. The results indicate that safflower oil shows a trend towards lowering serum lipids compared to rice bran oil in the rat.

## Introduction

For many years, research has been done on cholesterol and cardiovascular disease in order to establish a link between blood cholesterol level and the development of coronary atherosclerosis. Research supports a direct relationship between the plasma LDL cholesterol level and the development of atherosclerosis. Studies have shown that Americans who have high serum concentrations of cholesterol (over 220 mg/dL) are more likely than people with lower levels to develop cardiovascular disease. Increased serum cholesterol levels are associated with changes in dietary habits and decreased physical activity. These changes in dietary habits include an increase in the intake of dietary fat. There has been a great interest in dietary factors that lower serum cholesterol. It has recently been shown that plant-derived products (e.g., as psyllium, oat bran, and rice bran) can lower serum cholesterol. The active principle of rice bran is thought to be the unsaponifable fraction of the oil. This study compares dietary rice bran oil to safflower oil with respect to its effects on serum cholesterol, phospholipids and triglycerides because the effect of rice bran oil has not been compared safflower oil (polyunsaturated oil), which is recommended by the American Heart Association as part of a diet designed to lower serum cholesterol.

Table B-1

Serum Lipids (mg/dL) in Rats Fed Rice Bran Oil or Safflower Oil, N=7 per Group. Mean Values ± SD.

| | Group I 10% RBO | Group II 10% SFO |
|---|---|---|
| Total Cholesterol | 81 ± 14 | 89 ± 8.4 |
| Phospholipids | 187 ± 26 | 209 ±18.7 |
| Triglycerides | 85 ± 46 | 136 ± 7.4 |

Table B-2

Initial, Final Body and Change in Body Weights of Rats.

| | Initial Body WT. (g) | | | Final Body Wt. (g) | | | ΔBody Wt (g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | SD | SEM | $\bar{X}$ | SD | SEM | $\bar{X}$ | SD | SEM |
| Rice Bran Oil | 90.3 | 8.6 | 3.3 | 200 | 23 | 8.8 | 111 | 18 | 7 |
| Safflower Oil | 201 | 27 | 10 | 91.8 | 8.7 | 3.3 | 109 | 20 | 7.5 |

$\bar{X}$ = Mean

SD = Standard Deviation

SEM = Standard Error of Mean

## Methods

* 14 , 4-week-old, male Sprague-Dawley rats randomly assigned to two groups
* Group I received a 10% RBO
* Group II received a 10% safflower oil diet
* Diets were fed for 21 day period
* Food and tap water allowed ad libitum
* Room maintained at 22 $\pm$ 1 C
* Relative humidity of 50-60%
* Food intakes and body weights recorded weekly
* At 21 days, rats were fasted for 5-hours, anesthetized and exsanguinated by cardiac puncture
* Blood was collected and serum prepared
* Sera were analyzed for total cholesterol, triglyceride and phospholipid levels.

## Results

* No significant difference was found between the effect of rice bran oil and safflower oil on the serum level of cholesterol, phospholipids or serum triglycerides in male Sprague-Dawley rats.

* There was a trend for animals fed safflower oil to have lower serum lipids than those fed rice bran oil, with percentage difference being 9%, 10.4%, and 37%, for cholesterol, phospholipids, and triglycerides, respectively.