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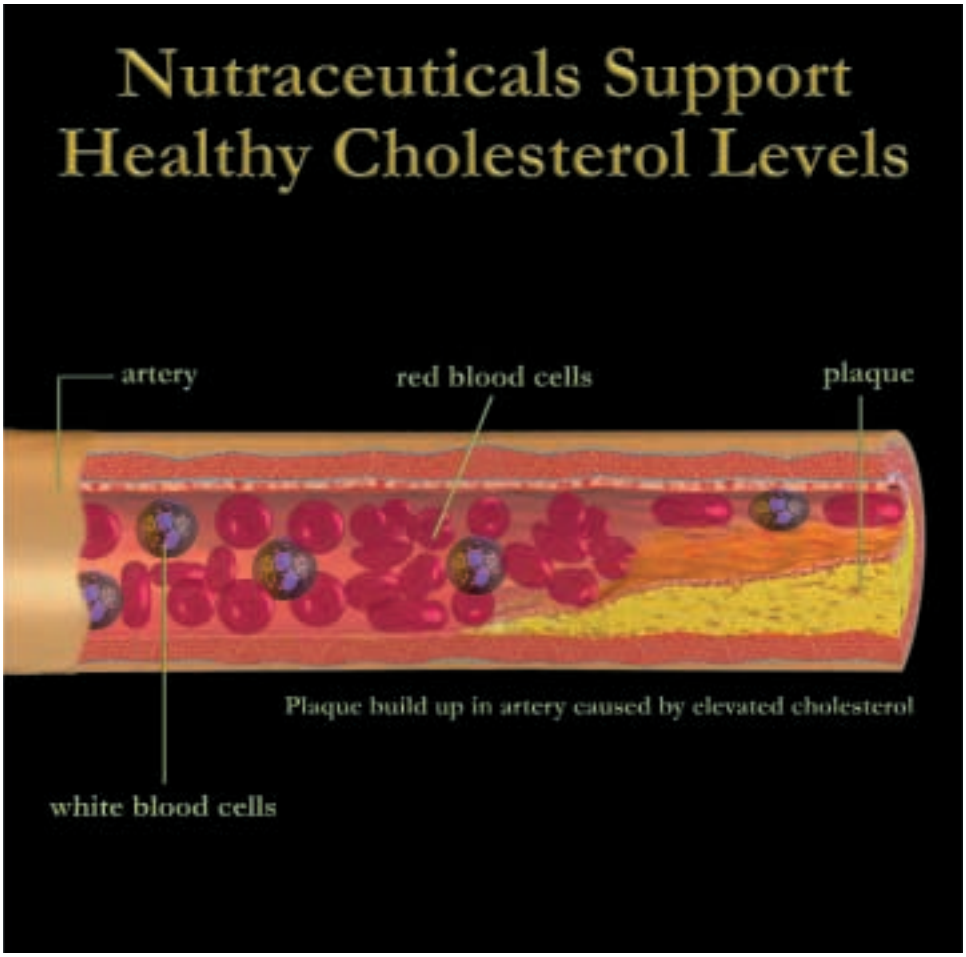

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REVIEW ARTICLE

The Role of Esterin Processed Alfalfa Saponins in Reducing Cholesterol

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CORONARY HEART DISEASE AND CHOLESTEROL REDUCTION

Coronary heart disease (CHD) is the major cause of morbidity and mortality throughout the United States and the industrialized world, accounting for more deaths annually than any other disease, including cancer and costs Americans over 150 billion dollars annually in lost wages, productivity, and treatment. A direct positive relationship between coronary artery disease and hypercholesterolemia has been documented in numerous epidemiologic and clinical studies. Reduction of cholesterol and LDLcholesterol by dietary and pharmacologic intervention reduces CHD and its clinical consequences.

It is known that high serum cholesterol levels may result from either an increase in cholesterol-rich low-density lipoprotein (LDL) commonly caused by high cholesterol or saturated fat intake, or through genetic predisposition. Oxidized LDL-C promotes atherogenesis through attachment to the endothelium of the vasculature, uptake by macrophages, and transmigration into the subendothelial area creating the initial foam cell. This extraluminal accumulation of cholesterol-dense plaque may eventually reduce luminal size, and result in a critical stenotic lesion that may reduce myocardial blood flow and produce ischemia. Activation of platelets and other clotting mechanisms may produce a thrombus, that completely obstructs an artery, leading to acute myocardial infarction.

Interventional prospective clinical trials targeting cholesterol-lowering have conclusively and consistently demonstrated both clinical reduction in CHD events and angiographic regression of CHD.[1] CHD events are reduced by approximately 2% for each 1% reduction in total or LDL-C.[1] Reductions that result in reduced risk for CHD can be obtained through both diet and drug therapy.[1]

EPIDEMIOLOGY

CHD and atherogenesis progress with age in both men and women in the US and other westernized societies primarily as a result of dyslipidemia, but also as a consequence of other CHD risk factors. Framingham and other studies have documented the additive or synergistic effects of hypercholesterdemia, hypertension, glucose intolerance or diabetes mellitus, tobacco abuse, left ventricular hypertrophy, and other CHD risk factors.[2,3,4]

INTRODUCTION

Cholesterol and triglycerides are transported through the bloodstream as spherical particles called lipoproteins, composed of a core and surface. Surface composition

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includes free cholesterol, phospholipids, and protein; the core is composed of mostly triglycerides and cholesterol esters. Lipoprotein abnormalities are associated with CHD, cerebrovascular disease, peripheral vascular disease, pancreatitis, and xanthomas. An inverse relationship exists between low-density lipoprotein (LDL) and high-density lipoprotein (HDL), and the advent of coronary artery disease and atherosclerosis. The total cholesterol (TC)/high density lipoprotein (HDL-C) ratio is a sensitive predictor of CHD.[5] A TC/HDL ratio of less than 4.0 is normal. However, an increase of one point in the TC/HDL ratio will increase the risk of CHD by about 60%.

METABOLISM AND TRANSPORT

Triglycerides and the major plasma proteins, including cholesterol, are essential substrates for cell membrane formation and provide a source of free fatty acids. Hyperlipidemia may be defined as an increase in one or more of the following: cholesterol or cholesterol esters, LDL-C phospholipids, or triglycerides, or reduced HDL-C. An increase in concentration of lipoprotein macromolecules responsible for the transportation of lipids in plasma is defined as hyperlipoproteinemia. Since the density of these lipoproteins is determined by their relative content of protein and lipid, they may be divided into four major classes based on density, composition, and electrophoretic mobility. Low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), and chylomicrons have been designated as the terminology for the major classes of lipoproteins. However, LDL may be further divided into an additional class known as intermediate-density lipoproteins (LDL_1), dense or "fluffy" LDL. HDL has also been divided into HDL_2 and HDL_3 classes of lipoproteins. Changes in HDL are most commonly related to alterations in HDL_2 rather than HDL_3 or HDL_1.[6]

Large triglyceride-rich particles known as chylomicrons are formed as a result of solubilized dietary bile salts in intestinal mucosal cells. These chylomicrons are normally not present in the plasma after a fast of 12 to 14 hours and are catabolized by lipoprotein lipase (LPL) in vascular endothelium and hepatic lipase (HL) to form chylomicron remnants. During this catabolism, chylomicrons are converted to free fatty acids and apolipoproteins (AI, AII, C1, C2, C3), and phospholipids are transferred to HDLs. During this stage, apolipoprotein E and cholesterol ester are transferred to chylomicrons from HDL. These chylomicron remnants are then taken up by the liver to bind to hepatic apolipoprotein B and E receptors. Hepatic VLDL synthesis is regulated partly by diet and by hormones and is inhibited by the uptake of these chylomicron remnants in the liver. At this point VLDL is catabolyzed by LPL, and HL to produce LDL, and, consequently, apolipoproteins C and E are transferred to HDL. VLDL triglycerides are the major triglyceride carriers in plasma.[6]

Similarly, the major cholesterol carrier, LDLis derived mostly from VLDL catabolism. However, in some patients with hypercholesterolemia, direct synthesis may occur. LDLs are metabolized as a result of the interaction of cell surface receptors, internalization and degradation, and receptor-independent mechanisms. Increased intracellular cholesterol that results from the catabolism of LDLinhibits the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase). This negative feedback system is a significant consequence of cholesterol synthesis as HMG CoA reductase is the rate-limiting enzyme for intercellular cholesterol biosynthesis.

TREATMENT

Because total cholesterol is composed of cholesterol derived from LDL, VLDL, and HDL, determination of HDLis critical in all patients even with normal cholesterol. HDL concentration as well as the ratio of LDL to HDL, or TC to HDL can be used to evaluate the degree of risk and necessity of treatment in patients with elevated or normal total cholesterol. The National Heart, Lung, and Blood Institute at the National Institutes of Health (NIH) has made the following recommendations regarding the treatment of patients with hypercholesterolemia.[7]

CLINICAL MANAGEMENT OF HIGH BLOOD CHOLESTEROL

Serum total cholesterol should be measured in all adults 20 years of age and over at least once every 5 years; HDL-cholesterol should be measured at the same time when accurate results are available. These measurements may be made in the nonfasting state. In individuals free of CHD, total cholesterol levels below 200 mg/dL are classified as "desirable blood cholesterol," those 200 to 239 mg/dL as "borderline-high blood cholesterol," and those 240 mg/dLand above as "high blood cholesterol." The cut-point that defines high blood cholesterol (240 mg/dL) is a value above which risk for CHD rises more steeply and corresponds approximately to the 80th percentile of the adult U.S. population (NHANES III). An HDL-cholesterol level below 35 mg/dL is defined as "low," and a low HDL-cholesterol level constitutes a CHD risk factor. Table 1 summarizes these categories.

For primary prevention in adults without evidence of CHD, initial classification is based on total cholesterol and HDL-cholesterol. For individuals with desirable blood cholesterol (<200 mg/dL), the level of HDL-cholesterol determines the appropriate follow-up. Those with HDL-cholesterol greater than 35 mg/dL are given general educational materials about dietary modification, physical activity, and

Initial Classification Based on Total Cholesterol and HDL-Cholesterol

Table 1. Guidelines of the National Heart, Lung and Blood Institute at the NIH

Total Cholesterol	
<200 mg/dL	Desirable Blood Cholesterol
200-239 mg/dL	Borderline-High Blood Cholesterol
>240 mg/dL	High Blood Cholesterol
HDL-Cholesterol	
<35mg/dL	Low HDL-Cholesterol

other risk-reduction activities, and are advised to have repeat total cholesterol and HDL-cholesterol analysis in 5 years. Those with HDL-cholesterol levels less than 35 mg/dL should proceed to lipoprotein analysis. For individuals with total cholesterol levels of 200 to 239 mg/dL, the level of HDL-cholesterol and the presence or absence of multiple other CHD risk factors determine the follow-up. Those with an HDL-cholesterol of 35 mg/dL or greater and fewer than two other risk factors are given instruction in dietary modification, physical activity, and other risk-reduction activities and are advised to repeat total cholesterol and HDL-cholesterol analysis in 1 to 2 years. Patients with total cholesterol levels of 200 to 239 mg/dL who have an HDL-cholesterol less than 35 mg/dL or two or more other risk factors should have a lipoprotein analysis. Lipoprotein analysis is also highly recommended for those whose total cholesterol is 240 mg/dL or greater. Lipoprotein analysis includes measurement of fasting levels of total cholesterol, total triglyceride, and total HDL-cholesterol. From these values, LDL-cholesterol is calculated as follows: LDL-cholesterol = total cholesterol - HDL-cholesterol - (triglyceride/5).

Levels of LDL-cholesterol of 160 mg/dLor greater are classified as "high-risk LDL-cholesterol," those 130-159 mg/dL as "borderline-high-risk LDL-cholesterol," and those <130 mg/dL as "desirable LDL-cholesterol." The clinical evaluation should include a complete history, physical examination, and basic laboratory tests. The aim is to determine whether a high LDL-cholesterol level is secondary to another disease or a drug, and whether a familial lipoprotein disorder is present. The patient's total coronary risk and clinical status, including age and sex, should be considered in developing a cholesterol-lowering program.

For primary prevention, subsequent classification is based on LDL-cholesterol. Individuals with desirable LDL-cholesterol levels (<130 mg/dL) do not need further evaluation and active medical therapy; they should be given information on diet and exercise designed for the general population and be reevaluated in 5 years. Those with borderline-high-risk LDL-cholesterol levels (130-159 mg/dL) who have fewer than two other CHD risk factors should be given instruction in dietary modification and physical activity and be reevaluated in 1 year. Patients with high-risk LDL-cholesterol levels (>160 mg/dL) and those with borderline-high-risk LDL-cholesterol (130-159 mg/dL) who have two or more risk factors should be evaluated clinically and begin active cholesterol-lowering dietary therapy. Assignment of patients to these last two categories should be done on the basis of the average of two LDL-cholesterol determinations to account for biologic variation.

For secondary prevention in adults with evidence of CHD or other clinical atherosclerotic disease, lipoprotein analysis is required and classification is based on LDL-cholesterol. For these patients, the optimum LDL-cholesterol is 100 mg/dL or lower. When a patient has an optimum LDL-cholesterol level, instruction on diet and physical activity should be individualized and lipoprotein analysis repeated annually. When the LDL-cholesterol level is above optimal (>100 mg/dL), appropriate clinical evaluation should be carried out and cholesterol-lowering therapy should be initiated.

Treatment Decisions Based on LDL-Cholesterol

Table 2. Guidelines of the National Heart, Lung and Blood Institute at the NIH

DIETARY THERAPY		
	Initiation Level	LDL Goal
Without CHD and with fewer than 2 risk factors	>160 mg/dL	<160 mg/dL
Without CHD and with 2 or more risk factors	>130 mg/dL	<130 mg/dL
With CHD	>100 mg/dL	<100 mg/dL
DRUG TREATMENT		
	Consideration Level	LDL Goal
Without CHD and with fewer than 2 risk factors	>190 mg/dL*	<160 mg/dL
Without CHD and with 2 or more risk factors	>160 mg/dL	<130 mg/dL
With CHD	>130 mg/dL**	<100 mg/dL

* In men under 35 years of age and premenopausal women with LDL-cholesterol levels 190-219 mg/dL, drug therapy should be delayed except in high-risk patients such as those with diabetes.

** In CHD patients with LDL-cholesterol levels 100-129 mg/dL, the physician should exercise clinical judgment in deciding whether to initiate drug treatment.

Table 2 summarizes the levels for initiating dietary therapy and considering drug treatment in patients with and without CHD, and the LDL goals in these patients.

For patients without CHD or other atherosclerotic disease, the LDL-cholesterol levels for initiation of dietary therapy are: (a) >160 mg/dLin patients with fewer than two other CHD risk factors, or (b) >130 mg/dLin patients with two (or more) CHD risk factors. The target goals of therapy are to lower LDL-cholesterol to levels below the cutpoints for initiating therapy: (a) to below 160 mg/dLwhen fewer than two other risk factors are present, or (b) to below 130 mg/dL when two (or more) CHD risk factors are present. Patients whose LDL-cholesterol exceeds these levels are candidates for dietary therapy and increased physical activity. However, if an elevated LDL-cholesterol persists after an appropriate trial of dietary therapy, drug therapy may be considered. Candidates for drug therapy include patients with multiple CHD risk factors or severe forms of hypercholesterolemia.

A limited number of patients with less severe elevations of LDL-cholesterol and fewer than two other CHD risk factors also may be candidates for drug therapy; examples are patients with diabetes mellitus or a family history of premature CHD. The LDL-cholesterol levels at which drug therapy may be considered after an adequate trial of dietary therapy are: (a) >190 mg/dL in patients with fewer than two other CHD risk factors, or (b) >160 mg/dL in patients with two (or more) CHD risk factors. Often drug therapy can be delayed in young adult men (<35 yrs) and premenopausal women who have LDL-cholesterol levels below 220 mg/dL and who are not otherwise at high risk.

In patients with CHD, therapy should be initiated when the LDL-cholesterol level is >100 mg/dL. The goal of therapy is to reduce LDL-cholesterol to 100 mg/dL or below. Maximal dietary therapy should be employed in patients in this category. When the LDL-cholesterol level remains >130 mg/dL with dietary therapy, drug treatment should be considered. However, when the LDL-cholesterol level is 100 to 129 mg/dL with maximal dietary therapy, clinical judgment must be used as to whether to use cholesterol-lowering drugs. Likewise, when one drug brings the LDL-cholesterol level to this range, clinical judgment is required as to whether to add a second drug.

DIETARY THERAPY

The general aim of dietary therapy is to reduce elevated serum cholesterol while maintaining a nutritionally adequate eating pattern. Dietary therapy should occur in two steps, the Step I and Step II Diets; these are designed to progressively reduce intakes of saturated fatty acids (saturated fat) and cholesterol and to promote weight loss in patients who are overweight by eliminating excess total calories and increasing physical activity. The appropriate use of physical activity is considered an essential element in the nonpharmacologic therapy of elevated serum cholesterol.

The National Cholesterol Education Program's (NCEP's) eating pattern recommendation for the general public is similar in nutrient intake to the Step I Diet.[7] Consequently, at time of detection of high blood cholesterol, many patients may already be adhering to the recommended diet. Therefore, an assessment of the patient's current dietary habits is necessary before prescribing a therapeutic diet. When the patient has not adopted the Step I Diet, this should be the first step of dietary therapy. It should be prescribed and explained by the physician and other involved health professionals. This diet involves an intake of saturated fat of 8 percent to 10 percent of total calories, 30 percent or less of calories from total fat, and intake of less than 300 mg/day of cholesterol. When the patient is already adhering to the Step I Diet at the time of detection, or when this diet proves inadequate to achieve the goals of dietary therapy, the patient should proceed to the Step II Diet. This diet calls for further reduction in saturated fat intake to less than 7 percent of calories and in cholesterol to less than 200 mg/day. The Step I Diet calls for the reduction of the major and obvious sources of saturated fat and cholesterol in the diet; for many patients this can be achieved without a radical alteration in dietary habits. The Step II Diet requires careful attention to the whole diet so as to reduce intake of saturated fat and cholesterol to a minimal level while maintaining an acceptable and nutritious diet. Involvement of a registered dietitian or other qualified nutrition professional is very useful, particularly for intensive dietary therapy such as the Step II Diet.[7]

The relationship between nutrition and disease prevention is not a newly recognized phenomenon. Antioxidants such as alphatocopherol, tocotrienols, ubidecarenone, ubiquinone, alpha lipoic acid, cysteine, ascorbic acid and others have been heavily scrutinized due to their ability to inhibit oxidation and lipid peroxidation and consequently, atherosclerosis. Recently discovered cell-regulating properties of vitamin E have been implicated in the function of platelets, monocytes and macrophages, endothelial cells and vascular smooth muscle.[8] Epidemiological studies have suggested a significant reduction in cardiovascular disease and cancer with the consumption of 7 to 9 servings of fruits and vegetables per day.

The effects of garlic as an antihypertensive agent, as well as a hyperlipidemia agent, have been explored by several researchers.[9] In addition, plant sterols found in a wide variety of foods, including soybeans and rice, possess a similar shape and structure as cholesterol. In fact, they mimic cholesterol so effectively that the human digestive tract cannot distinguish sterols from cholesterol.[9] As the body attempts to absorb the sterol compound (as opposed to the actual cholesterol) the cholesterol is largely unabsorbed and excreted in the feces[3], resulting in a decrease in cho-

lesterol absorption and the lowering of both LDL and total cholesterol levels by 10-15%.[10]

Similarly, Steinmetz and others reviewed 206 human epidemiology studies and 22 animal studies for the relationship between vegetable and fruit consumption for cancer risk.. Vegetables and fruits most commonly associated with protective effects include raw vegetables, allium vegetables, carrots, and tomatoes. Substances present in vegetables and fruit found to be protective included dithiolthiones, isothiocyanates, isoflavones, saponins, and others.[11]

Regardless of the intervention used (diet, surgery, medications), decreased levels of plasma cholesterol have consistently produced a reduction in CHD risk.[12,13]

CLINICAL EFFICACY OFALFALFA SAPONINS

The exact mechanism of action for the cardioprotective effects of saponins is not known. However, theories include a tissue plasminogen activator-like effect that reduces thrombosis,[14] a monoamine transmitter action,[15] calcium antagonism,[16] a digoxin-like effect,[17] and a nitric oxide action.[18]

Purmova and Opletal also reported that saponins may possess the ability to modulate the Na(+)K ATPase system and positively affect cardiac processes by inhibiting the formation of lipid peroxidases.[17,19] Additional positive effects on the immune system and glucose modulation were also reported. This is consistent with the work of Johns et al who reported a potential antioxidant role for saponins in relation to hypocholesterolemia.[20]

Of interest is the work of Wang et al who reported that saponin-treated patients attained angina pectoris remission rates of 82.3%, and 52.7% had an improvement in ECG results.[21] The authors concluded that saponins may improve angina pectoris through dilation of the coronary artery and thereby improve coronary circulation. This is consistent with other works by Liu et al, who concluded that patients with CHD-associated angina pectoris treated with saponins to be more effective as compared to a control group treated with Nifedipine. Improvements in ECG and impedance cardiogram were also reported.[21]

The ability of various sterols to form water-insoluble complexes with steroid saponins has been known for a long period of time. Saponins remain within the gastrointestinal tract. Some interact directly with cholesterol, producing an insoluble complex that prevents cholesterol absorption. Others appear to affect cholesterol metabolism indirectly by interacting with bile acids and increased fecal excretion of bile acids. As a result, bile acids reduced the enterohepatic recycling of cholesterol.[22]

Saponins are amphillic (partly hydrophilic, partly hydrophobic) compounds of sugars linked to a non-polar group. They occur almost exclusively in plants (soybeans, alfalfa, beans, spinach, and others as outlined in Table 3). When administered intravenously saponins produce a strong hemolytic effect. Since they are not absorbed by the gut, they are practically non-toxic when ingested orally. Several hundred different types of saponin molecules have been isolated from the plant kingdom. Some may have the ability to lower plasma cholesterol concentrations in a number of animal models. Therefore, some saponin-containing foods listed below may be important in designing cholesterol-lowering hypocholesterolemic products for human consumption.[22]

It has been established that saponins with acid sapogenins (ones that exhibit carboxylic groups) bind cholesterol in vitro, while saponins with neutral sapogenins do not form complex cholesterol. However, saponins with neutral sapogenins can form complexes with bile salts more easily than the acidic saponins. Therefore, there is a synergism with acidic and neutral saponins relative to the reduction of cholesterol. The only known edible plant to possess both kinds of saponins is alfalfa.

Table 3. Saponin content of some common foods.

Food Plant	Saponin content (g / Kg dry matter)
Chickpeas	56
Soyabeans	43
Alfalfa	56
Alfalfa sprouts	87
Navy beans	21
Green beans	13
Haricot beans	19
Red kidney beans	16
Peanuts	6.3
Mung beans	5.7
Mung bean shoots	27
Spinach	47
Silver beet	58
Lentils	4.6
Faba beans	4.3
Broad beans	3.5
Sesame seeds	3
Green peas	11
Asparagus	15
Garlic	2.9
Lima beans	1.1
Oats	1.0

Animal studies to explore the hypocholesterolemic effect of alfalfa were first conducted in 1976 by Rashaf et al. In these experiments, rats were fed 2% saponin extract. There was a trend towards a decrease in liver cholesterol production in the saponin-fed group. The endogenous cholesterol that passed from the liver to the gut interacted with the saponins and could not be reabsorbed, but did reappear as a surplus in the feces.[23] Malinow et al in 1977 conduct-

ed a similar experiment in monkeys and also concluded that saponins interfered with the intestinal absorption of cholesterol.[24] In 1983 Malinow investigated the atherosclerosis-inhibitory effect of saponins in animal models by evaluating the sclerotic changes of vessels for saponin-fed animals as compared with non-saponin fed animals. Animals who were fed saponin extracts did not exhibit fibrotic changes characteristic of regressed atherosclerosis.[25]

One of the earliest human studies was performed by Beaumont et al in 1970 where 15 patients with documented hyperlipoproteinemia were administered 40 grams of alfalfa seeds four times a day for 8 weeks. At the conclusion of the study, patients were divided into responders and non-responders. Patients with type II disorder had the most significant decrease in cholesterol (average of 25%). Patients with type IV disease had the least response to the alfalfa therapy. There were no clinical adverse effects observed. The authors concluded that patients with type II disease might benefit from alfalfa therapy to normalize plasma cholesterol concentration.[26]

Molgaard et al in 1987 investigated the efficacy of a regimen that called for 40 grams of heat-prepared alfalfa seeds given to patients three times a day for 8 weeks. Patients with type II disorder produced a 26% and 30% decrease in total cholesterol and LDL, respectively. The authors concluded that alfalfa seeds could be added to the diet to assist in the normalization of serum cholesterol in patients with type II hyperlipoproteinemia.[27]

Ivan Golub, MD, Chief Commissioner, Chronic and Rehabilitation Medical Wards, Budpapest, and Chief Physician, Pharmaceutical Committee of Budapest, conducted a clinical trial in 1992 to evaluate the efficacy and safety of Esterin processed alfalfa. Initially 30 patients were enrolled in the 16 week study at a dose of 1 gram twice a day.[28] Ten patients withdrew prior to study conclusion. Reasons given for withdrawal included: incorrect use of the product–3 persons; negligence in following the concurrent use of pharmaceutical products–3 persons; excessive alcohol consumption–1 person; and intercurrent illness–2 persons.

For the 20 patients who concluded the study, the breakdown of the patients according to the type of diabetes was:

Low CH-tolerance (IGT): 4 persons (20%)

Type II diabetic (NIDDM): 12 persons (60%)

Type I diabetic (IDDM): 4 persons (20%)

STUDY RESULTS

At the conclusion of the study Dr. Golub reported an average decrease of total serum cholesterol from 8.4 mmol/liter (15%), $p<0.01$, in 20 diabetic patients with concurrent hypercholesterolemia that occurred after 4 weeks of treatment with Esterin processed alfalfa dosed at 1 gram twice a day. At the end of week 8 of the study, the average drop of total serum cholesterol in all 20 patients was 24% to 6.73 mmol/liter ($p<0.01$). After another two months there was no further significant drop (27%), and the level of decrease actually became steady.

Following the administration of Esterin processed alfalfa, the LDL-cholesterol decreased from 6.82 mmol/liter to 5.38 mmol/liter (21%), $p<0.01$, at the end of week 4. At the end of week 8, this value decreased to 4.82 (29%), $p<0.01$, and by the end of the study it decreased to 4.61, a 32% total drop, $p<0.01$.

Following consumption of Esterin processed alfalfa, the average decrease of triglyceride (TG) was from 3.02 mmol/liter to 2.6 mmol/liter (14%), $p<0.05$. At the end of week 8, TG levels decreased to 2.56 mmol/liter (15%), $p<0.05$, and at the conclusion of the study, the average of the TG values decreased to 2.45 mmol/liter, an 18% change from the baseline. A significant decrease was noticed after 4 weeks, after that, the improvement was not significant.

At the beginning of the study the average of the HDL-cholesterol level was 1.04 mmol/liter. After week 4 the average increased to 28% to 1.33 mmol/liter, $p<0.01$. At the end of 8 weeks it was 1.40 mmol/liter (35%), $p<0.01$, and at the end of the study it increased by 43% to 1.4 mmol/liter, $p<0.01$.

The change in the "Atherogenic-index" (LDL/HDL) also indicated a favorable shift in the proportions due to a reduced LDL level and an increased HDL level. The start-

Table 4. Results of Golub study.

Parameter	% Change At Week 4 (mmol/L)	% Change At Week 8 (mmol/L)	% Change At Week 16 (mmol/L)
Serum Total Cholesterol	- 15% ($p<0.01$)	-24% ($p<0.01$)	-27% (not significant)
Serum Triglyceride	-14% ($p<0.05$)	-15% ($p<0.05$)	-18% (not significant)
Serum HDL Cholesterol	+28 ($p<0.01$)	+35% ($p<0.01$)	+43% ($p<0.01$)
Serum LDL Cholesterol	-21% ($p<0.01$)	-29% ($p<0.01$)	-32% ($p<0.01$)
Atherogene-index (LDL/HDL)	-38% ($p<0.01$)	-47.5% ($p<0.01$)	-53% ($p<0.01$)

ing index value of 6.56 decreased significantly after 4 weeks to 4.05 (38%), p<0.01; after 8 weeks it decreased 47.5% to 3.44, p<0.01, and by the time of study conclusion it was 3.09, for a total decrease of 53%.

Blood Sugar Values: The average of the patient profiles at the beginning of the study was 9.2 mmol/liter, the average of the fasting blood sugars was 8.3 mmol/liter, and the daily average of sugar discharge was 5.6 grams. These levels did not show significant changes during the study period, although these parameters were lower at study conclusion. The average of the blood sugar profiles declined to 8.7 mmol/liter, the fasting blood sugars were 7.7 mmol/liter, and the sugar discharge declined to 5 gram/day at the end of week 16.

Andrew Czeizel, MD, professor of human genetics and teratology, Semmelweis Medical University, Budapest, Hungary, and Head, World Health Organization (WHO) Collaborating Center for the Community Control of Hereditary Diseases, reported in 1992 positive changes in lipid levels in a placebo controlled study involving 40 patients. During the study, patients with serum total cholesterol > 6 mmol/L, serum triglyceride > 3 mmol/L, and serum HDL < 1 mmol/L were divided into a treatment group and a placebo group. Diet and exercise were monitored to ensure no activity changes occurred or medications were consumed during the study period. Serum total cholesterol, serum triglyceride, and serum HDL and LDL cholesterol were measured at the beginning, interim (42 days), and at study conclusion. Administration of 2 gm daily of Esterin processed alfalfa after a meal produced favorable serum effects on cholesterol in patients assigned to the treatment group; the average reduction in total serum cholesterol was 11.64%, p<0.01 with a maximum reduction of 22.8%, p<0.01.

The treatment group showed a significant increase in serum HDL, with an average increase of 25.72%, p<0.01, and a maximum increase of 52.2%, p<0.01; also there was a significant decrease in serum LDL of 21.17%, p<0.01, with a maximum reduction of 37.4%, p<0.01. The Atherogen index (Cholesterol/HDL) showed an average improvement of 29.08%, with a maximum improvement of 42.4% for the treatment group which was also significant (p<0.01). The HDL/LDL ratio also showed a favorable change, rising an average of 63.22%, with a maximum change of 96% for the treatment group at the end of the 12 week study (p<0.01). The average serum triglyceride level for the treatment group decreased 4.91% at the end of 12 weeks, and was not statistically significant (Table 5). There were no serious adverse reactions reported during the study.[29]

Eva Szigeti, MD, specialist in internal diseases and scientific secretary at the Medical Department of the Hungarian Rehabilitation Association, evaluated the efficacy of Esterin processed alfalfa following the registration and evaluation of the product by the National Pharmaceutical Institute of the Hungarian Ministry of Health and Welfare in 1992. Dr. Szigeti's study involved 22 patients (5 females and 17 males). Results demonstrated a decrease in total cholesterol and triglycerides and an increase in HDL in patients treated with Esterin processed alfalfa at 2 grams twice a day for 3 months (Table 6). Dr. Szigeti reported the following average changes in blood lipid levels according to sex: females: serum total cholesterol decreased 25.26%, serum triglyceride decreased 20.21%, and serum HDL-cholesterol increased 25.16%. Males: serum total cholesterol decreased 20.6% (significant), serum triglyceride decreased 25.09, and serum HDL-cholesterol increased 32.69%.

In addition to the changes in blood lipid levels indicated in table 6**,** 90% of patients experienced a 4.4 to 8.8 pound weight loss. The principal investigator concluded that Esterin processed alfalfa was very effective in reducing patient cholesterol and triglyceride levels and in increasing the HDL concentration.[30]

A study on 11 chronic hyperlipidaemic patients was conducted by Csak Ilona, MD, adjunct professor, Kutvolgyi Hospital, Semmelweis Medical University of Budapest, from March 1 to May 30, 1991. Patients with baseline serum cholesterol > 6 mmol/L, serum triglyceride > 3 mmol/L, and serum HDL < 1 mmol/L were evaluated. At study conclusion Dr. Illona reported that patients given

Table 5. Summary of results of the WHO study on lipid parameters conducted by Dr Czeizel in 1992

Average Evaluated During 12 week Study	Average Change week 12 (mmol/L)	Maximum Change week 12 (mmol/L)	Minumum Change week 12 (mmol/L)	P Values
Serum Total Cholesterol	-11.70	-22.80%	+2.30%	P<0.01
Serum Triglyceride	-4.19	-40.10%	+30.10%	not significant
Serum HDL Cholesterol	+25.72	52.20%	+6.30%	P<0.01
Serum LDL Cholesterol	-20.57	-37.4%	-1.50%	P<0.01
Atherogen Index (CHOL/HDL)	-29.08%	-42.40%	-7.70%	P<0.01
HDL/LDL Ratio	+63.22%	+96.00%	-18.20%	P<0.01

Table 6. Conclusions of lipid parameter changes from the Szigeti study.

Changes in Lipid Parameter	< 10%	Between 10 & 20%	> 20%
Serum Total Cholesterol (declined)	7 patients	6 patients	9 patients
Serum Triglyceride (declined)	7 patients	3 patients	12 patients
Serum HDL Cholesterol (increased)	5 patients	5 patients	12 patients

Table 7. Final changes of lipid parameters from Dr Ilona's study at Semmelweis Medical University.

Changes in Lipid Parameter	% change
Serum Total Cholesterol	-20.50
Serum Triglyceride	-16.60
Serum HDL Cholesterol	+13.3

Table 8. Results of Ilona's 1990 study on lipid parameter changes after supplementation with Esterin Processed Alfalfa.

Parameter	% Change At End Of Week 4 (mmol/L)	% Change At End Of Week 16 (mmol/L)
Serum Total Cholesterol	- 23.2	-28.6
Serum HDLCholesterol	+11.5	+11.1
Serum LDL Cholesterol	-24.3	-27.7

1 gram of Esterin processed alfalfa twice a day for 3 months produced favorable changes in lipid profiles as listed in Table 7. Hematological parameters that included quantitative and qualitative white blood cell count, hematocrit, hemoglobin, platelet, blood glucose, vitamins A and D, sodium, potassium, alkaline phosphatase, SGOT, SGPT, gamma GT, creatine, total protein, albumin, and uric acid were measured and remained within normal limits throughout the 3-month study period. There were no essential adverse effects observed during the trial period.[31]

This is consistent with Ilona's earlier works from 1990 where 60 patients aged 20 to 70 years with total cholesterol > 250 mg / 100 ml and triglyceride < 250 mg% were administered 1 gram of Esterin three times daily for 2 weeks, and then 1 gram twice daily after meals for a 4 month period of time (Table 8). Hematological parameters measured included quantitative and qualitative white blood cell count, hematocrit, hemoglobin, platelet, blood glucose, vitamins A and D, sodium, potassium, alkaline phosphatase, SGOT, SGPT, gamma GT, creatine, total protein, albumin, and uric acid. These were measured and remained within normal limits throughout the study period. Significant changes in blood lipid levels are shown below. Similar to the other studies, there were no significant adverse reactions to the Esterin processed alfalfa.[32]

SAPONIN TOXICITY

The saponins found in unprocessed alfalfa plants possess significant antilipidemic effects, but contain toxic substances coumesterol and canavanine. Many commercial alfalfa products contain from 20 to 190 ppm of coumesterol, which amounts to approximately 1 mg of this estrogenic hormone and may lead to pathological side effects. Canavanine has been associated with a systemic lupus-like erythematosus. Esterin processed alfalfa, however, is derived through a technically advanced patented extraction process which removes coumesterol and canavanine from the alfalfa leaf, and provides an extremely potent form of saponins from the alfalfa leaf that has been shown in clinical studies to reduce total serum cholesterol levels without serious adverse reactions.

DRUG THERAPYAND ADVERSE EFFECTS OF LIPID LOWERING AGENTS

Numerous cholesterol intervention trials have demonstrated that 3-hydroxy-3 methylglutaryl coenzyme A reductase inhibitors (statins) significantly reduce total serum cholesterol, total serum triglycerides, and low density lipoproteins.[33-39] Triglyceride and LDL reductions have been reported to range from 16-47% and 18-55%, respectively. Reductions in total cholesterol and increases in HDL average approximately 26% and 4%, respectively.[37] The most common reported side effects from patients taking statin products were mostly gastrointestinal in nature.[38] However, as many as 3% of patients may experience elevated liver enzymes. The majority of liver abnormalities present within 3 months of therapy initiation and is considered to be dose dependent.[40] An uncommon syndrome, rhabdomyolysis, is reported in only about 0.1% of patients who receive treatment with a statin. However, this incidence may be increased when these agents are used in combination with other agents that utilize the cytochrome P450 system for metabolism (fibric acid derivatives, erythromycin, cyclosporin, and fluconazole).[41] Therefore, caution is warranted when using these agents in combination with drugs metabolized by the CYP450 pathway, or in patients with existing hepatic impairment.

SUMMARY

Millions of people die each year as a result of coronary heart disease and its consequences. This disease remains a challenge for patients, family members, and clinicians as it is compounded by poor dietary habits in the United States and other westernized countries. Nonpharmacologic and pharmacologic reduction in cholesterol levels will significantly decrease CHD morbidity and mortality.

Though the studies on Esterin processed alfalfa have been limited, the outcomes demonstrated similar results: a lowering of total serum cholesterol, serum triglyceride, serum LDL cholesterol, an increase in HDL cholesterol, and an improvement in both the Atherogen Index (CHOL/HDL) and HDL/LDL ratio. These initial results are promising, and larger more well designed prospective clinical studies are warranted to confirm early studies on Esterin processed alfalfa.

Esterin processed alfalfa, a natural dietary supplement, may be selected when total serum cholesterol is mildly elevated, when the use of a HMG CoA reductase inhibitor is contraindicated, or in combination with numerous antilipid agents (i.e,statins, fibrates, niacin, resin binders) for overall cholesterol management.

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