

## *Cardiovascular Disease*

### **Dyslipidemia: Non-Pharmacologic Treatment**

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#### **INTRODUCTION**

Cardiovascular disease (CVD) is the number one cause of morbidity and mortality in the United States,<sup>1</sup> coronary heart disease (CHD) and myocardial infarction being the leading causes of death.<sup>1</sup> The five major risk factors for CHD – hypertension, dyslipidemia, diabetes mellitus, smoking, and obesity – account for 80% of the risk for CHD.<sup>1,2</sup> Interventions, both pharmacologic and nonpharmacologic, can improve all of these risk factors and decrease the incidence of CVD and its consequences, such as myocardial infarction, angina, congestive heart failure and stroke.<sup>3-6</sup>

Recent guidelines by the National Cholesterol Education Program (NCEP) recommend more aggressive control of serum lipids to reduce the incidence of CHD.<sup>7</sup> Nutritional and dietary therapy, weight loss, exercise, and scientifically-proven nutritional supplementation should be used initially in appropriately selected patients to manage dyslipidemia. Hypertriglyceridemia, which is frequently due to obesity, insulin resistance, metabolic syndrome and diabetes mellitus, deserves special attention.<sup>7</sup> Pharmacologic therapy should be administered in those cases that are at high or very high-risk for CHD and those who do not respond to non-drug therapy. Many patients prefer non-drug therapies for many reasons including adverse effects of anti-lipid drugs, contraindications or allergic reactions to drugs, perceptions of adverse effects of drugs, or personal preference for natural or alternative therapies. A more aggressive integrative approach to the management of dyslipidemia is recommended to improve CHD outcomes, minimize adverse effects, and reduce health-care costs.

#### **NUTRITION AND EXERCISE**

Optimal nutrition and proper aerobic and resistance exercise form the cornerstone for the management of dyslipidemia. Changes in weight and body composition can have a dramatic effect upon serum lipid levels. Indeed, loss of total body and visceral fat can improve serum lipid levels to a similar level that is obtainable with many pharmacologic therapies. This section will discuss the numerous modalities that have been studied in the literature which can provide an effective initial treatment plan for dyslipidemia.

##### *Nutrition*

Multiple approaches to diet therapy have been initiated for improvement of hyperlipidemia and reduction of cardiovascular disease. Dietary approaches extend from one extreme to another regarding fats, sugar, and protein content. Dean Ornish investigated 48 individuals with moderate to severe cardiovascular disease established by quantitative angiography in a randomized trial. Subjects were randomized to a usual-care control group or to an intensive lifestyle change group. Intervention in the intensive lifestyle group included: eating a 10% fat whole foods vegetarian diet, taking regular aerobic exercise, stress management training, smoking cessation, and group psychosocial support. In total, 20 of the 28 individuals assigned to the intensive lifestyle change group and 15 of 20 people assigned to the control group completed the 5-year-long trial. Results showed that those in the lifestyle change group had a decrease of 1.75 absolute percentage points in the diameter of stenosis at 1-year (a 4.5% relative improvement) and 3.1 after 5-years ( a 7.9% relative improvement). In contrast, the percent diameter in the control group increased by 2.3 percentage points in 1-year (a 5.4% relative worsening) and 11.8 percentage points (a 27.7% relative worsening) in 5-years. Even with this small cohort, these results were statistically significant.<sup>8</sup> In a subsequent trial that used the Ornish approach, 440 men and women with coronary disease and diabetes mellitus were evaluated for compliance to comprehensive lifestyle therapy for 1-year. Of the 440, 347 were men (55 with diabetes mellitus), and 93 were women (33 with diabetes mellitus). Results showed that adherence to the lifestyle changes prescribed led to significant weight reduction (average of -5 kg), body fat reduction, LDL reduction, functional capacity improvement and quality of life indices. No significant changes were noted in HDL or triglycerides. Within the diabetic cohort, 20% of the patients had a decrease in the amount of diabetic medication required to control their blood sugar.<sup>9</sup>

Overweight patients with type 2 diabetes present a difficult challenge to reduce their cardiovascular risk factors. The Look AHEAD trial involved 5,145 individuals with type 2 diabetes with a BMI greater than 25. Intensive lifestyle intervention with group and individual meetings were designed to achieve and maintain weight loss by decreasing caloric intake and increasing physical activity. The control group included diabetes support and education only. The lifestyle intervention group was designed to induce a minimum weight loss of 7% from initial body weight primarily by caloric restriction. The maximum caloric uptake from fat was 30%, saturated fat 10%, and the minimum from protein was 15%. Participants were prescribed portion-controlled diets utilizing liquid meal replacements and frozen entrees. The exercise program was graduated to a goal of 175 minutes of moderate activity per week. The 1-year data revealed the intensive lifestyle group lost an average of 8.6% of their total body weight versus 0.7% in the control group. Mean HbA1c decreased from 7.3% to 6.6% in the intensive lifestyle group and 7.3% to 7.2% in the control group. Systolic and diastolic blood pressure, triglycerides, HDL cholesterol, and the urine albumin to creatinine ratio all improved significantly more in participants in the intervention group than in participants in the control group. The authors concluded: "At 1-year, intensive lifestyle intervention resulted in clinically significant weight loss in people with type 2 diabetes. This was associated with improved diabetes control and CVD risk factors and reduced medicine use."<sup>10</sup>

The other dietary extreme extends the Atkins approach. Yancy *et al* compared the effects of a low carbohydrate (less than 20 g/day) ketogenic diet (LCKD) with those of a low fat diet (LFD) where less than 30% calories were obtained from fat and less than 10% from saturated fat. Exercise recommendations and group meetings were provided to both groups. In the patient population, 76% completed the study in the LCKD group as opposed to only 57% in the LFD group. In 24-weeks, there was greater weight loss in the LCKD group than in the low fat group (12.9% vs. 6.7%). The LCKD group had a greater decrease in triglyceride levels (74.2 mg/dl vs. 27.9 mg/dl). HDL increased in the LCKD group compared to the low fat group (5.5 mg/dl vs. 1.6 mg/dl). LDL cholesterol did not change significantly. The authors concluded: "Compared with a low-fat diet, a low-carbohydrate diet program had better participant retention and greater weight loss. During active weight loss, serum triglyceride levels decreased more and high-density lipoprotein cholesterol level increased more with the low-carbohydrate diet than with the low-fat diet."<sup>11</sup>

More detailed information concerning cardiovascular risk reduction can be obtained by evaluating lipoprotein subclass analysis. Studies have suggested that LDL particle concentration and HDL particle concentration are more predictive in assessing cardiovascular risk.<sup>12</sup>

There is discordance between LDL or even non-HDL cholesterol and LDL particle concentration (LDL-P). This is most pronounced in patients with type 2 diabetes mellitus, metabolic syndrome, or familial combined hyperlipidemia. In numerous studies,<sup>13-15</sup> NMR technology has been utilized and demonstrated superior predictive power in assessing cardiovascular risk. NMR utilizes the unique signal generated by each lipoprotein to identify the type of particle (LDL, HDL, and VLDL). The apo-protein components of each particle are unique and constant allowing the number of particles to be measured. The amount of triglyceride and cholesterol contained in each particle can vary significantly leading to the discrepancy between estimates of risk assessed by LDL-C and LDL-P. There can be as great as a 70% difference in particle concentration when the amount of cholesterol and triglyceride per particle is constant, but there is a change in particle size. At the same particle size, the concentration of triglyceride and cholesterol can vary leading to as much as a 40% difference of LDL-P at the same LDL-C.

Westman *et al* evaluated the effects of the LCKD versus LFD on NMR lipoprotein subclass analysis. Using standard analytical measures of lipid, there was a decrease in triglycerides in both groups, but it was significantly greater in the LCKD group. HDL increased significantly in the LCKD group, but not the LFD group. LDL did not change significantly in either group. Both diets had a positive effect on lipoprotein subclasses with less large, medium, and small VLDL particle concentrations with a greater change in the LCKD for medium and small VLDL particle concentration, which was statistically significant. There was an increase in VLDL particle size in the LCKD group versus the LFD group. LDL particle size increased in both groups with an increase in large LDL particle concentration and a decrease in medium and small LDL particle concentrations. Between groups, there was a statistically greater effect for large and medium LDL particle concentrations in favor of the LCKD group. Large and small HDL particle concentrations increased in both groups with no significant difference between the two diet groups. It was acknowledged that the LCKD group were given diet supplements containing omega-3 fatty acids, which are known to lower triglycerides and slightly raise HDL and LDL, but the daily dose was modest (1200 mg per day of fish oil and flax oil).<sup>16</sup> A study by Foster *et al* comparing LCKD and LFD

failed to demonstrate a maintenance of weight reduction over a 1-year period of time. Though weight loss was not maintained, lipid differences with HDL and triglycerides were maintained.<sup>17</sup>

The Portfolio diet, designed to lower LDL-C, consists of foods high in viscous fiber, soy protein, and plant sterols, such as nuts. These foods are known to reduce cholesterol. Jenkins *et al* compared a very-low-saturated fat control diet, the same diet plus lovastatin 20 mg (statin diet), and the Portfolio diet. The four major components of the Portfolio diet were: plant sterols, soy-protein foods, almonds, and viscous fibers (from oats, barley, psyllium, and the vegetables okra and eggplant). The three diets were essentially calorically equivalent. Within this 4-week trial, weight was maintained in the three groups. The results revealed a reduction in LDL-C in the Portfolio diet group and the statin group, with the latter group having a slight edge that was not statistically significant. More participants attained their NCEP goal on the Portfolio diet than on the statin group. HDL was not significantly affected.

Dietary therapy focusing on cardiovascular risk reduction incorporates whole foods rather than food components. Dietary studies suggest three strategies for the promotion of cardiovascular health:

1. The substitution of non-hydrogenated unsaturated fats for saturated and trans-fats.
2. Increasing dietary consumptions of omega-3 fatty acids from marine and plant sources.
3. Increasing consumption of low glycemic fruits and vegetables, nuts and whole grains, and reducing the consumption of refined grain products.

These three components are the essentials of the modern Mediterranean diet.

Numerous studies confirm the observations of Keyes and Hegsted that replacement of saturated fat with polyunsaturated fatty acids decrease total and LDL cholesterol.<sup>19-21</sup> Saturated, polyunsaturated and monounsaturated fats increase HDL levels modestly. When carbohydrates are substituted for saturated fat, the decrease in LDL and HDL stays constant and the ratio of total cholesterol to HDL does not change. When saturated fat is replaced with monounsaturated fats and polyunsaturated fats, LDL decreases and HDL stays the same or nominally increases and the ratio improves (total cholesterol:HDL or LDL:HDL). Additionally, changing to monounsaturated fats can assist in controlling insulin sensitivity and, thereby, improving control of type 2 diabetes.<sup>22</sup> Trans-fatty acids are found in stick margarine, vegetable shortening, commercially prepared baked goods and deep-fried foods. Trans-fatty acids increase LDL and decrease HDL, worsening the ratio (total cholesterol:HDL or LDL:HD).<sup>23</sup> Trans-fatty acids also increase triglycerides,<sup>24</sup> increase lipoprotein-a (Lp(a)),<sup>25</sup> and promote endothelial dysfunction.<sup>26</sup>

Omega-3 fatty acids have been shown to decrease fatal coronary heart disease in multiple population studies. The mechanism for that reduction was elucidated through the DART study<sup>6</sup> and the GISSI III Prevenzione trials.<sup>27</sup> The primary benefit is the reduction of sudden cardiac death. Subsequent studies have shown increased threshold of development of lethal ventricular arrhythmias.<sup>28</sup> The amount of omega-3 fatty acids (DHA and EPA) required for benefit is 850 mg daily. The most recent data suggests that higher doses of omega-3 fatty acids increase plaque stability, decrease inflammatory markers, and decrease inflammatory cells within the plaque.<sup>28</sup> In the Japanese JELIS trial, adding 1.9 g of EPA daily to the diet of a population that consumes 8-times more omega-3 fatty acids than their American counterparts, was shown to significantly reduce nonfatal myocardial infarction and overall coronary heart disease mortality.<sup>29</sup> The JELIS trial implies that our current recommendation of 2-5 fish servings weekly may not enough to obtain the full benefits that omega-3 fatty acids can deliver. There is uncertainty regarding optimal balance for omega-3 and omega-6 fatty acids. There are studies suggesting that omega-6 fatty acids reduce risk of developing coronary heart disease, but opposing studies suggests this not to be true. In fact, at the cellular level, omega-6 fatty acids have the opposing effect of omega-3 fatty acids. Presently, there is insufficient data to make firm recommendations regarding omega-6 fatty acids. Diets that increase omega-3 fatty acids can definitely be recommended and are supported by the current literature.

Joshiyura *et al* conducted a study examining the relationship between fruit and vegetable consumption and risk for CHD. The studied included 84,251 women and 42,148 men. Results revealed an inverse relationship between consumption of fruits and vegetables and the risk for CHD. Green leafy vegetables and vitamin C-rich fruits and vegetables were found to be most beneficial in terms of CHD risk reduction.<sup>30</sup> Studies demonstrating the benefits of whole grain consumption by Jacobs<sup>31</sup> and Liu<sup>32</sup> revealed that eating a diet rich in whole grain foods could reduce the risk of CVD by as much as 28-33%. The importance of using complex carbohydrates has been stressed. Simple carbohydrates (white starches such as white bread and potatoes) can be converted to simple sugar rapidly, producing higher glycemic states and insulinemic responses.<sup>33</sup> One method of ranking foods that have this potential is to know their glycemic index. The glycemic index is defined as the amount of a given food that will raise the

blood glucose level equivalent to a 50 g carbohydrate load. Foods with lower levels of starch (oatmeal) and higher levels of viscous fiber (barley, oats, rye and nuts) have slower rates of digestion and, therefore, lower glycemic indices. Several studies suggest that foods with low glycemic indices demonstrate improvement in glycemic control and lipid profiles in patients with type 2 diabetes mellitus, and thus presumably in patients with metabolic syndrome.<sup>34</sup> Multiple studies<sup>35-37</sup> have demonstrated an inverse relationship between nut consumption and risk of CHD. Of particular interest are nuts high in monounsaturated and polyunsaturated oils, which have been shown to lower LDL cholesterol (almonds and walnuts are examples). The recommended amount is three ounces or ¼ cup daily. Consideration must be given to total caloric intake in individuals on weight management programs.

Merit must still be given to a “whole food” nutrition diet. The Indian Heart Study<sup>38</sup> and the Lyon Heart Study<sup>39</sup> revealed a significant reduction in heart disease mortality despite lipid parameters that did not change dramatically. This suggests there is more to beneficial effects from dietary therapy than just lipid management.

In summary, a practical approach for reducing cardiovascular risk would be a modified Mediterranean approach. This incorporates replacement of saturated and trans fatty acids with mono and polyunsaturated fats, increasing omega-3 fatty acid consumption and consumption of components of the Portfolio diet, plant sterols, viscous fiber, vegetables, low glycemic fruits, soy protein, and nuts.

### *Exercise*

A preponderance of evidence suggests regular moderate exercise prevents development and progression of atherosclerosis and benefits dyslipidemia and reduces vascular symptomatology in patients with documented CVD. The mechanism of benefits is derived from maintenance of body weight, blood pressure control, and insulin resistance and dyslipidemia management, all of which promote endothelial stabilization and vascular health.<sup>40</sup>

Data suggests that 61% of Americans do not engage in regular physical activity.<sup>41</sup> Physical activity has multiple benefits. Related benefits are directly proportional to duration and intensity of physical activity. Recent analysis from Framingham data suggests that moderate physical activity increases longevity by 1-1½ years in men and women, and that vigorous activity increases longevity by 3½ years in both genders.<sup>42</sup> Moderate physical work is defined as 3-6 MET capacity or 40-60% of maximum aerobic capacity or VO<sub>2</sub> max (one MET is the amount of energy expended at rest breathing). Vigorous exercise is defined as greater than 60% of VO<sub>2</sub> max or 60% of the predicted maximal heart rate for age. Multiple components are incorporated for cardiovascular benefits. Benefits included are: weight management, decreased insulin resistance, glucose intolerance, postprandial hyperglycemia, and decreased systolic and diastolic blood pressure. Physical activity is an important adjunct to diet to achieve and maintain weight loss. In the National Weight Control Registry, over 3,000 individuals lost greater than 10% of body weight and maintained it for longer than 1-year. The average weight loss was 30 kg and it was maintained for 5.5 years. Increased activity was the mainstay for 81% of the participants in both genders.<sup>43</sup>

The Diabetes Prevention Program noted exercise to be the single best therapy for prevention of onset for type II diabetes. Exercise surpassed metformin and troglitazone for decreasing the onset of type 2 diabetes. Compared to usual care (managed by health care providers), exercise reduced new onset type 2 diabetes by 58%. Lifestyle intervention consisted of an 8 kg weight loss with an 8 MET hours/week increase in physical activity.<sup>44</sup> Reviewing nine trials with type 2 diabetic patients, the average reduction of HbA1c associated with regular physical activity was 0.5-1.0%.

In patients with established vascular disease, regular physical activity decreases the rate of cardiovascular events.<sup>45</sup> Multiple studies demonstrate the benefits of regular physical activity on systolic and diastolic blood pressure. The benefit is different for normotensive and hypertensive subjects. The average reduction in normotensive patients is 2.6 systolic/1.8 diastolic mm Hg; hypertensive subjects derive greater benefits with a reduction of 7.4 systolic/5.8 diastolic mm Hg.<sup>46</sup>

There is improvement in all parameters of dyslipidemia with regular physical activity including HDL, triglycerides, and LDL. Studies vary, but physical activity increases HDL (by an average of 4.6%), and decreases triglycerides and LDL (by an average of 3.7% and 5%, respectively).<sup>47</sup> The amount of change is proportional to baseline lipid parameters. The STRIDDE study was designed to evaluate the amount of exercise for cardiovascular risk reduction. A total of 111 sedentary overweight men and women with mild to moderate hyperlipidemia were randomized to four groups. A control group was observed and 3 exercise groups were randomized to exercise for 8-months. One group had a high amount and

intensity, equivalent to jogging 20-miles per week at 65-80% of VO<sub>2</sub> max. The second group exercised moderately, with a high intensity equivalent to jogging 12-miles weekly at 65-80% of VO<sub>2</sub> max. The final group was assigned a low amount of moderate intensity exercise equivalent to walking 12-miles weekly at 40-55% VO<sub>2</sub> max. All subjects were encouraged to maintain body weight throughout the study. A total of 84 subjects completed the trial. Exercise training had no significant effect on total cholesterol or LDL; however improvement was noted in LDL subfraction. LDL particle concentration was reduced in the high intensity group. The principal change noted was the reduction of small LDL particles. All three exercise groups noted improvements in LDL particle size with the greatest improvement seen in the high intensity group. A trend was detected in decreasing the intermediate density lipoprotein cholesterol for all exercise groups. The largest change was in the high intensity group, though it did not meet statistical significance. HDL changed in the high intensity group and reached statistical significance. HDL particle concentration, HDL particle size, and number of large HDL particles also improved. Triglycerides improved in every exercise group. The subfraction data revealed drops in large VLDL particles in all exercise groups, the greatest benefit being seen in the low-moderate intensity group.<sup>48</sup>

The recommendation for the amount of physical activity has changed over the last several years. The current recommendation from the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) for the healthy population (ages 18-64 years old) is a minimum of 30-minutes of moderate physical activity at least five days weekly, but preferably everyday, or 20-minutes of vigorous exercise.<sup>49</sup> Moderate physical activity is defined as 3-6 METS and vigorous exercise is defined as greater than 6 METS. Additionally, per ACSM and AHA guidelines, resistance training (weight training) should be incorporated at least 2 non-consecutive days weekly. All major muscle groups should be incorporated utilizing 8-10 exercises with 8-15 repetitions. Several articles suggest that greater amounts of physical activity produce more metabolic and cardiovascular benefit, thus suggesting that more is better.<sup>48,50</sup>

When the goal of exercise is weight maintenance, the amount of moderate exercise is 60-90 minutes daily or 40-60 minutes of physical activity daily. In older adults (65 years and older), the use of METS is difficult because of physical limitations, co-morbidities, obesity, and lower functional capacity. Structuring physical activities for this population needs special considerations. ACSM and AHA recommendations utilize a 10-point scale for exertion.<sup>51</sup> Sitting is zero and all out effort is 10. Moderate intensity activity is 5-6 correlating with increasing HR and respirations. Vigorous intensity is 7-8, which produces noticeable increase in HR and breathing. As individuals tend to overestimate or underestimate intensity, exercise should be prescribed by a trained practitioner.

Older adults benefit from resistance training. Recommendations are 8-10 exercises on non-consecutive days 2 or more days weekly utilizing all major muscle groups. The number of repetitions should be 10-15, and the amount of weight is based on the ten-point scale with zero being no movement and maximal movement being 10. Moderate strength training is defined as an effort exerted of 5-6 and vigorous strength training is defined as an effort of 7-8. Additionally, older adults benefit from balance and flexibility exercises. They have been shown to be beneficial to prevent falls and assist in ROM for daily activities.

### ***Concluding Remarks and Clinical Recommendations on Nutrition and Exercise***

1. Optimal daily dietary consumption of at least 10 servings of fruits and vegetables (4 servings of fruits and 6 servings of vegetables), whole grains, mixed soluble and insoluble fibers, low saturated fat, high monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and no trans-fat. In addition, the diet should include moderate-high protein (1.5 to 1.8 g/kg) and low refined carbohydrate intake.
2. Exercise 60-minutes daily with aerobic and resistance training.
3. Achieve ideal body weight, BMI, waist circumference and body composition (body fat). Ideal body fat for women is less than 22% and for men, it is less than 16%.

## NUTRITIONAL SUPPLEMENTS

The literature is replete with studies on the clinical use of nutritional supplements to improve the serum lipid profile. However, most of the clinical trials have been short-term, studied in small cohorts of patients, or poorly controlled with methodological flaws. Most nutritional supplements do not have long-term human clinical trials that demonstrate their efficacy in reducing cardiovascular endpoints.

The use of herbal medicine in the United States has become increasingly common as alternative forms of therapy integrate with standard drugs that are approved by the Food and Drug Administration (FDA). Some of these herbal and non-traditional agents are utilized in the management of hyperlipidemia and are considered unregulated dietary food supplements as a result of the 1994 Dietary Supplement and Health Education Act (DSHEA).

In this section, we review the scientific rationale for the use of nutritional supplements in improving both serum lipids and cardiovascular endpoints. Specific recommendations are made as to which nutritional supplements should be considered as adjunctive therapy to optimal nutrition, exercise, weight reduction, and pharmacological therapies.

### *Tocotrienols and Lipids*

Vitamin E is the generic name of a mixture of lipid-soluble phenols, tocopherols, and tocotrienols, which possess general structural features: an aromatic chromanol head and a 16-carbon hydrocarbon tail.<sup>52</sup> The amount of methyl substituents in the chromanol nucleus give rise to 4 tocopherol isomers: alpha, beta, gamma, and delta.<sup>52</sup> Tocotrienols are a naturally-occurring derivative of tocopherols in the vitamin E family and have the same four isomers, but differ in the number of double bonds in the side chains.<sup>52</sup> The tocotrienols have more potent antioxidant activity than tocopherols.<sup>52</sup> Tocotrienol and tocopherol concentrates, often referred to as “tocotrienol-rich fractions” or TRFs, are obtained from rice bran or palm oil and contain about 30% to 50% tocopherols.<sup>53</sup> If the TRFs contain more than 20% tocopherols, the cholesterol lowering effect is diminished.<sup>54-56</sup> Tocotrienols are more effective in reducing LDL and total cholesterol if the concentrations of tocotrienols are high and the tocopherols concentration is low.<sup>54-56</sup> The relative potency of the tocotrienols varies with the delta isomer being the best at reducing LDL, total cholesterol, and triglycerides.<sup>52,57,58</sup>

### *Mechanism of Action and Structure-Function Relationship*

The tocotrienols demonstrate one of the most important structure/function relationships in natural medicine. The tocotrienols are composed of a chromanol ring with a variable number of methyl groups that determine the lipid-lowering potency.<sup>52,59</sup> The gamma isomer is about 30-times more potent in lipid-lowering capability as compared to the alpha isomer.<sup>52,59</sup> The location of the double bonds and the structure of tocotrienols are very close to that of farnesyl (farnesylated benzopyran analogues), which is the compound preceding the formation of squalene in cholesterol synthesis.<sup>60</sup> Farnesyl is also the compound converted to ubiquinone (co-enzyme Q-10) via the formation of all-trans-garanylgaranyl pyrophosphate as well as to various prenylated proteins and dolichols. Tocotrienols increase the conversion of farnesyl to farnesol, which reduces the conversion of farnesyl to squalene and then to cholesterol.<sup>60</sup> In addition, the farnesol signals two post-transcriptional pathways suppressing HMG-CoA reductase activity.<sup>53,60,61</sup>

There is decreased efficiency of translation of the HMG-CoA reductase mRNA and a decrease in HMG-CoA reductase protein mass levels. In addition, the LDL receptor protein is augmented, increasing the number of LDL receptors and LDL removal as well as stimulation of apolipoprotein B degradation clearance.<sup>60,62,63</sup> There is a dose-dependent cholesterol reduction associated with tocotrienols. As the dose of tocotrienols increases, additional conversion to alpha-tocopherol may occur, which will limit the anti-lipid effects.<sup>59</sup> If the alpha-tocopherol concentrations are greater than 20%, this will inhibit the tocotrienol lipid-lowering effects.<sup>54-56</sup> The alpha tocopherol may compete for binding with the alpha tocopherol transfer protein (TTP) and, thus, interfere with the transport of tocotrienols in the circulation.<sup>56</sup> In addition, alpha tocopherol attenuates the inhibitory effects of tocotrienols on HMG-CoA reductase and actually induces enzymatic activity.<sup>54,59,64</sup> It is estimated that about 40% of plasma tocotrienols are carried in LDL.<sup>58</sup> The absorption of tocotrienols is greater when they are given with a meal than when they are given to subjects in a fasting state.<sup>65</sup>

### Animal Studies

Tocotrienols have been shown to provide significant lipid-lowering effects in experimental animals.<sup>66-71</sup> In a study of 5-week old female chickens, there was a dose response reduction in total and LDL cholesterol of 22% to 52%, respectively, with supplemental TRF (tocotrienol rich fraction of palm oil).<sup>66</sup> The alpha tocotrienol fraction reduced total cholesterol and LDL cholesterol 17% and 33% respectively. However, the more potent gamma and delta fractions reduced total and LDL cholesterol by 32% and 66% respectively. Triglycerides were also significantly reduced, but HDL was unchanged. In experimentally-induced hyperlipidemic rats, a TRF isolate from rice bran oil produced a dose-dependent reduction in total cholesterol (48%), LDL cholesterol (60%), and triglyceride (42%). No change in HDL cholesterol levels were observed, however an improvement in oxidative stress parameters at doses of 8 mg TRF/kg/day was noted.<sup>67</sup>

Feeding TRF to rats resulted in a significant decline of 30% in total cholesterol and 67% in LDL cholesterol.<sup>68</sup> In a swine study, administration of TRF with tocotrienols significantly reduced total cholesterol (32-38%), LDL (35-43%), apolipoprotein B (22-35%), triglyceride (15-19%), platelet factor 4 (12-24%), thromboxane B2 (11-18%), and glucose (22-25%).<sup>69,71</sup>

In rabbits fed a gamma tocotrienol complex of 80% with 20% alpha and beta tocotrienols, there was a significant reduction in lipid levels and reduction in lipid streaks and atheroma in the aorta.<sup>70</sup>

### Human Studies

Most prospective studies have demonstrated significant lipid-lowering effects of tocotrienols in humans.<sup>58,72-74</sup> Those studies that were negative generally have methodological flaws that may account for the discrepant results.<sup>75,76</sup>

A double-blind, 8-week, crossover study in 25 subjects compared the effects of TRF palm oil at 200 mg of Palmvitee capsules per day versus 300 mg of corn oil per day on serum lipids in hypercholesterolemic humans.<sup>72</sup> In those receiving TRF, total cholesterol fell by 15%, LDL by 8%, Apo B by 10%, thromboxane by 25%, platelet factor 4 by 16%, and glucose by 12% (all values  $p < 0.05$ ). HDL and triglyceride levels did not change significantly.<sup>72</sup>

Qureshi *et al* evaluated 36 subjects with dyslipidemia treated for 8-weeks with an AHA Step-1 dietary regimen, followed by administration of Palmvitee capsules or 200 mg of gamma tocotrienol for an additional 4-weeks. The Palmvitee capsules contained 40 mg of alpha-tocopherol, 48 mg of alpha-tocotrienol, 112 mg gamma-tocotrienol, and 60 mg delta-tocotrienol. In the Palmvitee group, total cholesterol was reduced by 10%, LDL by 13%, apolipoprotein B by 7%, thromboxane B2 by 10%, and glucose by 15%. In the gamma-tocotrienol group, total cholesterol fell by 13%, LDL fell by 15%, and apolipoprotein B fell by 8%. There were no significant changes in triglycerides, HDL, or apolipoprotein A1 in either group.<sup>73</sup>

Tomeo *et al* demonstrated regression of carotid artery stenosis with duplex ultrasonography in 7 of 25 subjects with known cerebrovascular disease treated over 18-months with a mixture of alpha-tocopherol and gamma-tocotrienols. There was also a reduction in platelet peroxidation in this group, but there were no significant changes in serum lipids. None of the subjects in the control group showed regression, and 10 of 25 showed progression.<sup>74</sup>

Mensink *et al* evaluated 20 men in a randomized, double-blind, placebo-controlled, parallel trial receiving 35 mg of tocotrienols with 20 mg of alpha-tocopherol versus 20 control subjects who received only 20 mg of alpha-tocopherol. As would be expected, there were no significant changes in any lipid measurement. The dose of tocotrienol used in this study was too low and the dose of alpha-tocopherol was too high, which inhibited any effect of the tocotrienols on lipid levels as noted previously in other studies.<sup>75</sup>

There may be differences among the tocotrienols in their ability to prevent the oxidation of LDL cholesterol. Subjects were administered placebo or purified alpha, gamma, or delta tocotrienyl acetates at 250 mg per day for 8-weeks, and followed a low-fat diet for 4-weeks. Serum levels were measured and indicated adequate hydrolysis, absorption, and retention in the circulation for each of the tocotrienols. However, the serum concentration of alpha-tocotrienol was twice that of gamma-tocotrienol and 10-times greater than that of delta-tocotrienols despite equivalent doses. Alpha-tocotrienyl acetate increased *in vitro* LDL oxidative resistance by 22% and decreased its rate of oxidation. The delta-tocotrienyl acetate resulted in significantly greater reductions in the rate of LDL oxidation and the amount of conjugated dienes formed. However, none of the preparations reduced serum lipids in the subjects.<sup>58</sup>

Mustad *et al* evaluated 3 commercially available tocotrienol supplements at a dose of 200 mg per day or a safflower oil placebo for 28-days in 67 hypercholesterolemic men and women. No significant differences in mean lipid or glucose concentrations were observed among the four treatment groups.<sup>76</sup> However, the composition analysis of the products indicated that all three had high concentrations of alpha-tocopherol, which reduced the tocotrienol effects, and that the gamma or delta concentration of tocotrienols was low in 2 of the products. Therefore, this study was not an adequate evaluation of the effects of purified tocotrienols on serum lipids.

Quershi *et al* demonstrated a dose response of TRF-25 in 90 subjects given 25, 50, 100, and 200 mg per day of the TRF while on an AHA Step-1 diet. TRF-25 is derived from stabilized and heated rice bran and contains alpha, gamma, delta, and desmethyl and didesmethyl tocotrienols. Results showed that 100 mg/day was the optimal dose, resulting in a 20% decrease in total cholesterol, 25% decrease in LDL, 14% decrease in apolipoprotein B, and 12% decrease in triglycerides.<sup>59</sup>

Baliarsingh *et al* evaluated tocotrienols as TRF 3 mg/kg body weight in 19 type 2 diabetic subjects for 60-days and found significant reductions in total cholesterol (30%) and LDL (42%). There were no changes in serum glucose or HDL. The TRF fraction was obtained from edible grade rice bran oil and contained 7.5% alpha-tocopherol, 14.6% alpha-tocotrienol, 2.2% B-tocotrienol, 38.8% gamma-tocotrienol, 29.9% delta-tocotrienol, 4.5% delta-tocopherol, and 2.4% unidentified tocotrienols.<sup>77</sup>

Wahlqvist evaluated 44 subjects over 20-weeks with hyperlipidemia treated with a Palmvitee oil containing 30% alpha-tocopherol as well as gamma and delta-tocotrienols and found no changes in serum lipids. The high percent of alpha-tocopherol may have inhibited the effects of the tocotrienols on lipids.<sup>78</sup>

Rice bran oil<sup>79-86</sup> has numerous components that improve the lipid profile, such as oryzanol and ferulic acid, which are organic compounds with both antioxidant and lipid-lowering properties. It contains unsaponifiables (up to 4.4%), including plant sterols (43%), 4-methyl sterols (10%), triterpene alcohols (29%), and less polar components such as squalene and tocotrienols (19%). Rice bran oil also contains 25% saturated fats, 40% PUFA and 40% MUFA. Oryzanol has a greater effect on lowering plasma non-HDL cholesterol and raising plasma HDL than ferulic acid, possibly through a greater ability to increase fecal excretion of cholesterol and its metabolites.<sup>83</sup> However, ferulic acid may have a greater antioxidant capacity due to its ability to maintain serum vitamin E levels.<sup>79</sup> The average reduction in LDL levels obtainable with rice bran oil is 7-14%.<sup>82,84</sup>

### **Summary and Concluding Remarks**

The tocotrienols are natural derivatives of vitamin E that demonstrate significant reductions in total and LDL cholesterol in humans. The gamma and delta isomers, as well as the desmethylated derivatives, have the most potent lipid-lowering effects with reductions in LDL of 8% to 27%. The tocotrienols reduce formation and increase the degradation of HMG-CoA reductase and increase LDL receptors. There is a dose-dependent effect that appears to be maximum at about 200 mg per day of gamma/delta tocotrienols and 100 mg per day of the desmethylated derivatives. The lipid-lowering efficacy is reduced in the presence of a concentration of tocopherols exceeding 20%. Tocotrienols are most effective if taken in conjunction with an AHA lipid-lowering diet *along* with other health lifestyle changes. Tocotrienols should be taken in the evening with a meal. In addition, the alpha and delta-tocotrienols exhibit reduction in LDL oxidation and reduce carotid artery stenosis progression. There is suggestive evidence that the gamma and delta-tocotrienols may also reduce serum glucose.<sup>87,88</sup> The annatto plant, which is a natural food color additive with high carotenoid content, also has the highest natural amount of delta-tocotrienols (90%) and gamma-tocotrienols (10%), compared to rice bran oil and palm oil.<sup>87,88</sup> Rice bran oil also contains oryzanol, which may reduce intestinal cholesterol absorption. A high grade extraction process of rice bran oil with TRF-25 may have advantages due to the higher concentration of desmethylated tocotrienols that appear to reduce LDL equal to or better than the gamma and delta isomers.<sup>59</sup> Comparative studies of these various forms of tocotrienols will be required to determine their relative potency. There are no adverse effects noted in any of the clinical studies in humans.



## ***Clinical Recommendations***

Dyslipidemic patients should follow the AHA Step-1 lipid-lowering diet and consume 200 mg of gamma/delta tocotrienols in a purified form, from the Annato plant with 90% delta and 10% gamma tocotrienols taken at night with food. If available, an alternative would be 100 mg of the TRF extracted forms of the two desmethylated tocotrienols also taken at night with a meal. The exact proportion of gamma, delta, desmethylated, and didesmethylated tocotrienols to maximally reduce total cholesterol, LDL, and triglycerides, will require additional human studies. Tocopherols should be less than 20% of the total vitamin E consumed per day and should be taken in the morning to avoid reduced efficacy of the lipid-lowering effect of the tocotrienols. The alpha tocopherol content should be less than 20% of the total gamma and delta tocopherol as well. The gamma-tocotrienol may be most effective when used in conjunction with a statin, but more studies are needed to document this use.<sup>57</sup> The addition of the alpha and delta tocotrienol in small doses to reduce LDL oxidation may also be of benefit.

## ***Pantethine***

Pantethine is a naturally-occurring disulfide compound, which is a derivative of pantothenic acid and a precursor of CoA. Human studies have shown significant improvement in lipid profiles with pantethine in dyslipidemic patients. Total cholesterol, triglycerides and LDL cholesterol are reduced and HDL cholesterol is increased without any known adverse effects (89,90,91). This paper will review the mechanism of action, clinical studies and recommended clinical use of pantethine.

## ***Mechanism of Action and Animal Studies***

Incubation of rat hepatic cells with pantethine increases coenzyme A (CoA) levels by 45%.<sup>92</sup> This increases bioavailability of CoA in the cytoplasm of the cells and stimulates the oxidation of acetate at the expense of fatty acid and cholesterol synthesis.<sup>93</sup> The increased availability of CoA increases Krebs cycle activity, reducing the utilization of acetate for cholesterol and fatty acid synthesis. Fatty acid synthesis is decreased by 50% and cholesterol synthesis by 80%.<sup>94</sup> In animal studies, there are significant reductions in LDL and VLDL, and increases in HDL cholesterol and apolipoprotein A-1.<sup>95</sup> Pantethine increases arterial cholesteryl esterase activity in cholesterol-fed rats, which enhances the removal of arterial cholesterol esters and reduces fatty streak formation, lipid deposition, endothelial dysfunction, and intimal thickening in the aorta and coronary arteries.<sup>96,97</sup> Pantethine also reduces oxidation of LDL, which is the most atherogenic form of LDL.<sup>98</sup>

## ***Human Studies***

As many as 28 clinical trials in humans have shown significant reductions in total cholesterol, LDL-C, VLDL, triglycerides, and apolipoprotein B, with increases in HDL-C and apolipoprotein A-1.<sup>89,91,99-126</sup> No adverse effects were noted in any of these clinical trials that were conducted between 1981-1991.<sup>99-126</sup> In all, a total of 646 patients were evaluated, with an average study time of 13-weeks. The mean dose of pantethine was 900 mg/day given as 300 mg TID. A time and dose-dependent improvement in serum lipids was observed. The optimal dose was 900 mg/day, and the maximal improvement in lipids occurred at 4-months.<sup>99-126</sup> However, in studies of longer duration, there appeared to be continued improvements in lipid levels for up to 6-9 months in total cholesterol (20.5%), LDL (27.6%) and triglyceride (36.5%), however HDL levels stabilized at 4-months.<sup>99,103,106-108</sup> At the end of 4-months, in the majority of studies, the reduction in total cholesterol was 15.1%, LDL was 20.1% and triglycerides were 32.9%. HDL increased by 8.4%. The only adverse effect was mild gastrointestinal side effects in 3.6% of the subjects. However, only 1 of the 28 trials was a double-blind, placebo-controlled study.<sup>102</sup> Of the remainder, 4 studies were double-blind with active control<sup>102,113,117,118</sup> and 3 were single-blind.<sup>100,111,112</sup> Other studies used parallel designs or crossover designs.

Pantethine was compared to fenofibrate in two separate parallel design studies.<sup>112,113</sup> Although fenofibrate reduced triglycerides better, the changes in total cholesterol and LDL were similar. In a comparative study with bezafibrate, the changes in triglycerides were similar; 44.4% for bezafibrate and 37.5% for pantethine.<sup>100</sup>

## ***Summary and Clinical Recommendations***

Clinical studies with pantethine indicate that it is an effective natural therapy for the treatment of dyslipidemia with minimal adverse effects. Pantethine is one of the few natural products that increase

HDL in addition to reducing LDL and triglycerides. The recommended dose is 300 mg TID. Maximal improvement in the lipid profile may not be evident for at least 4-months, but may improve over 9-months.

### ***Omega-3 Fatty Acids***

The ancient Greek physician Hippocrates famously said: “Let food be your medicine and let your medicine be your food”. Never has this been truer with omega-3 polyunsaturated fatty acids (PUFAs). Over 30-years ago, two scientists, J. Bang and D.O. Dyerberg began the studies that would first link low mortality from cardiovascular disease among Greenland Eskimos (when compared to age and sex-matched Danish controls) to the consumption of high concentrations of omega-3 PUFA in their diet.<sup>127-131</sup> These observations triggered extensive animal and human studies that focused on the role of marine oils in preventing CHD. Long chain omega-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in oily fish were determined to be the primary bioactive components that accounted for many of the health benefits of fish.

In 2001, results of the GISSI-Prevenzione Study (11,323 patients with recent myocardial infarction) convincingly demonstrated that omega-3 PUFA supplements significantly lowered all-cause mortality, resulting largely from a 45% reduction in sudden cardiac death during 3.5-years of follow-up.<sup>132</sup> Additionally, in the Nurses’ Health Study (n=84,688), women without prior CVD showed a lower risk of CHD, including fatal CHD and non-fatal myocardial infarction, with increased intake of fish or omega-3 PUFA.<sup>133</sup> A correlation between tissue concentrations of omega-3 PUFA and CVD risk was also reported in a prospective, nested case-control analysis of men enrolled in the Physicians’ Health Study, where blood levels of omega-3 PUFA were inversely related to risk of sudden death among men without prior evidence of CVD.<sup>134</sup>

More recently, a meta-analysis examining fish consumption and CHD in 13 cohort studies has confirmed the compelling evidence from previous studies, by showing an inverse relationship between fish consumption and CHD as well as sudden cardiac death. Additionally, this study suggests that for each 20 g/day increase in fish consumption, there is an associated reduction of 7% in fatal CHD.<sup>135</sup> We typically eat about 3½ ounces of fish in a single sitting; if we ate that every day, it would translate into a 35% reduction in fatal CHD.

Prospective studies and randomized clinical trials revealed death from CHD (documented or suspected fatal myocardial infarction and sudden death) is markedly reduced (by 25% or more) by modest consumption of fish oil (250-500 mg/d of EPA and DHA).<sup>6,132-134,136-152</sup> At intakes up to 250 mg/d, the relative risk of CHD death was 14.6% lower for each 100 mg/d of EPA and DHA, for a total risk reduction of 36%. This study concluded that higher intakes do not substantially further lower CHD mortality, suggesting a threshold of effect around 500 mg of EPA and DHA.<sup>153</sup>

This latter observation is somewhat controversial. Many investigators feel that evidence from Western countries – countries whose people do not consume substantial levels of EPA and DHA naturally in their diets – supports a continued reduction in CVD (including CHD deaths) up to 1000 mg/d. Lucas and Harris suggest that cohorts from Japanese studies that were used in the aforementioned analysis were not valid because of the marked differences in long chain omega-3 PUFAs in Western and Japanese diets.<sup>154</sup> They suggest that if the Japanese studies are eliminated from the analysis, the risk for CHD death continues to decrease, reaching a plateau at approximately 1000 mg/d. Data from our laboratory and others suggest that this is correct from a biochemical perspective: there are only small changes in plasma or inflammatory cell fatty acids at concentrations between 250-500 mg EPA and DHA and there is a clear dose-response in incorporation of these PUFA into cellular glycerolipids up to 1500 mg.<sup>155</sup>

Based on overwhelming evidence, including the studies described above, the AHA recommends that the general public eat at least 2-servings of fish per week, and that CVD patients consume 1 g of EPA and DHA per day, and that patients with hypertriglyceridemia consume 2-4 g of EPA and DHA per day.<sup>156</sup>

## ***A New Biomarker of Cardiovascular Disease***

Clemens von Schacky and William Harris have recently proposed that an “omega-3 index” (EPA + DHA as a percent of total red blood cell (RBC) fatty acid) be considered a new risk factor for death from CHD.<sup>157</sup> They suggest preliminary targets for those at low, intermediate, and high risk of CHD based on the % of these fatty acids in RBC's. For example, they feel that levels of 8% or above are cardioprotective, and that levels <4% are associated with an increased risk for CHD. Using data from the Physicians' Health Study, the omega-3 index was clearly related to risk in a dose-dependent manner. In addition, the risk reduction at the highest levels of the omega-3 index (90%) was greater than that associated with the lowest levels of CRP (65%). Thus, for the case of sudden cardiac death, their data indicates that the omega-3 index may be more informative than any other known risk factor.

## ***Effect of Fish Consumption on Mortality***

Given the benefits and risk outlined above, the big question is what would this mean for CHD mortality if people utilized this anti-inflammatory strategy? Given an approximate 36% reduction in CHD deaths as suggested by Mozaffarian and Rimm, intake of fish or fish oil would reduce total mortality by an average of approximately 14% in mixed populations. According to these authors, an analysis of placebo-controlled, double-blind, randomized trials performed since 2003, suggest that addition of long chain omega-3 PUFAs would reduce total mortality by 17%. Meta-analysis of statins suggests that they reduce total mortality 15%.<sup>157-160</sup>

The Harvard Center for Risk Analysis constructed models showing the health benefits that would result if Americans increased their fish consumption by a small amount, such as 8-ounces per week: Here, in brief, is what they found:

- CVD: One small serving of fish per week would reduce the risk of non-fatal heart attack by 27%. It also would lower the risk of death from CVD by 17%. Each additional serving would decrease the risk of death by a further 3.9%.<sup>161</sup>
- Stroke: One small serving of fish per week would reduce the risk of stroke by 12%. Each additional serving would decrease the risk by a further 2%.<sup>162</sup>
- Children's cognitive development: Although it might be possible that children born to women who eat high amounts of mercury-containing fish could have lower IQ scores ranging from 0 to 1.5 points, this risk would be far outweighed by the benefits of eating small amounts of fish. Children born to pregnant women who eat enough fish to get the equivalent of 1 g/day of DHA would be likely to have higher IQ scores ranging from 0.8 to 1.8 points.<sup>163,164</sup>

On a national level, this small amount (8-ounces) of the right type of fish would, according to the researchers, translate into some tremendous annual benefits. If everyone in the United States consumed 8-ounces of salmon (a low-mercury fish) per week, it would result in 20,000 fewer deaths from CVD, 4,000 fewer non-fatal strokes, and an aggregate increase of more than 2 million IQ points in newborn children.

Eating the right kinds of fish – which are high in long chain omega-3 PUFAs and contain low amounts of mercury – is essential to reducing inflammation and the risk of inflammatory disease. However, although some mercury is present in most fish, the levels are usually so extremely low that most people don't need to worry about it.

## ***Mechanisms of Action***

Long chain omega-3 PUFAs may influence cardiovascular risk factors utilizing several mechanisms.<sup>138,151,158-160,165-186</sup> These include altering eicosanoid biosynthesis in a manner which affects signaling, altering membrane fluidity in a manner which influences enzymatic reactions and receptor binding, and directly activating transcription factors in a manner which regulate tens to hundreds of critical genes affecting everything from hyperlipidemia to inflammation. These mechanisms are discussed in detail below. This diversity of mechanisms probably plays a critical role in giving omega-3 PUFAs their potency as well as affecting the dose and time required to get certain clinical affects. Mozaffarian and Rimm outlined these parameters in a very prescriptive manner: “At typical dietary intakes, antiarrhythmic effects predominate, reducing risk of sudden death and CHD death within weeks. At higher doses, maximum antiarrhythmic effects have been achieved, but other physiologic effects may modestly impact other clinical outcomes (possibly requiring years to produce clinical benefits). For instance, nonfatal

myocardial infarction may not be significantly affected by lower doses or shorter durations of intake, but may be modestly reduced by higher doses or prolonged intake (i.e. 1.8 g/d for 5-years)<sup>187</sup>

### Eicosanoid Biosynthesis

Leukotrienes, prostaglandins and thromboxanes are a class of lipid mediators of inflammation derived from the essential fatty acid, arachidonic acid (AA). The dietary concentrations of the long-chain omega-6 PUFA AA appears to play a key role in the quantities of eicosanoids that animals and humans produce.<sup>188-191</sup> In fact, the first reported study of oral administration of highly-enriched esterified AA to humans demonstrated a marked increase in urinary prostaglandin E metabolites, as well as a significant reduction in the threshold necessary to induce secondary, irreversible aggregation of platelets.<sup>189</sup> Other studies have shown dietary AA affects platelet reactivity and response to vaccination.<sup>188</sup> A more recent study by Dwyer and colleagues demonstrated a strong association between a polymorphism in the 5-lipoxygenase gene promoter and an increase in intima-media thickness (a common measurement of cardiovascular risk).<sup>192</sup> Interestingly, dietary AA was associated with enhanced atherogenesis in this genotype. In contrast, increased dietary intake of EPA and DHA blunted this effect. The diet-gene interactions observed in these studies were specific to these fatty acids.

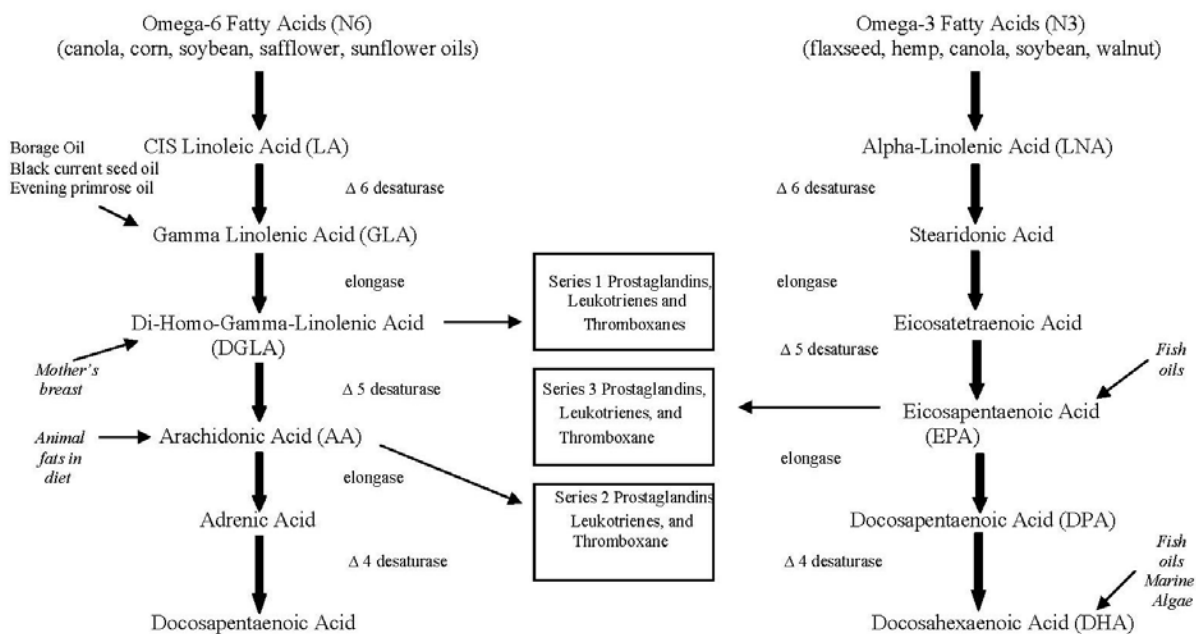


Figure 1. Essential Fatty Acid Pathway

In addition to the concentration of AA, the ratio of AA to very long chain omega-3 PUFAs (EPA and DHA) in human diets is an important factor in providing the anti-inflammatory effects of fish oils. Ingestion of fish or fish oil diets leads to a marked increase of EPA in membrane phospholipids and, in some cases, a concomitant decrease in AA. However, several fish species (especially those most intensively farmed) contain very high concentrations of AA and high ratios of AA to EPA and DHA. Wada and colleagues have recently reported that increasing the ratio of EPA to AA in cellular phospholipids likely dampens prostanoid signaling with its largest effects on cyclooxygenase-1 (COX-1) involving the production of prostaglandin D, E, and F.<sup>193</sup> Additionally, cells such as platelets can convert EPA to thromboxane A<sub>3</sub> via COX-1.<sup>194,195</sup> EPA may also increase production of prostacyclin, which has been shown to diminish platelet aggregations.<sup>196</sup> Another critical effect of increasing EPA/AA is that it enhances the formation of prostaglandin E<sub>3</sub> (PGE<sub>3</sub>) from EPA utilizing COX-2.<sup>197,198</sup> PGE<sub>3</sub> is thought to block inflammation, whereas AA-derived prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) may promote it. More recently, both EPA and DHA have been shown to be converted into anti-inflammatory mediators known as resolvins and protectins.<sup>199,200</sup> Biochemical data from Serhan and colleagues also predict that changes in AA to EPA or DHA ratios would shift the balance from proinflammatory prostaglandins, thromboxanes, and leukotrienes

to protective resolvins and protectins. Consequently, the ratio of AA to long chain omega-3 PUFAs in human diets is likely to be an important factor that regulates the balance of AA and fish oil-derived eicosanoids produced.

### Membrane Fluidity

There have been a large number of studies examining the effect of omega-3 fatty acids on membrane fluidity. Long chain omega-3 PUFAs are distributed in glycerolipids of cellular membranes throughout the body. The degree to which a fatty acid is desaturated determines its 3-dimensional structure, which affects membrane fluidity and function. Because long chain omega-3 PUFAs are so highly desaturated, they have a great capacity to influence membrane fluidity. Membrane fluidity is thought to be especially important for cognitive development and is also thought to play a role in several psychiatric disorders.<sup>201</sup> In terms of CVD, long chain omega-3 PUFAs reduce platelet aggregation, blood viscosity, plasma levels of fibrinogen, PF4, and beta-thromboglobulin, and increase capillary flow, all of which are thought to be functions of membrane fluidity.<sup>202</sup>

### Alterations in Gene Expression

Recent studies suggest that the anti-inflammatory effects of omega-3 PUFA are exerted at the level of altered gene expression. This regulation can be at the level of the inflammatory cytokines themselves. Curtis and colleagues found that when bovine chondrocytes were cultured with linoleic acid (LNA), EPA, or DHA, the expression of the genes for tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and interleukin (IL)-1 $\alpha$  were significantly reduced.<sup>203</sup> Mice fed fish oil have decreased levels of mRNA for several inflammatory cytokines, including TNF $\alpha$ , IL-6 and IL-1 $\beta$  in kidney, spleen, and peritoneal macrophages.<sup>204,205</sup>

In addition to altering cytokine expression levels, omega-3 PUFA exert their effects via activation of transcription factors, including nuclear factor kappa-beta (NF- $\kappa$ B), sterol regulatory element binding protein-1C (SREBP-1C), and peroxisome proliferator-activated receptors (PPARs). NF- $\kappa$ B induces many genes in response to inflammatory stimuli. These genes include COX-2, TNF $\alpha$ , IL-6, IL-1 $\beta$ , and acute phase proteins.<sup>206,207</sup> Recent studies have shown that omega-3 PUFA can inhibit NF- $\kappa$ B activation. Chen and Zhao showed that incubating human monocytes with EPA reduces LPS-stimulated activation of NF- $\kappa$ B and decreases phosphorylation of the inhibitor I $\kappa$ B.<sup>208</sup> Ross and colleagues showed that although incubation of pancreatic cells with the inflammatory cytokine TNF $\alpha$  causes increased degradation of I $\kappa$ B, preincubating the cells with DPA prevents this degradation, thereby inhibiting activation of NF- $\kappa$ B.<sup>209</sup>

SREBP-1c appears to be the critical genetic switch controlling lipogenesis. Dietary PUFAs suppress SREBP-dependent gene transcription by several mechanisms. Firstly, dietary PUFAs have been shown to reduce nuclear SREBP-1c in rats and in HEK293 cells.<sup>210,211</sup> Secondly, PUFAs reduce the stability of SREBP-1c mRNA.<sup>212</sup> Thirdly, PUFAs suppress SREBP-1c target gene transcription by reducing the active form of SREBP-1c.<sup>213</sup> The PPARs are activated in the absence of free fatty acids and lead to the transcription of genes for fatty acid  $\beta$ -oxidation (peroxisomal and mitochondrial). The overall effect of long-chain omega-3 PUFAs on SREBP-1c and the PPARs is to shift metabolism away from triglyceride storage and toward oxidation.

### Problems with Our Fish Supply

The increased awareness of the health benefits of omega-3 PUFAs in fish, coupled with dwindling supplies of fish in the wild, has spawned a dramatic expansion in aquaculture (an annual rate of increase of 9.2% compared with 1.4% for captured fish).<sup>214-216</sup> While a great deal of attention has been focused on the contamination of farmed fish populations with methyl mercury, polychlorinated biphenyls, and other organic compounds<sup>217</sup> little has been published with regard to the effects of rapid changes in the fish industry on PUFA or saturated fatty acid levels in emerging, intensively farmed species of fish.

In the U.S., tilapia has shown the biggest gains in popularity among seafood, and this trend is expected to continue as consumption is projected to increase from 1.5-million tons in 2003 to 2.5-million tons by 2010, with a sales value of more than \$5-billion U.S. dollars.<sup>218</sup> Based on this growth, tilapia is now the second most widely farmed fish in the world, second to farmed salmon, which has seen an increase in production from 0.5-million metric tons in 1980 to 2.7-million metric tons in 2003, and followed by catfish, which increased from 0.3-million metric tons in 1994 to 0.7-million metric tons in 2003.<sup>219</sup> The consumption of salmon in the U.S. has also increased, from 130,000-metric tons in 1989 to more than

300,000-metric tons in 2004, and 78% of this salmon is farmed.<sup>220</sup> This explains why, in spite of the marked increase in production of farmed salmon and tilapia in recent years, the amount of wild salmon capture and wild tilapia capture has remained unchanged – 0.75-million metric tons/year and 0.6-million metric tons/year, respectively – for the last ten years.<sup>219</sup>

A recent study in our laboratory has revealed that tilapia, as well as farmed catfish, have several fatty acid characteristics that would generally be considered by the scientific community as detrimental. Firstly, they have much a higher saturated fatty acid and MUFA to PUFA ratio than other farmed or wild fish. Ratios this high in diets have been shown to be directly associated with increases in saturated fatty acids and MUFA in cholesterol esters of LDL particles and increased atherogenesis in both humans and non-human primates.<sup>221-223</sup> While saturated fatty acids have long been associated with atherosclerosis, recent studies suggest that the desaturation of saturated fats, such as stearate (by stearyl-CoA desaturase to oleic acid), appears to be an essential step in mediating the induction of obesity, insulin resistance, and dyslipidemia.<sup>224-227</sup>

Secondly, the concentrations of n-6 PUFAs, and more specifically the long chain n-6 PUFA AA, are very high. In fact, these fish contain some of the highest levels of AA found in the human food chain. When the ratios of the two primary long chain omega-6 and omega-3 20-carbon PUFAs (AA and EPA, respectively) were examined, both farmed tilapia and catfish contained high AA/EPA. While there was a great deal of variability in the AA/EPA ratio in farmed tilapia, the average ratio of AA to EPA was approximately 11:1, and two fish samples harvested in Central America had over 20-times more AA than EPA. The ratios of PUFAs in these fish are high, predominantly because they contain high quantities of AA. Indeed, the average tilapia contained 134 mg of AA on average, while catfish contained 67 mg on average. However, some tilapia samples from Central America contained more than 300 mg of AA per 100 g portion. To put this in some perspective, a 100 g portion of hamburger (80% lean) contains 34 mg of AA, whereas a doughnut contains 4 mg of AA and 100 g of pork bacon contains 112 mg of AA.<sup>228</sup> For individuals who are eating fish as an alternative method of controlling inflammatory disease, it is clear from these numbers that tilapia and catfish are not the best choices. All other nutritional content aside, the inflammatory potential of hamburger and pork bacon is lower than the average serving of farmed tilapia. In contrast to tilapia and catfish, farmed-raised salmon and trout contained low and positive ratios of AA to EPA (approximately 0.2) largely due to their high concentrations of EPA. These data with farmed salmon are consistent with a recent study by Hamilton and colleagues, and are in contrast to those reported by the USDA which stated that farm-raised Atlantic salmon contains much higher levels of AA (1152 mg/100 g) and an AA to EPA ratio of 1.9 to 1.<sup>228,229</sup>

There are several factors that may contribute to the marked differences observed in fatty acids of tilapia. Tilapia is a very hardy fish that grows rapidly on formulated feeds that contain lower protein levels, higher carbohydrate levels, and a wide range of fat sources, compared with many other carnivorous farmed species.<sup>218</sup> They are easy to breed and can be cultured intensively and economically in systems ranging from rural ponds to situations where the nutrition is exclusively dependent on commercially formulated diets. Fish from the most intensively farmed system are typically fed higher levels of the 18 carbon n-6 fatty acid, linoleic acid from vegetable oils as part of the feed.<sup>230</sup> This, in turn, is efficiently converted through two desaturation steps and an elongation step to the AA that is found in tissues. Tilapia appears to represent an important example where an intensely farmed fish has a much higher content of saturated fatty acid, MUFA, and linoleic acid, thus leading to high concentrations of AA and high n-6 to n-3 ratios. Unfortunately, aquaculture, which holds such promise as a PUFA source from fish, can give rise to detrimental and potentially harmful PUFAs when fatty acid precursors of those PUFAs fed to fish are not taken into account.

### ***Risks of Methyl Mercury, Polychlorinated Biphenyls and Other Organic Compounds***

In 2004, the U.S. FDA and the U.S. Environmental Protection Agency (EPA) advised that pregnant women, women who may become pregnant, nursing mothers, and young children should avoid some types of fish and eat fish that are low in mercury. However, it did not recommend that these groups stop eating fish, and it encouraged everyone else to continue eating fish.<sup>231</sup> The advisory contained these recommendations:

- Do not eat shark, swordfish, king mackerel, and tilefish because they contain high levels of mercury.
- Eat up to 12-ounces (2 average meals) a week of a variety of fish and shellfish that are lower in mercury. Five of the most commonly eaten fish that are low in mercury

are shrimp, canned light tuna, salmon, Pollock, and catfish. Another commonly eaten fish, albacore (“white”) tuna, has more mercury than canned light tuna. You may eat up to 6-ounces (1 average meal) of albacore tuna per week.

- Check local advisories about the safety of fish caught by family and friends in your local lakes, rivers, and coastal areas. If no advice is available, eat up to 6-ounces (1 average meal) per week of fish you catch from local water, but don’t consume any other fish during that week.<sup>231</sup>

The advisory didn’t recommend that any other groups limit their fish consumption stating, “For most people, the risk from mercury by eating fish and shellfish is not a health concern”. The advisory also emphasized the health benefits of fish consumption stating: “Fish and shellfish are an important part of a healthy diet. Fish and shellfish contain high-quality protein and other essential nutrients, are low in saturated fat and contain omega-3 fatty acids. A well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children’s proper growth and development.”

Needless to say, the positive sections of the advisory did not receive much attention from the national media. The negative mercury angle received much wider play in newspapers, magazines, and on TV. While such government advisories are well-intentioned, they had unintended consequences. Because of the way they were trumpeted in the media, millions of Americans were scared into thinking that all fish is contaminated with high levels of mercury and that the risks of eating fish outweigh the benefits.

In November 2005, the *American Journal of Preventive Medicine* published a series of five review articles about fish and fish consumption by the Harvard Center for Risk Analysis.<sup>217</sup> Overall, the articles concluded that fish are an excellent source of omega-3 fatty acids, which may protect against CHD and stroke and help the neurological development of unborn babies. They also warned that if people inappropriately decrease fish consumption because of concerns about mercury, it may increase their risk of adverse health outcomes. The findings confirm a point that we emphasize throughout this paper: There may not be a better thing (from a dietary perspective) you can do for your health than to eat the right kind of fish. In fact, if you don’t eat such fish and you have a genetic susceptibility to inflammatory diseases, including heart disease, you may be placing yourself at great risk.

In America, fish consumption is low compared to many other countries and it hasn’t increased significantly in recent years. In 1999, per capita fish consumption in the United States was only 15.4 lb. It decreased to 15.2 lb in 2000 and to 14.8 lb in 2001, before increasing to 15.6 lb in 2002 and to 16.3 lb in 2003. When you compare that to per capita consumption of chicken (80 lb), beef (65 lb) and pork (50 lb), you can see that fish accounts for only a small fraction of the animal protein that Americans consume every year.

In some populations, fish consumption may actually be declining. After the FDA released a mercury advisory in 2001, one study showed a 17% decrease in fish consumption among pregnant women in the United States. Concerned that this trend could spread to the general population, the researchers from the Harvard Center for Risk Analysis calculated what might happen if everyone reduced their consumption of fish. They found, as predicted from the studies above, that even if the decrease were as little as 3-4%, it would translate into significantly higher rates of cardiovascular disease as well as more heart attacks and strokes.

### ***Plant Sources of Omega-3 PUFAs***

Despite the well-documented health benefits of long chain omega-3 PUFA, the approximate intake of omega-6 to omega-3 PUFA in the United States falls between 15 and 25 to 1, with the principle omega-3 PUFA consumed being  $\alpha$ -linoleic acid (18:3) (90% of omega-3 PUFA intake).<sup>232</sup> It is thought that our hunter-gatherer ancestors ate ratios close to 1 to 1 and these ratios were maintained until the industrial revolution, when subsequent urbanization radically changed our food supply. It is worth noting that for most of human history (100,000 generations) human beings lived as hunter-gatherers. In contrast, only 500 generations have lived since the dawn of agriculture, and just 3 generations have lived since industrial agriculture became the predominant means of food production.

There are multiple barriers to achieving the recommended omega-6 to omega-3 PUFA ratio in the American diet. To achieve this by fish consumption alone would require a 4-fold increase in the intake of fatty fish.<sup>232</sup> Given the relatively higher cost of fish compared to other sources of meat in the American diet, along with personal preferences in food, this option seems very unlikely. Supplementation of the diet with fish oil would be another means of increasing n-3 PUFA intake, but this option is unlikely to succeed

due to the organoleptic aversion (i.e., fishy aftertaste and smell) to fish oil supplements. Yet another possibility is to increase the consumption of foods and oils containing  $\alpha$ -linoleic acid, including flax seed oil, which can go through elongation and desaturation to EPA. However, studies have shown that  $\alpha$ -linoleic acid is poorly converted to EPA in humans and the degree of conversion depends on the amount of linoleic acid (18:2, omega-6) in the diet, since linoleic acid competes with  $\alpha$ -linoleic acid for  $\Delta 6$ -desaturation and diminishes the conversion of  $\alpha$ -linoleic acid to EPA.<sup>233-236</sup> Most Americans eat more than 15g/day of linoleic acid in primarily corn-based products. By comparison, the amount of  $\alpha$ -linoleic acid consumed is very small, thus it is unlikely it would be converted to long chain omega-3 PUFAs such as EPA.

Finding a solution to the problem of enriching the American diet in a meaningful way with long chain omega-3 PUFA would have an enormous impact on public health and would presumably reduce the incidence and severity of several complex human diseases, including CHD, stroke, hypertension, diabetes, and obesity.

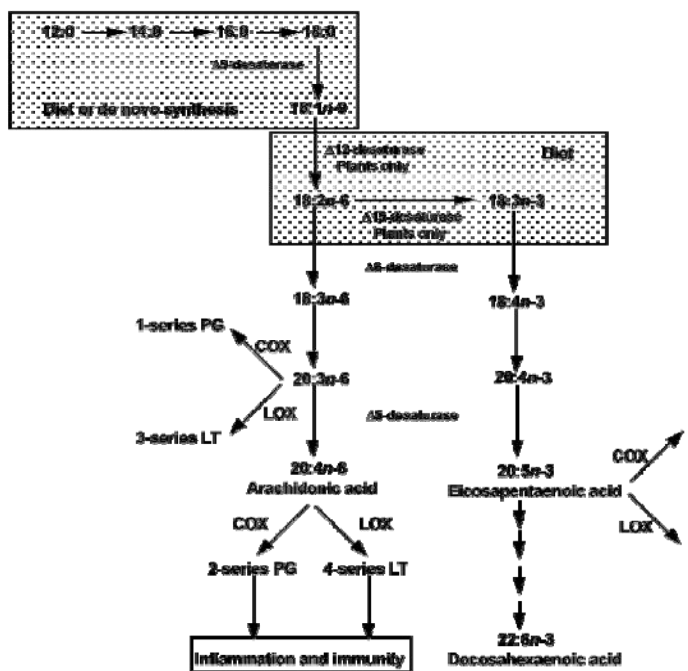


Figure 2. Outline of metabolic pathways for PUFA adapted from Calder and Grimble, 2002.

A novel idea that we have explored to enrich the American diet with EPA without the problems outlined in the previous paragraph is to use a botanical oil that is enriched in a fatty acid known as stearidonic acid (SDA, 18:4, n-3), which is the immediate product of  $\Delta 6$ -desaturation of  $\alpha$ -linoleic acid (Figure 2). Since  $\Delta 6$ -desaturase is the rate-limiting step in the formation of EPA from  $\alpha$ -linoleic acid, supplementation with SDA will enrich cellular membranes and plasma lipoproteins with SDA, which efficiently converts to EPA to reduce triglycerides and bestow the cardiovascular benefits of eating fish without the side effects mentioned above. It is worth noting that a UK company, Croda, has just received novel food status in the U.S. for echium oil, which is enriched with SDA.

### Summary

The benefits of consuming adequate quantities of long-chain omega-3 PUFA are clear. Studies have shown that these fatty acids decrease serum triglycerides, reduce the risk of CHD, as well as the risk of sudden death due to CVD and myocardial infarction. One study has even suggested that the amount of omega-3 PUFA in red blood cells could be a predictor for CHD.

However, despite the large body of research attesting to the therapeutic potential of omega-3 PUFA in the diet, there are barriers to adequate fish consumption. Stories of fish contaminated with mercury and other chemicals have made consumers wary, and even without that concern, it is difficult to



consume enough fish to provide the amount of omega-3 PUFA shown to be beneficial. Fortunately, innovative research on plant-based sources of omega-3 PUFA is progressing rapidly, ensuring greater consumption of omega-3 PUFA.

### ***Clinical Recommendations***

The omega-3 fatty acids, DHA and EPA are effective lipid-lowering agents. In doses of 4 g/day, DHA and EPA will reduce triglycerides by up to 45% and VLDL by up to 50%, with little change in HDL. In addition, they offer significant reductions in CHD events, improve endothelial dysfunction, improve the arachidonic acid/EPA ratio, and reduce body fat, body weight, and serum glucose. There does not appear to be any significant difference between EPA and DHA in terms of their triglyceride-lowering abilities. The omega-3 fatty acids provide a dose-dependent improvement in serum lipids in patients taking statins. It is recommended that patients take 2-4 g of combined EPA and DHA in a ratio of 3:2 EPA to DHA with GLA at 75-90% of the total DHA and EPA, along with approximately 100 IU of gamma/delta vitamin E.

### ***Guggulipid***

Guggulipid (Gugule) has been widely used as a medicinal agent in traditional Ayurvedic Indian medicine for more than two millennia to treat a variety of ailments including obesity and hyperlipidemia.<sup>237</sup> The active ingredient of this resin extract of the gugule or mukul myrrh tree (*Commiphora mukul*) is widely considered to be guggulsterone. This plant sterol has been found to be an antagonist ligand for the farnesoid X receptor (FXR) and leads to a reduced expression of bile acid activated genes.<sup>238</sup> Additionally, the (E) and (Z) stereoisomers of guggulsterone have greater binding affinity to the mineralcorticoid receptor and other steroid receptors, including both androgen and progesterone receptors, suggesting other potential medicated pharmacological effects.<sup>239</sup> The ethyl acetate extracted guggulsterones of the guggul tree, which is found commonly in arid regions of India and Pakistan, have been studied in several multicenter trials.<sup>240</sup> Early studies in 1966 by Satyavati found that guggul gum lowered cholesterol and prevented diet-induced atherosclerosis in rabbits.<sup>241</sup> On the basis of early human studies, guggulipid was approved for use as a lipid-altering drug in India in 1987; however, most of these human studies have been non-placebo controlled trials uniformly conducted in the Asian/Indian population. In the 1 trial that was placebo-controlled, standardized guggul extract reduced total cholesterol by 11%, LDL cholesterol by 12%, and triglycerides by 15%.<sup>242</sup>

A randomized, placebo-controlled, 8-week long trial, was conducted in the United States by Szapary *et al* in 103 hypercholesterolemic adults.<sup>243</sup> Short-term observation for safety and efficacy of 2 doses of a standardized guggul extract (containing 2.5% guggulsterones) in adults eating a typical western diet did not show cholesterol-lowering effects. Participants were randomly assigned to receive 3 daily doses of standard-dose guggulipid (1000 mg), high-dose guggulipid (2000 mg), or matching placebo, for 8-weeks. Results showed that LDL-cholesterol decreased by 5% in the placebo and actually increased by 4% and 5% in those receiving the standard-dose and high-dose guggulipid, respectively, resulting in a net positive change of 9-10%. Furthermore, 6 participants developed a hypersensitivity rash while taking the active supplement. Although it is possible that insufficient concentrations could have had an effect, each guggulipid tablet contained standardized 200 mg of bioactive E-Z guggulsterones, which is similar to the amount in previous positive studies of 75 mg per day and to the highest doses studied of 150 mg per day. A decrease of Lp(a) by 5-7% was also observed, but not statistically different from placebo. A secondary analysis found that high-dose guggulipid reduced levels of C-reactive protein (hs-CRP) by 29% compared to 25% in placebo. Since Asian/Indian and Western population studies appear to differ, there may be environmental or genetic explanations for the observed differences in response rates that should be given consideration. In this study, however, there was an overall 18% favorable responder rate, which is lower than response rates of 60-80% reported in Indian populations of previous trials. Guggulsterones are antagonists to the FXR and bile acid receptors, two receptors involved in bile acid regulation in cholesterol metabolism and bile acid synthesis, which are mediated by several enzymes, including the hepatic enzyme seven alpha hydroxylase (CYP7A). Thus, potential lipid modifying effects might be anticipated. FXR antagonism leading to up-regulation of CYP7A-facilitated cholesterol transport is corroborated by animal experiments in which FXR-null mice on high cholesterol diets had significant cholesterol lowering response in response to 100 mg/kg high-dose Z-guggulsterone in hepatic cholesterol content.<sup>243</sup>

HDL levels have been reported to increase in the 60% of cases of responders to guggulipid therapy, whilst total cholesterol and triglyceride levels have reportedly reduced by 11% and 16.8%.<sup>240</sup> Szapary *et al* reported paradoxical results in the western population with significant increases in LDL and small non-significant reductions in HDL of 2-3% in the randomized western trial.<sup>243</sup>

In clinical trials of guggulipid, generally 90% or greater compliance has been reported, with infrequent side effects of headache, mild nausea, and hiccups. However, concern has been raised about the bio-availability of concomitantly administered propranolol and diltiazem,<sup>242</sup> and a review by the National Standard Research Collaboration recommends avoidance of guggulipid in pregnant or breastfeeding women and children since the safety of its use has not been well studied. The medicinal use of guggul reportedly dates back to 600 BC and is of Biblical significance. This extract from the resin of the mukul myrrh tree merits further clinical research studies.<sup>242</sup> However, at this time, guggulipid is not recommended for the treatment of dyslipidemia.

## Soy

Replacement of ingested animal protein with vegetable-derived protein has been associated with a reduced risk of CVD and a reduction of serum cholesterol levels.<sup>244</sup> The beneficial effects of soy protein have been recognized in animals for more than a century. Studies have shown that laboratory animals given soy protein instead of animal protein are less likely to develop hypercholesterolemia and atherosclerosis.<sup>244</sup> Indeed, in 1999, based on clinical studies demonstrating that a minimum of 25 g of soy protein per day was beneficial in lowering total and HDL cholesterol, the U.S. FDA approved a food-labeling health claim for soy protein and cholesterol reduction, stating that 25 g/d of soy protein, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.<sup>245,246</sup> They required a serving to contain at least 6.25 g of soy protein, considered 25% of the necessary amount with expectations that soy protein foods would be ingested 4-times daily. In 2000, the American Heart Association concluded it was prudent to recommend including soy protein foods in a diet low in saturated fat and cholesterol. The American Heart Association Nutrition Council re-evaluated the evidence of soy protein in cardiovascular disease in 2006, stating that: "Earlier research indicating that soy protein has clinically important favorable effects [on improving risk factors for CVD] as compared with other proteins has not been confirmed. In contrast, many soy products should be beneficial to cardiovascular and overall health because of their high content of polyunsaturated fats, fiber, vitamins, and minerals and low content of saturated fat."<sup>246-248</sup>

Anderson *et al* reported a meta-analysis of the effects of soy protein on lipid levels in 1995.<sup>249</sup> This included 38 controlled studies, of which 34 demonstrated reductions in cholesterol levels with a variety in differing amounts of soy protein. In general, a 23 mg/dl reduction in total cholesterol (9%), 22 mg/dl reduction in LDL (12.9%), and a 13.3 mg/dl reduction in triglycerides (10.5%) were demonstrated, along with a non-significant 2.4% increase in serum concentrations of HDL cholesterol for an average ingestion of 47 g/day of soy protein intake.<sup>249</sup> In the 4 studies that did not find significant reductions, generally low levels of initial serum cholesterol averaging only 185 mg/dl were noted. A meta-analysis by Descovich *et al* followed encouraging studies in the 1980's, in which the soy protein hypothesis of replacing nearly all animal protein with soy protein reduced LDL by 20-30% in subjects with severe hypercholesterolemia.<sup>250</sup> The recognition that soy protein contains bio-active molecules such as phytoestrogens or isoflavones added enthusiasm to the soy protein cardiac hypothesis. When soy is washed with alcohol in preparation of a soy protein isolate, however, substantial amounts of isoflavones, which have biological properties of arterial vasodilatation, cholesterol-lowering and inhibition of atherosclerosis in monkeys, were lost. Major isoflavones are genisten, daidzin, formononatin, and bio kinen A. However, de-hulling, de-fatting, and flaking soybeans may reduce the isoflavone concentration of pure preparations of the protein. Sacks *et al* compared 22 randomized trials with isolated soy protein, casein protein, wheat protein, or other animal protein. Doses ranged from 25-135 g/day of soy protein (isoflavone content ranging from 40-318 mgs). Statistically significant changes in LDL or non-HDL cholesterol were seen in 8 of 22 studies, but with an overall average effect of only 3% reduction in LDL cholesterol. No significant effects on HDL-C, triglycerides or Lp(a) were identified.<sup>248</sup>

Soy isoflavones, because of their phytoestrogenic effects with weak estrogenic activity, have been reported to improve perimenopausal vasomotor symptoms and may be useful in treating hot flashes. Anti-androgenic and uterine anti-estrogenic effects have also been reported, peaking interest in their potential use in the treatment or prevention of cancer.<sup>248</sup>

Soybeans, however, are more than just isoflavones and protein calories. They also are rich in oligosaccharides. Compared to other legumes, soybeans are high in oligosaccharides, with a de-fatted soy meal containing 16-milligrams per gram. Alteration in human intestinal bacterial micro flora from this could potentially affect resorption of bile acids as well. Soy products containing much of the whey removed may have reduced oligosaccharides, such as seen in tofu. Fermented soy products, such as tempeh, mesol, and soy isolate powders, are often reduced in oligosaccharides.

Plant based phytoestrogens are structurally similar to 17 B-estradiol and are comprised of two groups, isoflavones and lignans. Bacterial micro flora in the colon produce enterolactone and enterodiol as active metabolites from the dietary lignans matairesinol and secoisolariciresinol. Lignans show minimal binding affinity; whereas, genisten and daidzin (2 of the major isoflavones present in soy) bind to the estrogen B receptor with high affinity and to estrogen A receptor with low affinity. Previous studies suggest lower cardiovascular risk in doses comparable to soy intake levels seen in Asia. Vanderschouw *et al* studied 16,165 women aged 49-70, who were free of cardiovascular disease at the start of the study, for a median 75-months. Results showed that neither isoflavones nor lignans were associated with decreased CVD risk.<sup>251</sup>

Other potential beneficial effects of soy have inconsistently been reported. In studies of the effect of soy protein with isoflavones on blood pressure, 1 mm/Hg decreases in systolic blood pressure were reported. Lp(a), a LDL-like lipoprotein that has been demonstrated to be an independent predictor of CVD risk, has been reported to be increased with soy protein in some studies, but not consistently. Meinertz *et al* reported that alcohol-extracted soy protein was associated with lower Lp(a) levels.<sup>252</sup> Data suggested that Lp(a) concentrations might be markedly reduced by dietary measures associated with alcohol extracted soy protein. Sacks *et al* and the AHA Science Advisory report from the AHA Nutrition Committee suggest that the evidence suggests that soy protein, rather than soy isoflavones, may be the responsible nutrient associated with the principal favorable effects of soy.<sup>247,248</sup> For this reason, many soy products such as tofu, soy nuts, soy butter, and soy burgers may provide beneficial effects to overall health because of their high polyunsaturated fat and fiber content and lower saturated fat content, as opposed to isoflavone supplementation in pill form. Studies are needed both on the potential impact of high protein diets on CVD and the effectiveness of isoflavones in prevention.<sup>248</sup> Soy protein has been popularized by its inclusion in the Portfolio Diet. Various forms of fermented soy at about 30-40 g/day would be an important addition to the diet of patients with dyslipidemia.

### ***Fenugreek***

Seeds of the Fenugreek (*Trigonella foenum-graecum*) plant have been used in Egyptian folk medicine and also as a spice and common dietary adjunct contributing to the taste and flavor of foods. Oil of Fenugreek has a maple-like flavor, and reportedly alters the color and odor of urine. In addition to use as a laxative, anti-pyretic and anti-inflammatory agent, Fenugreek is reported to be beneficial in the treatment of diabetes mellitus, and to have lipid-modifying effects.

In rats fed mucilage fiber of galactomannan isolated from Fenugreek seeds, a reduction in both cholesterol and triglyceride levels was reported, which led to reduced synthesis and secretion of apolipoprotein-B containing VLDL lipoproteins. This suggests a hypolipidemic effect of dietary fiber with glucomannan and a reduction of hepatic VLDL production.<sup>253</sup> Additionally, defatted portions of Fenugreek seed said to be rich in fiber (54%) and containing 4.8% of sterol saponins, significantly reduced plasma cholesterol in normal dogs and also caused a decrease in blood glucose levels.<sup>254</sup> In a comparative study by Evans *et al*, male adult rats were fed on diets containing 80 g/kg galactomannans with different galactose (G) and mannose (M) ratios/kg. The galactomannans were compared with purified cellulose (Solkaflok) and the animals were also fed on a basal diet free from fiber. The 3 galactomannans were fenugreek gum (1G:1M), guar gum (1G:2M), and locust-bean gum (1G:4M). In comparison with the fiber-free and Solkaflok diets, all 3 galactomannans lowered the concentrations of cholesterol in both liver and blood plasma and decreased the rate of hepatic cholesterol synthesis. The galactomannan with the highest caecal viscosity (Fenugreek) was the least effective in lowering plasma cholesterol and did not appear to have a direct effect on cholesterol absorption.<sup>255</sup> The effects of Fenugreek on glucose lowering appear promising.<sup>256-258</sup> The lack of good clinical human studies and minimal effect in animal studies would indicate that Fenugreek is not an important agent for the treatment of dyslipidemia.<sup>259</sup>

### ***Co-Enzyme Q-10***

Co-enzyme Q-10, or ubiquinone, has been evaluated in patients with dyslipidemia, but the effects on serum lipids are minimal.<sup>260-271</sup> The primary use of co-enzyme Q-10 in clinical practice is to support myopathic symptoms in patients treated with statins.<sup>262,264,270</sup> There is also evidence to suggest that co-enzyme Q-10 may reduce oxidation of LDL,<sup>268,269</sup> and improve endothelial dysfunction<sup>265</sup> and myocardial contractility.<sup>266,271</sup>

### ***Chromium***

Chromium, in its trivalent state, is a trace mineral important in the metabolism of glucose and is essential for health. Chromium is thought to potentiate the action of insulin in patients with impaired glucose tolerance by increasing insulin receptor-mediated signaling. Chromium deficiency has been reported to cause glucose intolerance, peripheral neuropathy, and confusion. The trivalent chromium found in supplements is largely non-toxic. The suggested intake of chromium for adults is 50-200 mcg/day. Chromium is found in many food sources including beer, cheese, meat, whole grains, and various herbal preparations including cat nip, horsetail licorice, nettle, elk straw, red clover, loud yam, and yarrow.

Supplemental chromium is best absorbed when administered as chromium picolinate, which enables chromium to be more readily absorbed. In a systemic review of randomized trials on chromium supplementation on glucose metabolism in diabetes care, 41 studies met criteria for review although half were poor quality. No benefit in individuals without diabetes was found; however, chromium supplementation significantly improved glycemic control in participants with type 2 diabetes, glycosolated hemoglobin levels improved by -0.6% and fasting glucose improved by -1.0 mmol/liter. Supplementation had no effect on lipid levels.<sup>272</sup> Chromium administration in the form of chromium yeast, however, was ineffective in improving glycemic control in western patients with type 2 diabetes taking oral hypoglycemic agents.<sup>273</sup>

In several studies, chromium picolinate was administered with biotin, a water-soluble vitamin with a bicyclic structure that reportedly plays a role in gluconeogenesis and fatty acid synthesis and serves as a CO<sub>2</sub> carrier on the surface of both cytosolic and mitochondrial structures, whilst also playing a role in the catabolism of certain amino acids. The recommended intake of biotin for adults is 30 mcg/day and 35 mcg/day in lactating females. Deficiencies have been reported in adults and infants. When a combination of chromium picolinate and biotin supplementation was administered in patients with type 2 diabetes mellitus with poor control, improved glycemic control was noted with a 9.7% reduction in 2-hour glucose levels compared to placebo.<sup>274</sup> Chromium picolinate and biotin combination administered as an adjunct to various diabetic medications in overweight or obese individuals with type 2 diabetes were well tolerated and improved glycemic control, reducing HgbA1c by 0.54%.<sup>275</sup> Additionally, Geohas *et al* conducted a study examining the effect of chromium picolinate and biotin supplementation in patients with type 2 diabetes mellitus. Results showed that just 4-weeks of supplementation led to a significant decrease in ratios of total to HDL cholesterol, LDL to HDL cholesterol, and non-HDL to HDL cholesterol.<sup>276</sup> Most recently, a study demonstrated that exposure of adipose tissue to chromium picolinate induces a loss of plasma membrane cholesterol. In addition, ABCA1, a transport mediator of cholesterol efflux was decreased gene. Activity of the membrane-bound sterol regulatory element-binding protein (SREBP) was also up-regulated by chromium picolinate.<sup>277</sup>

Although utilization of trivalent chromium to enhance glucose metabolism has gained more widespread acceptance, there are no definitive clinical outcome trials as yet to indicate the prevention of type 2 diabetes with chromium, nor the reduction of associated cardiovascular events with this supplement despite potentially beneficial effects on cardiovascular risk factors.<sup>277</sup> It is possible that adequate chromium supplementation may improve the lipid profile of selected patients with diabetes mellitus, glucose intolerance, metabolic syndrome, or insulin resistance, and who have concomitant dyslipidemia and are deficient in chromium. Further research is clearly indicated.

### ***Niacin (Nicotinic Acid)***

The term "niacin" is used to refer to both nicotinic acid and its amide form, nicotinamide. Both are precursors of nicotinamide adenine dinucleotide, the intracellular deficiency of which causes pellagra. These pellagra-preventing compounds are also classified as vitamin B<sub>3</sub>. The recommended daily allowance ranges between 14-18 mg. Because bread and cereal are supplemented with niacin, pellagra

is essentially non-existent in the United States. In much larger doses, nicotinic acid, but not nicotinamide, modifies the lipid profile.<sup>278</sup> The majority of clinicians use the term niacin to refer specifically to nicotinic acid, which might otherwise be confused with nicotine by patients.

In 1955, it was shown that nicotinic acid (in doses of 1000-4000 mg/day) reduced plasma cholesterol.<sup>278</sup> Subsequent studies have revealed that nicotinic acid:

- Reduces triglyceride levels by 20-50%.<sup>279,280</sup>
- Reduces LDL levels by 10-25%,<sup>279,280</sup> with a preferential decrease in the more atherogenic small, dense LDL.<sup>281</sup>
- Increases HDL by 10-30%, with a preferential increase in the HDL-2 subclass.<sup>282,283</sup> Note: niacin is the most effective currently available medication for raising HDL cholesterol.<sup>284</sup>
- Reduces Lp(a) levels by 10-30%.<sup>285,286</sup>

Nicotinic acid was the first lipid-lowering medication shown to reduce cardiovascular events. The Coronary Drug Project was a randomized, placebo-controlled trial of 3908 men with a history of previous myocardial infarction.<sup>287</sup> After a mean follow-up of six years, at an average daily niacin dose of approximately 2000 mg, there was a significant 26% reduction in nonfatal myocardial infarction and a 24% reduction in cerebrovascular events compared to placebo. After 9 more years of post-trial follow-up, total mortality was 11% lower in patients originally assigned to the niacin group versus the placebo-assigned patients.<sup>288</sup> The only other trial specifically designed to assess cardiovascular outcomes was the Stockholm Ischemic Heart Disease Secondary Prevention Study, which used niacin in combination with clofibrate, a bile acid-binding agent.<sup>289</sup> This 5-year study in men with a history of myocardial infarction demonstrated a 26% reduction in total mortality and a 36% reduction in ischemic heart disease mortality. There have also been 7 clinical trials of niacin that assessed vascular anatomic endpoints (coronary artery lesions in 6, carotid intima-media thickness in 1).<sup>290-298</sup> Five of these showed mean regression of the lesion dimensions from baseline.<sup>293-298</sup> All of these trials used niacin in combination with another lipid-lowering agent, either a bile acid sequestrant, statin, gemfibrozil or multiple agents. The dose of niacin used in these studies ranged from 1000-3000 mg/day. Several of these trials had statistically significant reductions in clinical events in the range of 50-70%,<sup>294-296</sup> although none were specifically designed with clinical endpoints as the primary outcome, nor had large enough numbers of patients to adequately and accurately assess the cardiovascular event reduction derived from the treatment regimens. However, 2 such clinical trials are currently underway.

There are many over-the-counter preparations of niacin, which can be divided into three categories: immediate release (marketed as immediate-release, crystalline or niacin), sustained-release (marketed as sustained-release, controlled-release or time-released niacin) and no-flush (marketed as no-flush, zero-flush or flush-free niacin). One study, which measured the nicotinic acid content in 29 over-the-counter preparations of niacin, revealed that none of the ten brands of no-flush niacin contained detectable free nicotinic acid.<sup>299</sup> The form of nicotinic acid supplied in each was inositol hexaniacinate.

### *Inositol Hexaniacinate (“No-Flush” Niacin)*

The purpose of using inositol hexaniacinate (IHN) is to make niacin therapy more tolerable. Since the cutaneous symptoms (skin flushing) associated with therapy correlate with levels of free nicotinic acid, it was reasoned that perhaps a pro-drug ester of nicotinic acid would be absorbed into the bloodstream, then slowly hydrolyze and release enough free nicotinic acid to reduce lipid levels without causing flushing.<sup>300</sup> Inositol hexaniacinate is a compound containing 6 molecules of nicotinic acid esterified to 1 molecule of inositol. Although treatment with inositol hexaniacinate showed promising lipid-lowering effects in a rabbit model,<sup>301</sup> this agent has shown little to no effect in lowering lipid levels in human studies.<sup>300,302,303</sup> These results are compatible with dose-response studies measuring blood levels of nicotinic acid in humans. After an oral dose of 1600 mg of inositol hexaniacinate plasma levels of free nicotinic acid peak at around 0.6 µmol/L.<sup>304</sup> In contrast, after an oral dose of 1000 mg crystalline niacin, plasma levels of free nicotinic acid peak at 224 µmol/L.<sup>305</sup> With 2 g/day of sustained-release niacin, plasma levels of free nicotinic acid reach a steady state between 22-40 µmol/L.<sup>306</sup>

Nicotinic acid is the preferred form of niacin to reduce lipids in doses of 500-3000 mg/day. IHN appears to be ineffective as a lipid-lowering agent and is not recommended.

## **Red Yeast Rice**

*Monascus purpureus* rice, popularly known as red yeast rice (RYR), is described as the fermented product of rice on which red yeast (*Monascus purpureus*) has been grown. Red yeast rice has been used both as a food preservative and for its medicinal properties in China since antiquity. However, in 1979 Endo discovered that a strain of *Monascus* yeast naturally produces a substance that inhibits cholesterol synthesis, which he named monacolin K (also known as mevinolin or lovastatin), and also a family of 8 monacolin-related substances with the ability to inhibit 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase.<sup>307</sup> Red yeast rice has also been found to contain sterols ( $\beta$ -sitosterol, campesterol, stigmasterol, and sapogenin), isoflavones and isoflavone glycosides, and MUFA.<sup>308</sup>

There have been 5 placebo-controlled, randomized trials published in English language journals, plus one poster presentation, involving a total of 961 subjects. All have demonstrated that extracts of red yeast rice, in varying concentrations, effectively lower cholesterol levels.<sup>309-314</sup> Statistically significant reductions of 16-31% were observed in total cholesterol and 22-32% reductions in LDL. Triglyceride reductions varied from small non-significant differences to 36%. HDL changes also varied, from no change to a 20% increase. Red yeast rice has been shown to reduced cardiovascular events.

The American studies used a proprietary preparation of red yeast rice known as Cholestin (Pharmanex, Simi Valley, CA), which is no longer commercially available in the United States. Cholestin was withdrawn from sale after the FDA decided that red yeast rice did not meet the definition of a dietary supplement according to the DSHEA of 1994, which stated that any product marketed as a dietary supplement cannot contain an agent that has been approved as a new drug unless the product was marketed before the drug's approval. Lovastatin (monacolin K), the main active ingredient in red yeast rice, was approved as a new drug by the FDA in 1987 under the brand name Mevacor. Cholestin is still available as a red yeast rice supplement in Canada, Europe, and Asia. In the United States, Cholestin has been reformulated and no longer contains red yeast rice, but rather polymethoxylated flavones extracted from citrus fruits, geraniol, and marine fish oils.<sup>315</sup> It is unknown whether this new preparation has any effect on lipid parameters.

There are other preparations being sold as Chinese red yeast rice, but these products have not been studied for lipid-lowering effectiveness or safety. One study analyzed 9 of these commercially available dietary supplements for monacolin amounts, and for citrinin, a toxic fermentation byproduct.<sup>316</sup> Total monacolin content varied from 0% to 0.58% by weight or 0.15 to 3.37 mg of lovastatin. By contrast, the original Cholestin preparation used in the study by Heber *et al* contained 4.8 mg of lovastatin.<sup>311</sup> Only 1 of 9 preparations contained the full complement of monacolin compounds, and citrinin was found at measurable concentrations in 7 of the 9 preparations. Aside from FDA regulatory issues with red yeast rice, this compound should not be recommended until standardized manufacturing practices are established which insure equivalence of content of active ingredients and limit the production of harmful byproducts of fermentation such as citrinin. Some of the standardized products contain about 0.4 mg HMG-CoA reductase inhibitors, with 0.3 mg coming from lovastatin equivalents. A dose of 2.4 g/day delivers about 9.6 mg of HMG-CoA reductase inhibitors, including about 7.2 mg of lovastatin equivalents. Such a dose would reduce LDL cholesterol about 8-10%.

## **Policosanol**

Policosanol is the commonly used name for a mixture of long-chain aliphatic alcohols originally derived from purified sugarcane wax.<sup>317</sup> The purported ability of this drug to lower cholesterol without significant side effects has made it one of the fastest growing over-the-counter supplements in the United States.<sup>318</sup> Policosanol has been used in Cuba since 1991, and until 2004, virtually all of the published medical literature on policosanol had been conducted by 1 research group based in Havana.<sup>319-333</sup> Funding for the Cuban studies was provided by Dalmer Laboratories, a commercial enterprise founded by the Center of Natural Products, National Center for Scientific Research, La Habana, Cuba, to market policosanol. Their studies have uniformly reported that sugarcane-derived policosanol has similar efficacy to statins in its ability to lower total and LDL-cholesterol, and even greater efficacy in raising HDL without any significant side effects.<sup>334</sup> The cholesterol-lowering response has been reported to be dose-dependant within the range of 2-40 mg.<sup>335</sup> The underlying mechanism of action of policosanol to lower cholesterol has not been definitively elucidated, but is proposed to include inhibition of cholesterol synthesis by down-regulating the cellular expression of HMG-CoA reductase.<sup>336,337</sup>

In contrast to the Cuban data, several recent animal and human studies from outside of Latin America have demonstrated a lack of efficacy for policosanol to favorably alter the lipid profile.<sup>338-344</sup> Animal studies from Canada and Australia tested policosanol derived from sugarcane, sunflower seed, and rice, results showed that it had no effect on lipid levels.<sup>338,339</sup> The first negative clinical trial in humans was published by a research group from the Netherlands, who tested a 20 mg dose of wheat germ-derived policosanol.<sup>340</sup> The second negative human trial was a study of 10 mg rice-derived policosanol from Croatia, which showed no significant changes in LDL after 8-eight weeks of treatment.<sup>341</sup> In 2006, 3 groups (2 from the United States and 1 from Germany) published randomized, placebo-controlled trials assessing the lipid altering effects of sugarcane-derived policosanol in doses ranging from 10-80 mg/day.<sup>342-344</sup> These studies all failed to find any significant lipid-altering effects of policosanol.

Ethnic and nutritional differences between European white and Latin American populations are extremely unlikely to account for the discrepant results in the Cuban and non-Cuban based trials since other lipid-altering drugs, such as statins<sup>345</sup> and ezetimibe,<sup>346</sup> have been shown to have no ethno-specific effects. Policosanol cannot be recommended for the treatment of hyperlipidemia.

### ***Ginseng***

Several varieties of ginseng exist; however, the majority of clinical studies have used either *Panax ginseng* (commonly known as Asian, Chinese, Korean, or Radix ginseng) or *Panax quinquefolius* (commonly known as American ginseng). These varieties contain ginsenosides, a diverse group of saponins (or glycosides) that are thought to be the main active components of ginseng.<sup>347</sup>

Nine studies which reported lipid-altering effects of ginseng, at highly variable doses, over periods ranging from 7-days to 3-months have been published.<sup>348-355</sup> The quality of most of these studies was poor. Only 3 were randomized, placebo-controlled trials, but none of these were designed to evaluate lipid changes as the primary outcome. Thus, they were underpowered to reveal small, but significant, effects such as a 10% lowering of LDL. The only one which reported an impact on lipid parameters was a 4-week trial of 15 men designed to evaluate the effect various antioxidant supplements on lipid peroxidation in smokers: the ginseng arm consisted of just 3 people. The other 2 studies showed no statistically significant effect on any lipid parameter. The first was an 8-week trial of 36 persons with type 2 diabetes (12 ginseng 100 mg, 12 ginseng 200 mg, 12 control). The second was a 12-week cross-over trial of 24 patients with liver disease, given *Panax ginseng* extract at a dose of 40 mg twice daily.

The remaining studies were non-randomized or had no control group. Dose ranges varied greatly (from 9-80 mg/day) as did frequency (from once daily to three times daily). Only 2 studies used ginseng products with known ginsenoside contents, and there was a 186-fold difference between these 2 studies in total ginsenoside content per daily dose. Currently, there is insufficient data to judge if ginseng has any effect on lipids, much less the magnitude of an effect.

### ***Green Tea***

Green tea and its active ingredient, epigallocatechin gallate (EGCG), reduces cholesterol levels and atherosclerosis in experimental animal models, and its consumption is associated with reductions in CVD in study populations by lowering fasting and postprandial serum cholesterol.<sup>356-367</sup> The mechanisms by which green tea may reduce the incidence of CVD include: reduction in gastrointestinal absorption of cholesterol, upregulating the hepatic LDL receptor, stimulation of fatty acid synthase gene expression in the nucleus, stimulation of cell energy expenditure in the mitochondria, and reducing LDL oxidation.<sup>356-367</sup>

A rat model study showed a significant 2.7-fold increase in LDL receptor binding activity and a 3.4-fold increase in LDL receptor protein, which reduced cholesterol absorption by 24% despite no change in serum cholesterol.<sup>356</sup> While another study in a rat model confirmed the improved LDL receptor binding associated with reductions in cholesterol of 60% and LDL by 80%, with reductions in hepatic and aortic cholesterol.<sup>360</sup> This suggests that green tea reduces liver cholesterol concentration by increasing the efflux of cholesterol from liver cells.<sup>356,360</sup>

Green tea catechins, especially EGCG, interfere with the emulsification, digestion, and micellar solubilization of lipids, which are critical steps involved in the intestinal absorption of dietary fat, cholesterol, and other lipids.<sup>357,359,365</sup> A green tea extract was found to significantly reduce serum glucose, total and LDL cholesterol, triglycerides, and free fatty acids, and increase HDL, in diabetic rats. In addition, myocardial levels of lipids were reduced, thus improving myocardial function.<sup>358</sup>

In a small human study of 22 subjects, administration of 7 cups of green tea daily for 2-weeks decreased serum MDA-LDL (oxidation of LDL) concentrations, but had no significant effects on serum cholesterol, platelet aggregation, platelet thromboxane B (TXB), or metalloproteinases (MMP's).<sup>362</sup> However, in another human study, 2 cups of green tea per day reduced serum LDL cholesterol by 13 mg, increased plasma total antioxidant activity, decreased plasma peroxides, and decreased DNA oxidative damage in lymphocytes.<sup>363</sup> Unno *et al* found that supplementation of human subjects with a low-dose (224 mg) or high-dose (674 mg) of green tea catechins attenuated the postprandial increase in plasma triglycerides by 15% (low-dose) and 29% (high-dose) following a fat load. However, no significant differences were observed in the postprandial responses for plasma total cholesterol.<sup>364</sup>

A Japanese study of 13,916 men and women found a direct relationship of green tea consumption and reduction in serum cholesterol levels. For each cup of green tea participants drunk, total cholesterol fell by 0.015 mmol/L, however there were no changes in triglycerides or HDL. The effect appeared to level off at about 10 cups of green tea per day.<sup>366</sup>

It is recommended that humans consume about 60 ounces of green tea per day or take a green tea extract standardized with EGCG at 500 mg once or twice per day.

### ***Plant Sterols***

Plant sterols and stanols (phytosterols) are natural fatty substances found in all plants, which have proven benefit in lowering total cholesterol and LDL with variable effects on HDL.<sup>368-382</sup> The sterols are beta-sitosterol, campesterol, and stigmasterol (4-desmethyl sterols of the cholestane series). The stanols are saturated sterols. Plant sterols are similar in structure to cholesterol, but are bound to plant fiber, which makes them difficult to absorb. In addition, storing, freezing, and cooking can destroy their activity, thus supplementation is preferred in order to get adequate intake.

The mechanism of action of phytosterols and phytostanols is to reduce intestinal absorption of cholesterol via competition with incorporation into the micelle. The phytosterols and phytostanols share the same mechanisms of absorption with the cholesterol molecule and influence the cholesterol metabolism inside the enterocytes. They prevent cholesterol absorption from the gut lumen and slow the esterification rate of phytosterols and phytostanols inside the enterocytes.<sup>372</sup>

In hypercholesterolemic subjects, consumption of 1.6 g/day of phytosterols with low-fat fermented milk reduced LDL by 9.5% over 6-weeks, with a 35% increase in plasma sitosterol concentration, but no change in campesterol concentrations and no change in biomarkers of oxidative stress.<sup>368</sup> A combination with low-fat margarine or milk enriched with plant sterols significantly reduced total cholesterol by 5.5%, LDL by 7.7%, and apolipoprotein B by 4.6%, but had no effect on hsCRP or Lp(a).<sup>369</sup> The lipid-lowering effect of phytosterols is also improved with the simultaneous consumption of canned tuna in olive oil (MUFA with omega 3-fatty acids),<sup>370</sup> PUFA,<sup>372</sup> fiber,<sup>372</sup> psyllium,<sup>376</sup> beta glucan,<sup>379</sup> statins,<sup>372,373</sup> and other combined portfolio approaches.<sup>378</sup>

Consumption of 7.28 g of psyllium with 2 g of plant sterols decreased LDL-1 from 2.46 mmol/L to 2.26 mmol/L and LDL-2 from 0.63 mmol/L to 0.54 mmol/L. Supplementation also increased LDL peak size, reduced cholesterol ester transport protein activity by 11%, and increased LDL receptors in circulating mononuclear cells by 26%.<sup>376</sup>

A dose response of phytosterols indicated that 1.6 g/day reduced LDL by 10.4% and 3 g/day reduced LDL by 14.7% within 3-weeks, with increased serum levels of beta sitosterol and campesterol.<sup>371</sup>

A study on hypercholesterolemic subjects administered 1.6 g/day plant sterol esters in capsule form over 4-weeks, results showed that supplementation lowered LDL levels by 7% and raised HDL levels by 9%.<sup>377</sup>

The Dutch Doetinchem Cohort Study examined the effects of chronic ingestion of plant sterols over 4-years in 80 subjects. Result showed that an average intake of 1.1 g/day of phytosterols in enriched margarine increased serum levels of sitosterol and campesterol and led to a 4% reduction in total cholesterol. No adverse effects of the increased serum levels of phytosterols were noted.<sup>375</sup>

A large meta-analysis of 6 studies evaluated the effects of phytosterols and phytostanols at an average daily dose of 2.3 g over 1 to 4-months in familial hypercholesterolemic patients. Total cholesterol fell by 7-11% and LDL fell by 10-15%, while triglycerides and HDL remained unchanged.<sup>382</sup>

Concerns have been raised that chronic increases in serum levels of plant sterols could have adverse cardiovascular consequences and actually increase the risk of CHD despite lower lipid levels.<sup>380</sup> This is based on the observation that patients with the rare genetic condition phytosterolemia overabsorb phytosterols and develop premature atherosclerosis. It is well documented that plant sterols supplementation produces an increase in blood phytosterol concentration in humans.



The evidence from human studies is mixed and does not prove or disprove an increase in atherosclerotic risk from serum phytosterols levels. However, a recent prospective nested case-control study of 373 cases and 758 controls did not appear to indicate that phytosterols would be adversely related to CHD.<sup>381</sup> Individuals in the highest tertile of the sitosterol concentration were found to have an unadjusted odds ratio (OR) of future CHD of 0.75 and an adjusted OR of 0.79. While in individuals in the highest tertile of campesterol concentration, unadjusted OR was 0.95 and the adjusted OR was 0.97. Additional studies should address this possibility, but there does not appear to be any increased risk of atherosclerosis with chronic ingestion of phytosterols at this time.

It is recommended that patients with dyslipidemia consume plant sterols at 1.6 to 3.0 g/day in supplemental capsule form, or in the form of phytosterols-enriched foods. For optimal results, the plant sterols should be taken with mixed fiber, MUFAs such as olive oil or nuts, and omega-3 fatty acids.

### ***Probiotics and Lipids***

Both animal and human studies have documented a modest but significant reduction in serum lipids with chronic consumption of oral probiotics.<sup>383-394</sup> Probiotics consist of various strains of beneficial bacteria that are known to have numerous health benefits.<sup>383-394</sup> Some of these bacteria include *Lactobacillus acidophilus* (1.5-2.0 billion per day), *Bifidobacterium animalis* (BB-12) (1.5-2.0 billion per day), *Streptococcus thermophilus* (0.50-0.60 billion per day), and *Lactobacillus delbrueckii* subspecies *bulgaricus* (0.20-0.30 billion per day). Probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis or redistributing cholesterol from plasma to the liver.<sup>393</sup>

In human studies over a period of 4 to 6-weeks, reductions in total cholesterol range from 4% to 12%, LDL from 5% to 8%. Triglyceride levels fell by approximately 10%.<sup>384-386,394</sup> However, some studies show no effect on lipids.<sup>389,390</sup>

Probiotics have been shown to reduce lipids via a number of mechanisms, including: co-precipitation with bile salts, deconjugation to bile salts, incorporation of cholesterol into the cellular membrane, and microbial assimilation of cholesterol.<sup>388</sup> In addition, probiotics have numerous other non-lipid benefits, including: an increase in antioxidant potential; lowering of blood pressure, leptin, fibrinogen, F-(2) isoprostanes, and IL-6; and decreased monocyte adhesion to endothelial cells.<sup>387,392</sup>

It is recommended that humans consume a high-quality mixed probiotic on a daily basis in the doses as mentioned above.

### ***Garlic and Lipids***

Recent well designed randomized, controlled clinical trials in humans do not demonstrate any significant reductions in serum lipids from the consumption of garlic.<sup>395,396</sup> Van Doorn evaluated 90 normolipidemic obese smokers treated with garlic powder and found no significant effect on inflammatory biomarkers, endothelial function, or lipid profile.<sup>395</sup> Gardner evaluated 192 moderate hypercholesterolemic patients treated for 6-months with raw garlic (4 g clove per day) or 2 commonly used garlic supplements, one a garlic powder and the other a garlic extract. There were no significant changes in total cholesterol, LDL, triglycerides, or HDL during the study period.<sup>396</sup> Garlic does not appear to have clinical effects on lipids and is not recommended as an effective natural lipid-lowering agent.

### ***Dietary Fiber and Lipids***

Dietary fiber is a collective term for a variety of plant substances that are resistant to digestion by human gastrointestinal enzymes. Dietary fibers are classified into two major groups depending on their solubility in water.<sup>397,398</sup> In humans, the structural or matrix fibers such as lignins, cellulose, and some hemicelluloses, are insoluble, whereas the natural gel-forming fibers, such as the pectins, gums, mucilages, and the hemicelluloses, are soluble.<sup>397,398</sup>

The mechanisms by which fiber lowers cholesterol include: binding of bile acids or cholesterol during the intraluminal formation of micelles; upregulation of LDL hepatic receptors; increased clearance of LDL; inhibition of hepatic fatty acid synthesis by products of fermentation, such as short chain fatty acids like acetate, butyrate, and propionate; changes in intestinal motility; reduced absorption of macronutrients; improved insulin sensitivity; and increased satiety with lower overall energy intake.<sup>397</sup>

Studies have focused on soluble fibers such as oats, psyllium, pectin and guar gum, and qualitative reviews suggest that these fibers lower total and LDL cholesterol.<sup>399,400</sup> Water-insoluble wheat fiber and cellulose have no effect unless they displace foods that supply saturated fats and cholesterol from intestinal absorption.<sup>401</sup>

There continues to be debate about the degree of cholesterol reduction caused by soluble fibers. The range of effects on total cholesterol varies, from 0-18% reduction in trials with oat products, 3-17% reduction in trials with psyllium, 5-16% reduction in trials with pectin, and a 4-17% reduction in trials with guar gum.<sup>397</sup> The reasons for such variations include small sample sizes, variable fiber doses, different concomitant diets, changes in body weight, unstable dietary control, and different study populations. Hyperlipidemic patients tend to have better reductions in lipids than normolipidemic patients.

In one of the largest meta-analysis, Brown *et al* reviewed 67 controlled trials to quantify the cholesterol-lowering effect of the major dietary fibers. Soluble fiber at 2-10 g/day reduced total cholesterol by 1.75% and LDL cholesterol by 2.2%. The effects on plasma lipids of soluble fiber from oat psyllium or pectin were not significantly different and were very minimal.<sup>397</sup> However, other meta-analysis of fibers or various fiber components conducted since Brown's meta-analysis indicate a more significant reduction in total cholesterol (4-5%) and LDL (5-7%).<sup>402-406</sup> Nevertheless, fiber clearly reduces the risk of CVD, CHD, myocardial infarction, and peripheral artery disease.<sup>407,408</sup>

Based on the cumulative data, it is recommended that dyslipidemic patients consume a mixture of soluble fibers at a dose of at least 10 g/day.

### ***Curcumin and Lipids***

Curcumin is a natural polyphenolic compound and the most active component of tumeric (*Curcuma longa*). Curcumin has been shown to improve serum lipid levels in experimental animal studies,<sup>409-412</sup> but only 1 human study has been conducted to evaluate its lipid-lowering effects.<sup>413</sup> Curcuminoids, the yellow pigments of curcuma, are obtained from the rhizomes of *Curcuma longa* and are commonly used as a spice and food coloring.<sup>409,411,414</sup> Curcumin and turmeric extracts exhibit anticarcinogenic, anti-inflammatory, antioxidative, anti-infectious, hypoglycemic, and hypocholesterolemic activities, as well as activities similar to recently discovered TNF blockers, vascular endothelial cell factor blockade, and epidermal growth factor blockers.<sup>409,411,414</sup> Curcumin increases LDL receptor mRNA 7-fold, slightly increases HMG CoA reductase and farnesyl diphosphate synthetase, and induces changes in the expression of genes involved in cholesterol homeostasis.<sup>409</sup> In ApoE/LDL-R double knockout mice, curcumin demonstrated anti-atherogenic effects despite no change in lipid levels.<sup>414</sup> Curcumin has also been shown to improve antioxidant activity by increasing levels of the potent antioxidants superoxide dismutase and glutathione peroxidase in the liver,<sup>410</sup> and reduce oxidation of LDL.<sup>411</sup>

The effect of curcumin administration in reducing the serum levels of cholesterol and lipid peroxides was studied in 10 healthy human volunteers. Subjects received 500 mg/day of curcumin for 7-days. A significant decrease in the level of serum lipid peroxides (33%), increase in HDL (29%), and a decrease in total serum cholesterol (11.6%) were noted.<sup>413</sup>

Phase 1 clinical studies with curcumin in doses of 3600 to 8000 mg/day for 4-months did not detect discernable toxicities except mild nausea and diarrhea. In general, pharmacokinetic studies show a low bioavailability of curcumin following oral administration.<sup>415</sup> Curcumin may have a protective effect against alcohol and PUFA-induced hyperlipidemia.<sup>416</sup>

Additional randomized controlled clinical trials in humans with larger sample sizes will need to be conducted to confirm these findings on lipids in humans.

It is recommended that patients consume approximately 500 mg/day of high-quality curcumin (turmeric extracts). There appears to be no adverse effects with chronic use, however it is important to note that curcumin may aggravate bleeding in patients taking anticoagulants.

### ***Sesame Oil***

Sesame oil (*Sesame indicum*) has been demonstrated to have lipid-lowering effects on both animals and humans.<sup>417-421</sup> Sesame oil is rich in both MUFA and PUFA (47% oleic acid and 39% linoleic acid), and contains lignans (e.g. sesamin and sesamol) and several antioxidant compounds (e.g. sesaminol).<sup>417</sup> Sesamin was found to reduce serum lipid levels and increase fatty acid oxidation in rodents.<sup>417</sup> Rodent studies indicate reductions in serum lipids, as well as decreases in atherosclerotic lesions, over a 3-month period.<sup>421</sup>

Sesame oil was administered to 40 hypertensive diabetics for 45-days. Results showed that sesame oil supplementation was associated with a drop in systolic and diastolic blood pressure, decreased levels of glucose, HbA1c, total cholesterol, LDL, triglycerides, and increased antioxidant activity.<sup>417</sup> In a study of 24 postmenopausal women administered 50 g/day of sesame seed powder, total cholesterol fell by 5% and LDL fell by 10% over the 5-week study period.<sup>418</sup> In 530 patients given 35 g/day of sesame oil for 60-days, significant reductions in blood pressure, total cholesterol and LDL, and an increase in HDL was recorded.<sup>419</sup>

Given the findings documented in the medical literature, it is recommended that sesame oil should be included in the diet of dyslipidemic patients at a dose of at least 35 g/day.

## THE FUTURE OF NUTRITIONAL COMPOUNDS FOR DYSLIPIDEMIA

As of this writing, the following non-pharmacologic agents are under evaluation in human clinical trials.

### *Resveratrol*

Resveratrol reduces oxLDL, inhibits ACAT activity and cholesterol ester formation, increases bile acid excretion, reduces TC, TG and LDL, increases PON-1 activity and HDL, inhibits NADPH oxidase in macrophages and blocks the uptake of modified LDL by CD36 SR (scavenger receptors)<sup>422</sup>. N Acetyl Cysteine (NAC) has this same effect on CD 36 DR and should be used in conjunction with resveratrol<sup>422</sup>. The dose of trans resveratrol is 250 mg per day and NAC is 1000 mg twice per day.

### *Wogonin*

Wogonin is a flavonoid and one component of *Scutellaria baicalensis Georgi* extract that enhances reverse cholesterol transport.<sup>426</sup> Wogonin increases the protein expression, level and half life of the ABCA-1 transporter (ATP binding cassette transporter A-1). This effect is linked to its ability to stimulate PP2B which is a protein Ser/Thr phosphatase that regulates the stability of ABCA1 protein. In addition, Wogonin inhibits the expression of pro-atherogenic molecules in endothelial cells and vascular smooth muscle cells. Other flavonoids such as procyanidins, quercetin, catechins, red wine, resveratrol, grape seed extract, tangerine extract and anthocyanins may have similar effects.

### *Citrus Bergamot*

Citrus bergamot has been evaluated in several clinical prospective trials in humans. In doses of 1000 mg per day this compound lowers LDL up to 36%, TG 39% and increases HDL 40% by inhibiting HMG CoA reductase, increases cholesterol and bile acid excretion, reduces ROS and oxLDL<sup>425</sup>. The active ingredients include naringin, neoeriocitrin, neohesperidin, poncerin, rutin, neodesmin, rhoifolin, melitidine and brutelidine<sup>98, 99</sup>.

### *Pomegranate*

Pomegranate increases PON 1 binding to HDL and increases PON 2 in macrophages. It is a potent anti-oxidant, increases total anti-oxidant status (TAS), lowers oxLDL, decreases antibodies to oxLDL, inhibits platelet function, reduces glycosylated LDL, decreases macrophage LDL uptake, reduces lipid deposition in the arterial wall, decreases progression of carotid artery IMT and lowers blood pressure especially in subjects with the highest oxidative stress, known carotid artery plaque and the greatest abnormalities in TG and HDL levels<sup>423, 424</sup>. Consuming about 8 oz of pomegranate juice per day is recommended.

## **PROTOCOL FOR NON-PHARMACOLOGIC MANAGEMENT OF DYSLIPIDEMIA**

### ***Mechanism 1. Address Dyslipidemia- Induced Vascular Disease.***

1. Decrease endothelial permeability, gap junctions, endothelial dysfunction and improve endothelial repair.
2. Modify caveolae, caveolin -1, lipid rafts, membrane microdomains, unesterified cholesterol and cholesterol crystals.
3. Increase eNOS and nitric oxide
4. Modify pattern recognition receptors (PRR) activation and toll like receptors
5. Decrease cholesterol crystals, LDL phospholipids, ox LDL, APO –B and 7 ketosteroids that activate PRR.
6. Decrease LDL burden
7. Reduce cholesterol absorption
8. Increase cholesterol bile excretion
9. Decrease LDL particle number
10. Decrease APO B
11. Decrease LDL modification
12. Inhibit LDL glycation
13. Increase LDL size
14. Modify LDL composition
15. Upregulate LDL receptor
16. Regulate sortilins and SORLA
17. Deactivate the LOX-1 receptor
18. Decrease modified LDL macrophage uptake by scavenger receptors
19. Decrease native LDL macrophage uptake by pinocytosis
20. Decrease LDL signaling
21. Decrease macrophage recruitment and migration
22. Alter macrophage phenotype
23. Modify signaling pathways
24. Increase reverse cholesterol transport
25. Increase HDL and increase HDL size
26. Improve HDL function
27. Increase APO-A1
28. Increase PON 1 and PON 2
29. Reduce inflammation
30. Reduce oxidative stress
31. Modulate immune dysfunction
32. Decrease VLDL and TG
33. Lower Lp(a)
34. Reduce foam cell and fatty streak formation
35. Reduce trapping of foam cells in the subendothelium
36. Stabilize plaque
37. Reduce LpPLA2
38. Reduce plaque burden, progression and increase regression.

### ***Mechanism 2. Inhibit LDL Oxidation***

- Niacin
- EGCG and catechins
- Quercetin
- Pantethine
- Resevertrol
- Red Wine
- Grape Seed extract
- MUFA
- Curumin
- Pomegranate

- Garlic
- Sesame
- Gamma/delta tocotrienols
- Lycopene
- Polyphenols
- Flavonoids
- Oleic acid
- Glutathione
- Citrus Bergamot
- Tangerine extract
- Policosanol
- RBO (Ferulic acid gammaoryzanol)
- Coenzyme Q10
- Vitamin E

***Mechanism 3. Inhibit LDL glycation***

- Carnosine
- Histidine
- Myricetin
- Kaempferol
- Rutin
- Morin
- Pomegranate
- Organosulfur compounds

***Mechanism 4. Lower LDL***

- Niacin
- RYR
- Plant Sterols
- Sesame
- Tocotrienols(gamma/delta)
- Pantethine
- Citrus Bergamot
- EGCG
- Omega 3 fatty acids
- Flax Seed
- MUFA
- Garlic
- Resveratrol
- Curcumin
- Orange Juice
- Soluble fiber
- Soy
- Lycopene

***Mechanism 5. Convert Dense LDL B to Large LDL A***

- Niacin
- Omega 3 Fatty Acids
- Plant Sterols

***Mechanism 6. Reduce Intestinal Cholesterol Absorption***

- Plant Sterols
- Soy
- EGCG
- Flax Seeds
- Sesame
- Garlic
- Fiber

***Mechanism 7. Inhibit HMG CoA Reductase***

- RYR
- Pantethine
- Gamma/Tocotrienols
- Sesame
- EGCG
- Omega 3 Fatty Acids
- Citrus Bergamot
- Garlic
- Curcumin
- GLA
- Plant Sterols
- Lycopene
- Soy

***Mechanism 8. Lower Lp(a)***

- Niacin
- NAC
- Gamma delta tocotrienols
- Omega 3 Fatty acids
- Flax Seed
- CoQ 10
- Vitamin C
- L Carnitine
- L-Lysine
- L-Arginine
- Almonds

***Mechanism 9. Lower Triglycerides***

- Niacin
- RYR
- Omega 3 Fatty Acids
- Pantethine
- Citrus Bergamot
- Flax Seed
- MUFA
- Resveratrol
- Orange Juice

***Mechanism 10. Increase Total HDL and HDL 2 b levels and convert HDL 3 to HDL 2 and 2 b***

- Niacin
- Omega 3 Fatty Acids

- Pantethine
- Red yeast rice
- MUFA
- Resveratrol
- Curcumin
- Pomegranate
- Orange juice
- Citrus Bergamot

***Mechanism 11. Alter Scavenger Receptor NADPH Oxidase and oxLDL Uptake into Macrophages***

- Resveratrol
- NAC ( N Acetyl Cysteine)

***Mechanism 12. Increase Reverse Cholesterol Transport***

- Lycopene
- Niacin
- Plant Sterols
- Glutathione
- Wogonin
- Resveratrol
- Various flavonoids and anthocyanins

***Mechanism 13. Decrease LDL Particle Number***

- Niacin
- Omega 3 fatty acids

***Mechanism 14. Reduce Inflammation***

- Niacin
- Omega 3 fatty acids
- Flax seed
- MUFA
- Plant Sterols
- Guggulipids
- Resveratrol
- Glutathione

***Mechanism 15. Lower APO B Lipoprotein***

- Niacin
- Omega 3 fatty acids
- Plant Sterols
- EGCG

***Mechanism 16. Increase APO A- 1 Lipoprotein***

- Niacin

***Mechanism 17. Decrease LDL Particle Number***

- Niacin
- Omega 3 fatty acids

***Mechanism 18. Upregulate the LDL receptor***

- EGCG
- Sesame
- Tocotrienols
- Curcumin
- Policosanol
- Plant Sterols

***Mechanism 19. Increase PON 1 and PON 2***

- EGCG
- Ouercetin
- Pomegranate
- Resveratrol
- Glutathione

***Mechanism 20. Increase Bile Acid excretion***

- Resveratrol
- Citrus Bergamot
- Fiber
- Probiotics
- Plant Sterols
- Sesame

**CONCLUDING REMARKS AND CLINICAL RECOMMENDATIONS**

The foundation for the treatment of dyslipidemia is optimal nutrition, diet, and the achievement/maintenance of ideal body weight, combined with an aerobic and resistance exercise program in all patients. Depending on the degree of CV risk, nutritional supplements or drug therapy is the next step. For the low to moderate-risk patient, nutritional supplements are the second cornerstone of therapy. In the high and very-high-risk patients, pharmacologic agents are needed and should be used in conjunction with diet, nutrition, exercise, weight loss, and scientifically proven nutritional supplements. Clinical studies support the ability of diet, lifestyle modifications, and nutritional supplements, to reduce serum cholesterol, LDL, and triglycerides by 30-40%, in the majority of patients. Details of the NCEP diet, portfolio diet, DASH diet and Mediterranean diet, are discussed in detail in this paper, as well as the type and duration of exercise required to achieve significant and clinically relevant reductions in serum lipids.

Nutritional supplements provide additional therapeutic interventions for lipid-lowering. Those supplements that have the best clinical data in humans for improving the lipid profile include: niacin, omega-3 fatty acids, rice bran oil, gamma/delta tocotrienols, pantethine, red yeast rice, plant sterols, soluble fibers, probiotics, soy, and mixed nuts with MUFA and PUFA (e.g. almonds). Agents that do not appear to have significant effects on lipids based on recent randomized controlled trials are: guggulipid, policosanol, garlic, IHN, ginseng, fenugreek, co-enzyme Q-10, and chromium. Additional studies are needed to evaluate the role of green tea (EGCG) and curcumin (turmeric) as effective lipid-lowering agents in humans.

In addition to cholesterol and LDL reductions, several nutritional supplements have other anti-atherogenic effects. Reduced oxidation of LDL cholesterol is documented with niacin, EGCG, pantethine, resveratrol, garlic, policosanol, RBO, Co-Q-10, gamma/delta tocotrienols, vitamin E, MUFA, polyphenols and curcumin. While niacin, omega-3 fatty acids, plant sterols and psyllium, have been shown to convert type B dense LDL to the larger type A LDL, which is not atherogenic.

Intestinal cholesterol absorption is reduced with plant sterols, soy, EGCG, sesame, and fiber. Inhibition of HMG-CoA reductase is seen in the presence of pantethine, gamma/delta tocotrienols, red yeast rice, and sesame. Triglycerides are especially lowered with niacin, omega-3 fatty acids, and pantethine, and to a lesser extent, with red yeast rice, and soy. HDL is increased in size from HDL 3 to HDL 2 by niacin, omega 3-fatty acids, pantethine, and soy.

The best clinical data for reduction in cardiovascular events with nutritional supplements is with omega-3 fatty acids and, to a lesser extent, with niacin and fiber. This includes CHD, myocardial



infarction, and overall cardiovascular events for each of these, as well as reductions in stroke and sudden death for omega 3 fatty acids, and decrease in peripheral artery disease with fiber.

### ***Summary of Nutrition Guidelines for Dyslipidemia Treatment***

1. Mediterranean and portfolio diets are recommended.
2. Reduce saturated fats to about 10% of total fat intake.
3. Eliminate trans fats.
4. Increase MUFAs to 40% of total fat intake.
5. Increase PUFAs (omega-3) to 40-50% of total fat intake.
6. Increase viscous fiber to 50 g/day.
7. Increase vegetables to 6 servings per day.
8. Increase fruits to 4 servings per day.
9. Add plant sterols and nuts to diet.
10. Reduce refined carbohydrates and use low glycemic foods. Use more complex carbohydrates.
11. Consume high quality protein with cold water fish and organic lean meat and poultry.
12. Maintain ideal body weight and body composition.

### ***Summary of Recommended Nutritional Supplements for Dyslipidemia Treatment***

1. Gamma/Delta Tocotrienols: 200 mg/night with food.
2. Pantethine: 300 mg 3-times per day (or 450 mg 2-times a day).
3. Omega-3 Fatty Acids: at 3-5 g/day at a ratio of 3-parts EPA, 2-parts DHA and GLA at 75-90% of the total DHA and EPA.
4. Vitamin E at 100 IU/day with mostly gamma/delta tocopherol (80%) should be added to reduce oxidative stress.
5. Niacin (nicotinic acid): various forms at 500-3000mg/day.
6. Red Yeast Rice (high-quality and standardized): 2400 mg/night. Doses of 4800 mg may be safe and even more effective.
7. Probiotics: standardized to provide the optimal bacterial count.
8. Curcumin: 500/day.
9. Green Tea Extract: standardized to 250-500 mg of EGCG twice per day.
10. Plant Sterols: 1.6-3.0 g/day in divided doses with food.

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