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• The Effects of Australian Tea Tree and Jojoba Essential Oils with Minerals for Treatment of Nail Fungus
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The Fructose Controversy: Separating Fact from Fiction

Jeffrey S. Bland, PhD, FACN*
President and Chief Science Officer
Metagenics, Inc., Gig Harbor, Washington

ABSTRACT
Fructose is a monosaccharide found in many natural foods, and its modest consumption has been demonstrated to have beneficial effects on blood sugar and lipid parameters. Paradoxically, several recent studies have reported that fructose consumption is associated with adverse physiological effects including hypertriglyceridemia, reduced HDL cholesterol, increased uric acid levels, and increased blood postprandial insulin and glucose, which has led to considerable misunderstanding and confusion among clinical nutrition and dietetics professionals. Many factors influence the response to fructose, such as glycemic load, caloric percent of sugars, the delivery form of the sugar and, most notably the actual amount of intake, which has increased considerably over the past few decades. This increase has primarily resulted from the increased consumption of high fructose corn syrup-containing beverages like soft drinks and sweetened fruit drinks. Unfortunately, these specifics are often not included in the evaluation process, and in many cases, are not even reported so that study results can be kept in context. This lack of consistent reporting about fructose has led to the labeling of fructose as detrimental. This paper reviews the metabolism of fructose, and discusses some of the recent data in order to place this debate in perspective.

Key Words: fructose, HFCS, triglycerides, cholesterol, metabolic syndrome, diabetes

INTRODUCTION
Fructose: A Common Dietary Monosaccharide
Fructose, a 6-carbon sugar found in fruit, vegetables and honey is the most common naturally occurring monosaccharide.1 Because early data indicated that fructose does not induce an insulin response but attenuates the blood glucose peak when taken with glucose, and that fructose is known to produce the same sweetness for less energy, researchers and diabetes care organizations initially recommended fructose as a preferred sweetener for individuals with diabetes.2,3 Over the past three decades, however, the consumption of fructose has increased 120-fold—from an estimated 0.5 lb/year per person in 1970 to 62.4 lb/year per person.4 This increase is correlated with increased incidences of obesity, diabetes, and metabolic syndrome; therefore, some researchers have proposed that fructose is a factor in promoting these conditions.5,6 Taken together with controversial data suggesting that fructose can lead to hypertriglyceridemia,7 in the mid-1990s, the American Diabetic Association (ADA) indicated that the use of added fructose as a sweetening agent may have "no overall advantage over other nutritive sweeteners," even though they went on to state that there is no reason to avoid naturally occurring fructose in fruit, vegetables and other foods.8
Developers of nutraceuticals, supplements, and medical and specialty food products have limited sweetener choices. Glucose has inherent concerns of leading to increases in blood insulin and glucose levels; whereas, sucrose (table sugar) also contains glucose and can increase insulin and glucose responses. Synthetic sweeteners are available, but not acceptable for a natural food-based product. And while some natural products may be allowable in a supplement, they may not be generally recognized as safe (GRAS), and thus are not allowable in a food product (e.g., stevia). Fructose is a preferred choice in some cases because it can be used at lower amounts than other natural sweeteners to achieve the same sweetness level, and to help attenuate blood glucose and insulin levels. Therefore, this debate on the benefits and adverse effects of fructose is particularly relevant to the natural foods market.

In an attempt to tease apart fact from fiction within this controversy, and to better understand the effects of fructose in the diet, it is important to review some of the basic physiological and nutritional aspects of fructose-containing sweeteners. This paper briefly reviews the basic biochemistry and physiology of fructose and discusses the controversial fructose data with respect to human physiology and medical relevance.

**Metabolic Fate of Fructose**

Many types of sugars are found in food, and different terms have been identified to describe them (Table 1). The most common monosaccharides are fructose, glucose and galactose. Disaccharides that are also common sweeteners in foods include sucrose (glucose + fructose), the milk sugar lactose (glucose + galactose), and maltose (glucose + glucose). Once consumed, these disaccharides are broken down into their respective monosaccharides by specific enzymes (disaccharidases). Glucose, if present in the disaccharide, is released and absorbed across the intestinal mucosa by way of a sodium-glucose transport system. Similarly, when fructose is present in the disaccharide, it is released and available for absorption. In contrast to glucose, however, fructose is transported across the intestinal barrier of the duodenum and jejunum by a sodium-independent facilitated diffusion process. Studies indicate that fructose is absorbed more slowly than glucose, but its absorption is enhanced when glucose is present.4

The metabolism of fructose has been extensively reviewed.4,6,10-12 Briefly, it has been noted that its metabolism differs from that of glucose in several ways. Glucose enters the body's cells by way of the glucose transporter GLUT-4, which requires insulin in all tissues except the eyes, nerves and kidneys.6 In this process, insulin activates the insulin receptor, which then results in an increased number of GLUT-4 transporters on the cell surface. In contrast, fructose enters cells via the GLUT-5 transporter, which does not depend on insulin.10 The beta cells of the pancreas lack the GLUT-5 transporter, and therefore, fructose does not directly stimulate insulin secretion.6 It is interesting to note that the GLUT-5 transporter is also absent from cells in the brain, which may be the reason why fructose does not produce the same type of satiety signal as does glucose.

In humans, at least half of the absorbed fructose is rapidly taken into the liver, where it is phosphorylated to form fructose-1-phosphate by the enzyme fructokinase (Figure 1).11,12 Fructose-1-phosphate (a 6-carbon sugar) is readily cleaved by the enzyme aldolase B to form two triose (3-carbon) molecules. These 3-carbon molecules become the backbone for triglyceride synthesis, and are also used for the generation of the phospholipids that are necessary for healthy cell membranes. Fructose can also be converted into glucose-6 phosphate via the phosphofructokinase pathway, which leads to an increase in liver glycogen levels. Although the data are somewhat equivocal, the balance of

<table>
<thead>
<tr>
<th>Table 1. Definitions of Sweeteners.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sugar</strong></td>
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<tr>
<td><strong>Added sugar</strong></td>
</tr>
<tr>
<td><strong>Naturally occurring sugar</strong></td>
</tr>
<tr>
<td><strong>Total sugars</strong></td>
</tr>
<tr>
<td><strong>Free Fructose</strong></td>
</tr>
<tr>
<td><strong>Total Fructose</strong></td>
</tr>
<tr>
<td><strong>High fructose corn syrup (HFCS)</strong></td>
</tr>
</tbody>
</table>
studies indicate that fructose together with glucose promotes glycogenesis better than glucose alone.\textsuperscript{11} The increase in blood glucose and insulin is much smaller after a dose of fructose than when a similar amount of glucose is consumed.\textsuperscript{12} For this reason, fructose has been considered valuable as a glucose substitute in diets for individuals with diabetes. Clinical studies have demonstrated that a small amount of fructose, when ingested with glucose, also reduces the glycemic response and increases liver synthesis of glycogen in patients with type 2 diabetes. \textsuperscript{4,12,13} Glucose metabolism is under the control of phosphofructokinase, an enzyme that is regulated by ATP and glucose. \textsuperscript{12} In contrast, fructose can bypass the phosphofructokinase regulatory step; therefore, excessive fructose can continue to be metabolized (Figure 2), which ultimately produces an increase in lactate with only a small increase in diet-induced thermogenesis.\textsuperscript{4,11,12}

High Fructose Corn Syrup: the "Other" Fructose

In a 2004 \textit{Time} magazine article entitled "The Corn Connection," Eric Roston pointed out that technological advancements in the processing of corn have resulted in a substantial decrease in the cost of goods for corn products over the past several decades.\textsuperscript{14} Furthermore, he noted that these advancements, coupled with the mass amount of corn grown in the United States, have resulted in increased availability of inexpensive corn-based industrial products, the major uses of which are as food ingredients for human consumption. The sweetener high fructose corn syrup (HFCS) accounts for about five percent of these processed corn products in the United States.

The production of HFCS begins with the removal of protein and fiber from cornstarch, after which the purified starch is hydrolyzed to form glucose, and then some of this glucose is isomerized to yield fructose. The most common forms of HFCS are HFS-42 and HFS-55. Although the name suggests this is "fructose," in fact, fructose comprises only around half of these products: HFS-42 is 42% fructose and 53% glucose; HFS-55 is 55% fructose and 42% glucose.\textsuperscript{15} The final HFCS product also contains negligible amounts of other sugars, such as various oligosaccharides. HFCS is cheaper than many other sweeteners, is sweeter than sucrose and glucose on an equal weight basis (e.g., some reports indicate HFS-42 and HFS-55 are 1.16 and 1.28 times as sweet as sucrose, respectively),\textsuperscript{6} and is easily formulated into beverages.\textsuperscript{6,15}
In its pure form, fructose (i.e., crystalline fructose) is not the same as HFCS; it is not a combination of glucose and fructose, nor does it contain other sugars. Crystalline fructose can be purified from HFCS using a process by which the non-fructose substances are removed. In contrast to HFCS, crystalline fructose is not a primary source of fructose in processed food, but is mainly used in powdered drinks and specialty food products.

HFCS and Fructose Consumption

Because fructose is the most common monosaccharide in fruit, vegetables, and other foods, it has been consumed at moderate levels for thousands of years. It is estimated that, prior to the 20th century, the average intake of fructose was around 16 to 20 g per person per day. Table 2 summarizes the fructose that is delivered in commonly consumed fruit. The dietary fructose intake from one serving of fruit can range from ~4 g (e.g., one peach) to ~10 g (e.g., banana). Traditional dietetic and nutritional wisdom would suggest that the ingestion of two or three portions of fruit per day is considered valuable as part of an overall dietary program. This intake of fruit would be expected to deliver from 10 to 35 g of fructose daily. Natural sweeteners such as honey and molasses are also natural sources of fructose. For example, taking into account the free fructose and the fructose liberated after digestion of the disaccharides in these foods, 100 kcal of honey (~30 g serving) delivers 13.6 g fructose, and 100 kcal of blackstrap molasses (~47 g serving) delivers 11.8 g fructose.

The availability of a commercial process for making inexpensive HFCS has led to a considerable shift in the food production system. At one time, sucrose derived from sugar cane or beets was the primary sweetener. Currently, HFCS sweeteners are often used in the absence of sucrose. The extra sweetness of HFCS and its ability to be used in beverages such as soft drinks have resulted in HFCS accounting for approximately 50% of sweetener intake, and 20% of total dietary carbohydrate intake. As pointed out by Dr. George Bray and his colleagues at the Louisiana State University's Pennington Biomedical Research Center, the development of the inexpensive, sweet, corn-based syrup made it profitable to replace sucrose (table sugar) and simple sugars with HFCS in the American food supply so that HFCS now represents a major source of added sugars in the diet. The primary factor in this increase in daily fructose intake has come from the increased consumption of HFCS in such products as processed drinks (e.g., colas). In fact, the sugars from processed drinks and desserts total about two-thirds of all caloric sweeteners consumed in the United States. As stated by Basciano and colleagues: "In 1992, the USDA recommended that only 40 g of extra sugar should be added to a standard 2000 calorie a day diet. The amount of HFCS found in only one 12-oz soft drink equals this total proportion of daily intake." This increase of sweeteners not only represents empty carbohydrate calories, but also accounts for the increase to a typical consumption of 50 g to 100 g fructose per day per person in the United States in 2002.

In June, 2005, the Center for Science in the Public Interest (CSPI) published the second edition of the report entitled "Liquid Candy: How Soft Drinks are Harming Americans’ Health." In this report, it was noted that carbonated beverages are the single biggest source of refined sugars in the American diet. Youths and young adults appear affected the most: among 12- to 19-year-olds, carbonated soft drinks provide 9% and 8% of boys’ and girls’ daily caloric intake.

Table 2. Fructose content in 100 g (3.5 oz) portions of some commonly consumed fruit. Values are an average and may vary somewhat during different growing seasons and with specific subspecies of fruit.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Total kcal/serve</th>
<th>Glucose (%)</th>
<th>Fructose (%)</th>
<th>Sucrose (%)</th>
<th>Total Sugar (g)</th>
<th>Fructose* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td>85</td>
<td>26.5</td>
<td>30.1</td>
<td>43.4</td>
<td>19.6</td>
<td>10.2</td>
</tr>
<tr>
<td>Figs, dried</td>
<td>274</td>
<td>50.2</td>
<td>34.4</td>
<td>15.4</td>
<td>64.2</td>
<td>27.0</td>
</tr>
<tr>
<td>American Grapes</td>
<td>69</td>
<td>51.6</td>
<td>46.2</td>
<td>2.2</td>
<td>9.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Orange</td>
<td>49</td>
<td>29.4</td>
<td>21.2</td>
<td>49.4</td>
<td>8.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Peach</td>
<td>38</td>
<td>13.3</td>
<td>13.3</td>
<td>73.3</td>
<td>7.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Pear</td>
<td>61</td>
<td>28.7</td>
<td>57.4</td>
<td>13.8</td>
<td>8.7</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*Sum of free fructose and the fructose present in sucrose
daily caloric intake, respectively.\textsuperscript{18} Teenage boys alone get 44\% of their daily refined sugar intake from soft drinks. These intakes should take into account that a considerable number of individuals consume no soft drinks, and therefore, a specific individual’s consumption could be much higher than the average value. Moreover, as noted by Barry Popkin of the University of North Carolina-Chapel Hill, who has investigated food intake patterns among the United States population, “Sugar-loaded beverages are really just empty calories that block out healthy foods.”\textsuperscript{18,19}

Therefore, there are different sources of fructose in the American diet, the most common being HFS-42, HFS-55, and sucrose, with crystalline fructose, fruit, and natural sweeteners like honey and molasses also moderately contributing to fructose intake. Because each of these sources have different compositions, they may have differing effects; therefore, it is important to separate the data obtained with HFCS from the data obtained with other sources of fructose. Moreover, it is important to critically evaluate whether HFCS-containing products are replacing nutrients in the diet, and to consider the amount of total sugar consumption in various studies with HFCS to understand if the effects seen following its consumption can be extrapolated to the consumption of fructose alone before conclusions are drawn. Unfortunately, many of the studies that have been published have either failed to clearly define the form of fructose that was studied, or have generalized their conclusions from one form of sweetener to all fructose-containing foods.

**HEALTH EFFECTS OF FRUCTOSE**

**Effect of Fructose on Glucose Tolerance and Insulin Secretion**

Daly and colleagues have provided extensive, in-depth reviews of data collected on the influence of fructose on glucose and insulin metabolism and note that, although animal studies have provided convincing evidence for an effect of high-fructose diets in decreasing insulin sensitivity, research in humans has produced very conflicting results, with limited evidence for an effect.\textsuperscript{20,21} Much of the data accumulated in animal studies have represented diets in which fructose comprised at least 35\% of energy, or have used sucrose at >50\% of energy.\textsuperscript{21} In an average 2,000 kcal human diet, for example, this would constitute 125 g to 175 g fructose per day. With the high fructose content of products such as soft drinks, this data may represent some intake levels, but is higher than most individuals consume.

Fructose is known to have a low glycemic index, to attenuate the glucose peak, and does not increase insulin secretion after acute doses. For example, Moore et al. reported that consumption of 7.5 g fructose dose with 75 g glucose solution reduced the glycemic response in both healthy and type 2 diabetic subjects.\textsuperscript{22,23} Heacock et al. confirmed this effect on glycemic peak, reporting that a small (10 g) dose of fructose taken 30 or 60 minutes before a starch challenge (potatoes providing 50 g available carbohydrate) significantly attenuated the glucose peak by 27\% and 25\%, respectively, but they saw no change in triglycerides when the fructose was given with the starch.\textsuperscript{24} As shown in Table 3, studies in humans have provided conflicting results with no clear demonstration of an adverse effect of fructose on blood glucose and insulin levels. In discussing this disparity in the human research. Daly summarizes by saying, “Overall, despite the wealth of interest that sucrose has attracted, research has failed to show a consistent effect of dietary sucrose or fructose on insulin sensitivity.”\textsuperscript{21}

Tournian et al. investigated the effects of fructose infusion on hepatic insulin and glucose metabolism, and proposed that stimulation of hepatic glycogen synthesis and inhibition of net glycogenolysis by fructose are involved in maintaining a constant glucose production.\textsuperscript{25} In a follow-up study, these researchers found that acute fructose infusion induced hepatic insulin resistance in humans.\textsuperscript{26} It is important to point out that the infusion was performed in the presence of exogenous insulin to provide a consistent blood glucose level of ~144 mg/dL, and to stimulate an exaggerated metabolic response. As noted by the authors, it remains to be determined whether fructose ingestion as part of the diet will exert the same result.\textsuperscript{25,26}

These observations have left dietitians wondering if fructose should be advised as a sweetener choice for the insulin-resistance/glucose-intolerant patient. In a 2003 review, McGuinness and Cherrington address this issue and note that catalytic doses of fructose (defined as <10\% of total carbohydrate flux) in a glucose-containing meal lead to improved glucose tolerance.\textsuperscript{27} They conclude this review stating: “This improvement [in glucose tolerance] is primarily mediated by the activation of hepatic glucokinase and consequent facilitation of liver glucose uptake. The improvement in glucose tolerance is most evident in insulin resistant settings (e.g., type 2 diabetes and infection). …Thus, activating glucokinase during a carbohydrate meal may serve to minimize the hyperglycemia seen in insulin resistant settings where underlying defects in hepatic glucose disposal are present.”\textsuperscript{27} This mechanism suggests that the effect of fructose on glucose tolerance requires first pass metabolism in the liver, and would not be seen from intravenous delivery of the fructose.

**Effect of Fructose on Triglyceride Synthesis**

The effect of fructose consumption on blood triglyceride levels is probably the most discussed and controversial area of fructose research today. This area has been extensively reviewed, and studies have shown conclusively that diets high in fructose (>25\% energy) increase serum triglycerides.\textsuperscript{4,12,20,21,28,29} The mechanism leading to this
increase is proposed to involve the increase in triglyceride production with excess fructose (Figure 1) and may also be influenced by a decrease in turnover due to a shift in VLDL production and, hence, decreased turnover of triglycerides.

That excess dietary fructose can contribute to triglyceride production is not controversial. The controversial aspects of this discussion relate to whether this is a clinically relevant observation. For example, although some researchers have reported increases in triglycerides in clinical studies using diets with 20% of energy as fructose, others have reported no change in triglycerides with 20% of energy as fructose. Moreover, Reiser reported dose-dependent increases in blood triglyceride levels in male but not female subjects using diets with 5%, 18% and 33% energy as sucrose, and Osei reported no change in triglycerides with a diet of approximately 12% energy as fructose. Several studies have indicated that males and females respond differently as well. For example, after a diet containing 17% fructose (as added free fructose) was provided to healthy men and women, only the men showed an increase of plasma triglyceride levels; no increase was seen in the women. Therefore, the influence of fructose on

| ISOENERGETIC EXCHANGE OF SUCROSE FOR STARCH AT 30% OF ENERGY | CROSSOVER | 6 WK | HEALTHY, WITH HYPERTRIGLYCERIDEMIA SUBGROUP (N = 19) | GREATER IN SUCROSE GROUP | ___ |
| SUCROSE AT 5%, 18%, AND 33% OF ENERGY | CROSSOVER | 6 WK | HYPERINSULINEMIA (N = 24) | INCREASED AS SUCROSE CONTENT ROSE | ___ |
| FRUCTOSE AT 0%, 7% AND 15% OF ENERGY | CROSSOVER | 5 WK | HEALTHY WITH HYPERINSULINEMIA SUBGROUP (N = 23) | NS, BUT INCREASED POSTPRANDIAL INSULIN | ___ |
| ADDITION OF 250 G FRUCTOSE OR GLUCOSE | CASE CONTROL | 1 WK | HEALTHY (N = 7) | NO CHANGE FROM BASAL | 25% FALL IN FRUCTOSE GROUP AS ASSESSED BY IVITT (INTRAVENOUS-INSULIN-TOLERANCE TEST) |
| FRUCTOSE SUBSTITUTED FOR 24% OF TOTAL CARBOHYDRATE (I.E., 13.2%) | SINGLE FACTOR | 2 WK | NIDDM (N = 7) | NS | ___ |
| FRUCTOSE SUBSTITUTED FOR 20% OF CARBOHYDRATE DURING 45% OR 85% STARCH | CROSSOVER | >2 WK | HYPERGLYCEMIA WITH OR WITHOUT NIDDM (N = 6) | NS | ___ |
| FRUCTOSE SUBSTITUTED FOR 20% STARCH | CROSSOVER | 4 WK | NIDDM (N = 10) | INCREASED SENSITIVITY IN FRUCTOSE GROUP AS ASSESSED BY EUGLYCEMIC CLAMP | ___ |
| FRUCTOSE AT 13% | SINGLE FACTOR | 3 MO | NIDDM (N = 6) | NS | NO CHANGE DURING EUGLYCEMIC CLAMP |
| SUCROSE (32%) FOR STARCH AT 70% OF ENERGY | CROSSOVER | 4 WK | HEALTHY (N = 9) | NS | ___ |
| REPLACEMENT OF 45 G STARCH WITH SUCROSE | CROSSOVER | 6 WK | NIDDM OR IDDM (N = 12 OF EACH) | NS | ___ |
| EXCHANGE OF STARCH AND SUCROSE AT 23% OF ENERGY | CROSSOVER | 14 D | HEALTHY (N = 9) | NS | ___ |
| LOW GLYCEMIC COMPARED WITH HIGH GLYCEMIC (25% SUCROSE COMPARED WITH 1% SUCROSE) | CROSSOVER | 28 D | HEALTHY MEN (N = 7) | NS, DECREASED INSULIN SENSITIVITY WITH LOW-GLYCEMIC DIET | ___ |

Table 3. Human studies – effects of sugars compared with starch on insulin sensitivity.


increase is proposed to involve the increase in triglyceride production with excess fructose (Figure 1) and may also be influenced by a decrease in turnover due to a shift in VLDL production and, hence, decreased turnover of triglycerides.
triglycerides at moderate or low intakes is equivocal.

The early human studies suggesting an effect of fructose on hypertriglyceridemia, such as those performed by the group at the USDA Research Center in Beltsville, Maryland, used diets high in fats (e.g., 42% fat, 43% carbohydrate, 15% protein).\textsuperscript{20,21,32} Frayn and his colleagues at the Burnlee General Hospital, UK, have investigated the effect of a bolus fructose dose (0.75 g per kg body weight) with a high-fat meal (1 g fat per kg body weight) and found that the elevation of triglycerides was positively related to fasting insulin concentration, suggesting that insulin-resistant individuals might be more sensitive to this effect.\textsuperscript{33} Daly, who has extensively reviewed this literature, commented that reproducible effects of fructose on postprandial hypertriglyceridemia have only been shown in the context of a significant fat load.\textsuperscript{21} He further added, "We should not ignore the possibility that sucrose may affect insulin sensitivity only with a high fat intake, particularly given the high fat intake in the positive studies by the Beltsville group. Furthermore, these studies should be done in different subject groups [patients with a body mass index \textgreater{} 25 kg/m\textsuperscript{2} but without diabetes, patients with hypertriglyceridemia, and patients with diabetes (type 1 and type 2 separately)]. Results should be interpreted not only as results of the dietary intervention itself but also as changes from the subjects' habitual diet."

The dietary context of fructose is exemplified by a study from Yves Rayssiguier and colleagues at the Centre de Recherche en Nutrition Humaine d'Auvergne in France.\textsuperscript{34} Although the title of this report indicates that the effect of honey was compared to that of a fructose diet on triglyceride levels ["Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidant effect of fructose"], the comparator diets consisted of the "fructose diet" that delivered 34 g fructose and 31 g glucose per 100 g dry matter, and the "honey" diet of 65 g honey (34 g fructose, \textasciitilde{}27 g glucose, and \textasciitilde{}4 g other components) per 100 g dry matter. Both diets provided 68% of energy as simple sugars (as the fructose + glucose, or the honey), with 21% and 12% of energy as protein and fat, respectively. These researchers reported that the hypertriglyceridemic effect of fructose was not observed when the fructose was provided in the honey diet, but was observed with the "fructose" diet, which consisted of the refined carbohydrates.\textsuperscript{34} These observations indicate that the total diet appears to influence this response. Furthermore, the hypertriglyceridemic effect reported for fructose appears to be individualized and may not be seen in all patients.

**Effect of Dietary Fructose on Appetite**

One of the more intriguing differences between fructose and glucose metabolism may be the diverse influences that these monosaccharides have on central nervous system function and, therefore, on appetite. Fructose, unlike glucose, does not stimulate insulin secretion. For example, in rhesus monkeys, an 8-hour intravenous fructose infusion resulted in markedly reduced insulin secretion and no increase in circulating leptin concentrations; however, infusion of the same amount of glucose increased plasma leptin levels by more than 50% above baseline fasting levels.\textsuperscript{35}

Results from the laboratory of Dr. Karen Teff and her colleagues from the Monell Chemical Senses Center and Department of Medicine at the University of Pennsylvania support this finding. Teff et al. studied women who were administered different isocaloric diets containing 30% of energy as either free fructose or glucose and report a significant decrease of \textasciitilde{}20% to 30% in secretion of leptin and the orexigenic gastrointestinal hormone ghrelin.\textsuperscript{36} The investigators concluded that the changes with the fructose-containing diet could promote overeating through the reduction of the satiety signals, thereby contributing to obesity.

In an August 2004 report in *JAMA*, Walter Willett, MD, DrPH, (Department of Nutrition, Harvard School of Public Health) and his colleagues report the findings of an analysis on consumption of sugar-sweetened beverages and type 2 diabetes.\textsuperscript{5} In this study, a higher consumption of sugar-sweetened beverages was associated with higher body mass index (BMI) and an increased risk of type 2 diabetes. Interestingly, fruit juice consumption was not associated with diabetes risk, whereas fruit punch consumption was. These researchers commented that, "...vitamins, mineral, soluble fiber and phytochemicals in fruit juices may have beneficial effects counterbalancing potential adverse effects of sugars." Moreover, they noted that women who increased their sugar-sweetened soft drink consumption also increased energy intake from other foods. They suggest that consumption of these beverages either induces hunger and food intake, or may reflect detrimental accompanying dietary and lifestyle changes.

**The Effects of Fructose as Part of a Low Glycemic Load Diet**

In a year-long study comparing a low-carbohydrate diet with a conventional low-fat diet that had no specific restriction of sugars and refined starch, participants on the low-carbohydrate diet were seen to fare better with respect to changes in atherogenic dyslipidemia values and glycemic control even though caloric intake and weight loss were similar between the two groups.\textsuperscript{37} Willett commented on this finding in an editorial, in which he discussed how nutritionists and dietitians have disparaged the very-low-carbohydrate ("Atkins") diet because it requires a high saturated fat content and the fact that its purported benefits had not been tested.

He pointed out, however, that four randomized trials in adults have compared a very-low-carbohydrate diet with a
low-fat diet and all of these trials have supported the conclusion that the low-carbohydrate diet is associated with improved insulin response and lowered triglycerides. Therefore, Dr. Willett has stated that "we can no longer dismiss very-low-carbohydrate diets." Diets that are called "low-carbohydrate" represent a low glycemic load and, therefore, provide for a low glycemic index.

Studies in humans are difficult to fully control, however, and, as noted by Wood and colleagues in a recent review on the nutrigenomics of insulin resistance and type 2 diabetes, diets are often vaguely described in many of the studies on metabolic syndrome. The diets are often termed "high-fat" or "high-sugar," yet often the specific dietary constituents are not precisely defined. The composition of the total diet includes not only the relative amount of the simple carbohydrate (e.g., sucrose, glucose, and fructose), but also the amount and type of complex carbohydrates like starch (e.g., amylose and amylopectin) and fiber. The type and amount of phytonutrients, vitamins and minerals, as well as the proportion of protein and fat, are also important factors in the effect of sugars on metabolic function.

Rigden and Lerman have recently described a series of case histories of complicated, high-risk, obese patients with insulin resistance/metabolic syndrome who consumed a low glycemic load diet with a fructose-sweetened supplement delivering 30 grams of fructose daily. In this report, the individuals who consumed the fructose-sweetened soy protein supplement had reduced triglycerides and stabilized postprandial blood sugar levels. Additionally, these patients lost weight without feeling overly hungry.

A randomized, controlled prospective trial compared a similar fructose-sweetened soy protein and phytosterol beverage and a low glycemic index diet to the American Heart Association (AHA) Step 1 diet in postmenopausal women with hypercholesterolemia. Individuals who consumed the fructose-containing beverage showed significantly reduced triglycerides, LDL and total cholesterol, and increased HDL cholesterol. Both programs incorporated weight management by setting caloric intake goals to result in weight loss, but a significantly greater improvement was observed in cardiovascular disease risk factors with the low glycemic index diet and fructose-sweetened soy beverage [29% protein, 43% carbohydrates, 29% fat] as compared to the AHA Step 1 diet [20% protein, 54% carbohydrates, 27% fat]. A significantly greater improvement was also seen in the triglyceride-to-HDL cholesterol ratio, a known marker for insulin sensitivity. These data show that clinical programs with modest intakes of fructose in the context of a low glycemic index diet are effective for the management of lipids, insulin sensitivity, and excess body fat in postmenopausal women with hypercholesterolemia and/or obesity, and do not appear to promote increased triglycerides. On the contrary, this approach has been demonstrated in these studies to support improved blood lipids.

Other Benefits of Fructose

Fructose consumption is associated with lowered risk of cardiovascular disease. A commonly accepted hypothesis to explain this observation is that the flavonoids in fruit provide beneficial, protective antioxidant support. Recently, however, Balz Frei and Silvina Lotito from the Linus Pauling Institute at Oregon State University have questioned whether flavonoids are the main substances responsible for the benefit of fruit consumption, especially given that many flavonoids are poorly absorbed and require much higher doses for efficacy than may be predicted from average fruit consumption. These researchers tested the antioxidant capacity in plasma of subjects after consumption of various test foods and concluded that fructose mimicked the effects of fruit (apples) with respect to supporting plasma antioxidant reserve. It was suggested that the effect of fructose on urate promotes this higher antioxidant capacity.

Excessive Consumption of Fructose

Intakes of fructose beyond that of 30% of calories can promote undesirable metabolic consequences. For example, because fructose can bypass the phosphofructokinase regulatory step that is the major controlling step in the intermediary metabolism of glucose in the liver, excessive fructose can continue to be metabolized into substances such as lactate and triglycerides, and can also result in elevations of uric acid. The pathway showing the influence of a high fructose intake on uric acid is provided in Figure 2, and involves the depletion of ATP and the increased catabolism of adenosine to uric acid. Because elevated uric acid and triglyceride levels have been associated with insulin resistance and metabolic syndrome (syndrome X), the suggestion has been made that high fructose intake may precipitate insulin resistance and hyperinsulinemia.

Fructose and Gastrointestinal Symptoms

Consumption of large amounts of fructose in some individuals may be associated with symptoms of Irritable Bowel Syndrome (IBS), such as bloating, flatulence and diarrhea. For example, Johlin et al. have investigated fructose intolerance in 256 patients referred to their clinic because of the severity and idiopathic nature of their abdominal pain and found evidence that 47 of these patients had small bowel overgrowth (SBO), and 75.6% of those without SBO (158 patients of the 256 referred) had an intolerance to fructose as the major contributor to their symptoms. The mechanism of this intolerance relates to the amount of unabsorbed fructose that then traverses through the intestinal tract, which can serve as an osmotic load and draw fluid into the intestinal lumen and, after reaching the lower intestine, be fermented by intestinal bacteria, producing excessive amounts of hydrogen, methane, carbon dioxide and other gases. The resulting symp-
toms may include bloating, pain and discomfort, distention of the small intestine, flatus and diarrhea. Choi et al. report similar findings, in which 73% of 183 patients with unexplained gastrointestinal symptoms had evidence of unabsorbed fructose.49

Upon reviewing the literature on the contribution of dietary fructose to gastrointestinal symptoms, Skoog and Bharucha indicate that incomplete fructose absorption can be seen in many healthy individuals at high doses of fructose. For example, incomplete absorption of fructose after a 50 g load (delivered as a 10% solution) was observed in 37.5% of healthy subjects.50 A higher percent of people show unabsorbed fructose when more concentrated fructose solutions are used, such as 20% solutions, even at the same total load, but a 10% solution closely approximates most carbonated beverages. Fructose malabsorption is considered higher in patients with functional bowel disorders, although the prevalence is not well established.50 Hereditary fructose intolerance has also been recognized as a cause for some cases of fructose intolerance.51,52

In her book, *Breaking the Vicious Cycle, Intestinal Health Through Diet*, Elaine Gottschall counsels that a specific diet restricted in fruits and certain vegetables and increased in animal products is very successful in reducing the problems associated with IBS for many patients.53 Other authors have noted that, because glucose enhances the absorption of fructose, when glucose and fructose are taken at equal amounts, the facilitation of fructose’s absorption by the glucose can prevent the intolerance symptoms. Skoog and Bharucha, in particular, have investigated this facilitation process and advocate that the fructose intolerant patient should consume products that contain glucose at an equal or higher amount to the fructose to avoid symptoms.50 Certain amino acids also appear to facilitate the absorption of fructose. It is important to note that sorbitol, a sugar alcohol found naturally in foods, has the opposite effects and inhibits fructose absorption.50 Sorbitol and fructose occur together in many fruits, including apples, cherries, peaches and pears. Interestingly, bananas and strawberries do not contain sorbitol. Understanding these subtleties of fructose absorption may allow for more choices for patients with functional bowel disorders like IBS.

**SUMMARY**

The USDA has recommended that, depending on calorie intake, people consume no more than 6% to 10% of their calories from added sugars, and the World Health Organization has recommended that people limit their intake of added sugars to 10% or less of total calories. The Institute of Medicine, which provides nutritional advice to the United States Food and Drug Administration, has advised that people get 25% or fewer of their calories from added sugars. For a 2,000 Kcal/day diet, these guidelines constitute anywhere from 30 g to 125 g added sugars. It is interesting to note that one soft drink alone delivers from 40 g to 52 g of added sugar, while sweetened fruit beverages may have even more added sugar. Studies should identify what is actually contributing to the substantial health concerns of today – that is, whether it is the specific sugar, fructose, or whether it is the impact of processed foods with high amounts of added sugars and no nutritional value–, as well as provide clear details on the context within which the fructose is consumed.

Given the evidence presented over the past several years, it is apparent that a principal issue in this debate is the displacement of “quality calories” by empty calories coming from such products as HFCS-sweetened beverages–where added sugars in one beverage constitute the entire amount of added sugar recommended by world health organizations. Modest intake (<20%) of fructose has not been demonstrated to have adverse effects on either serum lipids or insulin sensitivity. Quite the contrary, modest intake of fructose as part of a low glycemic load diet has been associated with improved hepatic glycogen storage, reduced postprandial insulin and glucose, and a lower triglyceride-to-HDL cholesterol ratio, all of which indicate improved insulin sensitivity rather than reduced insulin sensitivity.

Much of the difficulty of making conclusions about the effect of fructose relates to the varying compositions of the diets in different studies, the differing magnitudes of fructose doses and the specific source of fructose. These differences are often extraordinary, leading to difficulty in a direct interpretation of the adverse metabolic effects of fructose. Furthermore, the lack of standardization of a test diet with respect to glycemic load complicates analysis considerably. While it is clear that excessive fructose consumption can have adverse metabolic effects in some individuals, fructose is considered an important part of the overall dietary intake. Fructose is a low-glycemic index alternative sweetener source and may provide benefit as an antioxidant. When consumed at reasonable dietary levels, fructose does not lead to significant adverse events.

As has been pointed out by Fried and Rao from the Department of Nutritional Science at Rutgers University, the magnitude of the effect that sugars have on triglyceride concentrations depends on aspects of the total diet.54 Too often, studies take one principal out of context, study it in an exaggerated intervention trial, and then form general conclusions from it. Such is often the case with regard to studies that have been done with fructose-enriched diets, where the amount of fructose that is added is well beyond that which an individual normally consumes. In order to truly understand how a specific diet containing fructose influences metabolic function, an appropriate intervention trial against a control needs to be accomplished. It is only through this type of clinical evidence that reasonable con-
clusions can be drawn. Until then, clinicians might consider these guidelines with their patients:
1. Minimize the intake of total sugars.
2. Remove sweetened fruit drinks and soft drinks, and do not use table sugar to sweeten food.
3. Promote a diet with a low glycemic load, moderate fat intake, and high in complex carbohydrates.
4. Allow for moderate intake of sugars, including fructose, when included as part of a complex food.

In conclusion, modest consumption of fructose has been demonstrated to have beneficial effects on blood sugar and lipid parameters. High intakes of fructose, however, have resulted from the increased consumption of processed sweeteners like HFCS over the past few decades. This increase in fructose consumption is much greater than what the human body has experienced and managed over the past centuries. It appears that the availability of high processed foods is a primary issue in this supranormal fructose intake. Therefore, focusing on the quality of the diet, not just the removal of a specific sugar that has value in moderate amounts, should be considered during nutritional counseling.

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REFERENCES


ABSTRACT

Background

The use of non-pharmacologic dietary supplements is of potential benefit in addressing hypercholesterolemia, a well-established risk factor for ischemic heart disease.

Objective

Two herbal products were investigated for their cholesterol-lowering effects.

Design

This investigation was a 24-week, double-blind study with 20 participants. U85-2 contained tannin, cayenne pepper and vanillin; U100-3 contained turmeric, ensian root, hot paprika and vanillin. At inclusion, plasma cholesterol was > 6 mmol/l, or with one or more risk factors: > 5 or 4.5 mmol/l respectively. Previous self-reported dietary patterns (mean fat/energy %: 34) and physical activity levels were maintained as estimated by indirect methods: no changes were seen in weight or hip/waist measure.

Results

U100-3 had a significant effect with a decrease of 16% on non-HDL cholesterol, a close to significant effect with a decrease of 13% on total cholesterol and a borderline significant effect with a decrease of 16% on LDL cholesterol. Ratios between the atherosclerotic (total and LDL) cholesterol and the protective HDL-cholesterol improved by 11% (TC:HDL ratio) and 14% (LDL:HDL ratio). The U85-2 showed no effects.

CONCLUSION

The U100-3 preparation has a beneficial and clinically relevant long-term effect and should be investigated further for treatment of mild to moderate hypercholesterolemic individuals.

Key Words: hypercholesterolemia, spice preparation, paprika, turmeric, vanilla, gentian.

BACKGROUND

Cardiovascular disease is one of the most significant health problems in the western world, causing loss of both quality of life and life years as well as being a socio-economic burden. Cardiovascular disease develops as an interaction between a number of factors – including heredity, lifestyle, diet and physical activity.

Hypercholesterolemia is a known risk factor for ischemic heart disease. Approximately half the Danish population has plasma cholesterol levels over 5 to 6 mmol/l. Mild to moderate hypercholesterolemia is presumably due, for the most part, to dietary and lifestyle factors.

The therapeutic targets most often suggested are a total plasma cholesterol level of ≤ 5.0 or 5.2 mmol/l, LDL cholesterol (low-density lipoprotein cholesterol) ≤ 3.5 mmol/l,
HDL cholesterol (high-density lipoprotein cholesterol) ≥ 1.0 mmol/l, and plasma triglyceride ≤ 2.0 mmol/l ([www.americanheart.org](http://www.americanheart.org)). For each 1% reduction in LDL cholesterol, the risk of a blood clot in the heart is reduced by at least 2%, and for each 1% increase in HDL cholesterol, the risk is reduced by 3% to 4%.1,2,3

An important risk factor of thromboembolism related to cholesterol levels is the ratio between total or LDL cholesterol and HDL cholesterol – a ratio reflecting whether cholesterol is deposited (LDL) or transported for decomposition and elimination (HDL).4,5 It is desirable to achieve a ratio between total cholesterol and HDL ≤ 4.2 and a corresponding ratio between LDL and HDL cholesterol of < 2.5.6 The reduction in cholesterol levels and an enhanced ratio between the different types are best achieved by adjustments in diet and lifestyle combined with dietary supplements, and in severe cases where these measures prove inadequate, with medication. In addition, prevention of ischemic heart disease requires modification of other risk factors such as smoking, diabetes, overweight, inactivity, hyperhomocysteinemia, hypertension, and the elevation of plasma lipoprotein (a), depending on the individual risk profile. Various dietary supplements have exhibited a cholesterol-lowering effect by different mechanisms of action. These supplements include guar gum, psyllium, red rice and garlic. Diet alone has been found to reduce blood cholesterol, and from a health-economic point of view, it compares well with medication.3,7,8 However, a strict diet can be difficult to maintain, and in those individuals who are unable or unwilling to fully comply, the effect on cholesterol levels is inadequate.

Guar gum and psyllium are rich in fibers and bind to water in the intestinal contents, thereby inhibiting intestinal cholesterol uptake and enhancing fecal excretion of dietary cholesterol.9 Psyllium also upregulates cholesterol turnover by increasing enzyme activities related to both cholesterol synthesis and catabolism.10 In clinical trials with humans, guar gum has been shown to reduce total cholesterol by 10% to 15% and LDL cholesterol by 10% to 20%,11 while psyllium may lower total cholesterol by about 4% and LDL cholesterol by about 7%.12

Red rice (rice fermented with a special yeast, *Monascus purpureus*) acts at an earlier stage of cholesterol metabolism by inhibiting cholesterol synthesis. This cholesterol synthesis inhibition has produced reductions in total cholesterol of 11% to 32%, and in LDL cholesterol of about 22%.13 Red rice has a mechanism of action identical with that of statins, a group of pharmaceutical products approved to treat hypercholesterolemia. In fact, it has been shown that one of its active ingredients is lovastatin, a selective inhibitor of HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis. This being the case, there have been disputes about whether red rice should be approved as a dietary supplement or a pharmaceutical product.

The mechanism of action in garlic preparations is unknown, but presumed to be due to allicin, which is formed by conversion of the compound allin.14 Results from human clinical trials show considerable inconsistency regarding garlic and its cholesterol-lowering effect. Some short term clinical studies of up to 3 months duration15,16 show a reduction in total cholesterol of about 10%, and in LDL cholesterol of 10% to 16%. Contrary to these results, the last three years have produced six well-designed studies that failed to prove a beneficiary effect of garlic after three to six months of treatment.17,18

U85-2 and U100-3 are two different spice preparations with potential cholesterol-lowering effects. U85-2 contains tannin, vanillin and cayenne pepper, while U100-3 contains turmeric, gentian root, hot paprika, and vanillin. Table 1 specifies the composition of the two preparations. Tannin is available from grapefruit seeds, tea and wine, while turmeric, cayenne, vanilla and paprika are well-known spices and gentian is an ancient medicinal plant.

Animal trials have shown that both cayenne and tannin significantly lower blood levels of cholesterol and triglycerides and improve the HDL:LDL ratio.19,20,21 Conversion into bile salts is the body’s primary route of eliminating cholesterol,22,23 and the mechanisms of action of cayenne, vanilla and turmeric work by stimulating the formation and secretion of bile salts from the liver. Furthermore, cayenne enhances hepatic metabolism of lipids by increasing the enzyme levels, thereby reducing fatty deposits in the liver.19

| Table 1. Composition of the two tested herbal/spice mixtures (mg per daily dosage, 4 tablets) |
|---------------------------------|-------|-------|
| **U85-2**                       | **U100-3** |
| Cayenne (*Capsicum spp)*        | 80    |       |
| Tannin                          | 120   | 100   |
| Hot paprika (*Fructus Capsici aedelsuss sharp*) | 300  |         |
| Gentian root (*Radix Gentiana*) | 500   |       |
| Turmeric** (*Rhizoma Curcumae javan.*) | 20   |       |
| Vanillin                        |       | 20    |

* Capsaicin content 0.4%
** Cucurmin 1.0% to 1.3% (double determinations), essential oils 4% to 5% (double determination)
Tannin has a different mechanism of action and acts by binding to cholesterol in the intestine. In this way, it reduces cholesterol absorption significantly and increases the amount of cholesterol excreted with the feces.24

MATERIALS AND METHODS

Objectives and Design

The objective of this study was to investigate U85-2 and U100-3 for their potential cholesterol-and triglyceride-lowering effects in a 24-week double-blind, prospective study. The project was approved by the Danish Data Protection Agency (File no. 1999-1200-343) and the regional Ethics Committee (File no. (KF) 07-00-043/02). Participants were informed, verbally and in writing, and signed informed consent documents.

Inclusion Criteria

Included in the study were adult individuals, at least 18 years of age and with no upper age limit, of both sexes with total cholesterol values:

- > 6 mmol/l without any other risk factors
- > 5 mmol/l with one other risk factor
- > 4.5 mmol/l with at least two risk factors

Risk factors were smoking, overweight/obesity, hypertension, diabetes, hereditary disposition and vascular disorders/atherosclerosis.

Exclusion Criteria

- known, familial, severe hypercholesterolemia
- treatment with cholesterol-lowering medication or cholesterol-lowering food supplements
- children, pregnant women and alcohol and drug abusers

Recruitment took place in the clinic and in general practices in the local area of Copenhagen.

A total of 21 individuals were randomised, double-blind, to one of two treatments. Randomisation lists were generated by the use of a table with random numbers, by a pharmacist trained in these procedures who was not dealing with the data acquisition, thus neither the subjects nor the staff handling data acquisition knew which group subjects were included. The food supplements to be tested were of similar shape, color and smell (vanilla), rendering the contents indistinguishable. The dosage of the dietary supplements was 2 tablets bid (morning and evening), with the possibility of reduction in case of adverse reactions. Dispensing more tablets than needed for each period and counting the number of tablets left over assessed compliance.

Subjects were asked not to change their diets and physical activities, or their administration of dietary supplements and existing medication throughout the study.

Dietary intake (food) was recorded/weighed daily at the start of the study for seven days on the electronic scales supplied (Soehnle Domino) and subsequently analysed electronically in Dankost 3000 on the basis of the national food-composition tables.

ANALYSIS

Analysis of blood lipids was performed on freshly drawn blood at weeks 0 and 24 following overnight fasting. Total cholesterol, HDL cholesterol and triglycerides were measured by a standard enzymatic technique at a precision of 2%, 6% and 3%, respectively, at Nova Medical Medilab (Copenhagen, Denmark), which has GLP status. LDL cholesterol values were calculated by the Friedewald equation

$\text{LDL} = \text{TC} - \text{HDL} - (\text{TG}/5)$.25 Data were entered into Excel® and analysed by the SAS® 8.2 statistics package (SAS INC, Cary, NC) and StatXact 5. The effect of dietary supplements on cholesterol values was evaluated by a non-parametric test, the Wilcoxon test for paired differences, as data were not normally distributed. A two-sided p value < 0.05 was considered significant. The randomisation code was not broken until after the final analysis.

RESULTS

Twenty subjects completed the study, and one of these subjects was excluded from the analysis because of a change in existing medication (hormone replacement therapy) during the study. Two subjects received antihypertensives and continued with their regimens. One individual with known multiple allergies was an early drop-out because of a potential hypersensitivity reaction.

Table 2 shows baseline characteristics for the two analysis groups. As seen from table 2, the two analysis groups were comparable with regards to baseline characteristics (age, height, weight, BMI, number of current or previous smokers, use of antihypertensive medication and dietary energy intake). The fat intake was quite low because the subjects in both groups had been previously diagnosed with hypercholesterolemia years prior to the study. In response to this diagnosis, most of them had already changed to a more low-fat diet, but without adequate effect.

Adverse reactions were few and mild. In the U85-2 group, one subject became nauseous from the tablets and one suffered mild colic for a few weeks at the start, which abated without dose reduction or interruption. In the U100-3 group, one subject suffered colicky pain, which ceased after dose reduction to 2-3 tablets a day. One subject reported dizziness, headache, flushing of the face, impaired concentration, fatigue and joint stiffness. These reactions appeared within two weeks from the start in an individual with known multiple allergies, were dose-dependent and tapered off a few days after the dietary supplement was discontinued without any sequelae. The capsaicin content of hot paprika has a vasodilatatory effect, which may lead to the reactions described in sensitive individuals. The subject...
One subject in each group took more tablets than scheduled (137% and 129%, respectively), with no adverse reactions. The absence of any major discomfort or adverse reactions, apart from those mentioned during the six months of the study, indicates that both preparations are non-toxic and well tolerated.

Table 3 shows the results of the lipid analyses at the start of the study (week 0) and after 24 weeks.

U85-2: A non-significant decrease was seen in all parameters except for HDL, where a non-significant increase was observed.

U100-3: A statistically significant decrease was seen for the non-HDL cholesterol of 1.64 mmol/L (p=0.035). The decrease in non-HDL cholesterol corresponds to 16%.

For total cholesterol, a non-significant decrease of 1.69 mmol/L (p=0.055) was observed. The decrease in total cholesterol corresponds to 13%. For LDL cholesterol, a borderline significant decrease of 0.95 mmol/L (p=0.051) was observed. The decrease in LDL cholesterol corresponds to 16%. For HDL cholesterol, no change was observed during the study period. For Triglyceride, a non-significant decrease of 0.27 mmol/L (p=0.24) was observed. The decrease in Triglyceride corresponds to 16%. No study subjects had excessively elevated triglyceride values, but the highest values tended to decrease the most (by 40% to 60%). For the ratio between total cholesterol and HDL cholesterol, a non-significant decrease of 0.52 (p=0.098) was observed. The decrease in ratio corresponds to 11%.

For the ratio between LDL and HDL cholesterol, a non-significant decrease of 0.52 (p=0.074) was observed. The decrease in ratio corresponds to 14%.

The effects of the preparations bore no relation to body weight, age or compliance, which supports the choice of dosage. There were no clinical or statistically significant changes in blood pressure, body weight, BMI, waist or hip circumference and waist:hip ratio during the study (data not shown), indicating that the subjects maintained their dietary patterns and physical activity levels. Consequently, it is less likely that the differences observed in lipid values are due to changes in diet or physical activity levels during the study.

DISCUSSION

The ingredients in U100-3 inhibit intestinal absorption of cholesterol. In addition, they act by direct stimulation of hepatic formation and secretion of bile salts from cholesterol, which means that they inhibit cholesterol absorption while increasing elimination (McCaleb 1993, Sambaiah 1980, Srinivasan 1992 and 1993).

A review of literature shows that a change in diet alone can reduce serum total cholesterol by 10–15% and LDL

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**Table 2. Baseline characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>U85-2 (n = 10)</th>
<th>U100-3 (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.5 ± 14.5 (33 - 74)</td>
<td>64.0 ± 8.8 (47 - 77)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.1</td>
<td>1.74 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.2 ± 11.2</td>
<td>77.7 ± 14.6</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.9 ± 3.4 (22.4 - 32.8)</td>
<td>25.6 ± 3.4 (21.1 - 30.5)</td>
</tr>
<tr>
<td>Current smokers (number)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Previous smokers (number)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Antihypertensive therapy (number)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Energy consumption (KJ/day)</td>
<td>8946 ± 3077 (5,340 – 14,562)</td>
<td>9902 ± 2403 (7,470 – 13,814)</td>
</tr>
<tr>
<td>Energy %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>33.9</td>
<td>33.8</td>
</tr>
<tr>
<td>Protein</td>
<td>15.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>46.2</td>
<td>41.6</td>
</tr>
<tr>
<td>Alcohol</td>
<td>4.1</td>
<td>9.0</td>
</tr>
</tbody>
</table>

1. mean + SD, range in parenthesis. The two groups showed no statistically significant difference between the variables.
In studies of dietary supplements, it is important that the subjects do not change their dietary patterns during the study period; therefore, we instructed the subjects in the present study to strictly maintain their dietary patterns and physical activity level. U100-3 therapy led to a reduction in total cholesterol of 13% and in LDL cholesterol of 16%. This reduction corresponds to the levels obtained by guar gum\textsuperscript{11} and in the most optimistic studies, by garlic.\textsuperscript{15,16} However, guar gum has rarely been investigated in studies of similar long duration, which is relevant in evaluation of cholesterol-lowering food supplements and medications where a sustained effect is highly important. A comprehensive meta-analysis of studies investigating the cholesterol-lowering effect of garlic has shown that the studies found a reduction in total cholesterol at one and three months, but not at six months.\textsuperscript{17,18} It is possible that the body adjusts to a cholesterol-lowering therapy so that the effect may be transient.

This study is somewhat limited due to the small number of subjects; however, the results are statistically significant and of a clinically interesting magnitude.

It can be argued that the initial group differences in the total and LDL cholesterol values is of clinical importance, but these differences arise from one subject with high initial values in the U100-3 group and one subject with rather low initial values in the U85-2 group. As the distribution of subjects was due to chance because of the randomisation, it is not appropriate to exclude any of these subjects from the analysis and the use of a non-parametric test minimizes the impact of outliers.

It could also be stated that other factors were in play, such as dietary changes. As we did not undertake a second dietary registration at the end of study, this factor cannot entirely be ruled out. If the diet had changed, some of the indirect indicators of dietary changes, such as weight, BMI or anthropometric measures of waist and waist:hip ratio, would most likely have changed. However, these statistics remained unchanged. Additionally, the subjects had dietary counselling and had changed their diets long before the onset of this study, and it is unlikely that dietary changes would have occurred in the U100-3 group only.

It was not possible to examine other potential physiological effects of the products such as the effect on the cell membranes and platelet aggregation, or the anti-oxidative effect. Animal trials have demonstrated that both turmeric and capsaicin (present in cayenne and paprika) stabilize cell membranes and reduce their cholesterol contents,\textsuperscript{27} and capsaicin also inhibits platelet aggregation and displays anti-oxidative properties.\textsuperscript{28} Turmeric has been fairly thoroughly investigated for cancer prophylaxis, although not yet in humans,\textsuperscript{29} and both turmeric and capsaicin inhibit cyclooxygenase-2, an enzyme involved in various inflammatory disorders such as arthritis, migraine and pain disorders.\textsuperscript{30} These findings from in-vitro and animal research suggest that spice preparations may have other favourable long-term effects apart from those investigated here.

### Table 3. Lipid concentrations in subjects treated with the spice preparations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Start Mean</th>
<th>Start SEM</th>
<th>Week 24 Mean</th>
<th>Week 24 SEM</th>
<th>Difference from start to week 24 Mean</th>
<th>Difference from start to week 24 SEM</th>
<th>p value two-sided exact</th>
<th>Test statistic\textsuperscript{2}</th>
<th>p value\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U-100-3, n= 9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>7.77</td>
<td>0.54</td>
<td>6.77</td>
<td>0.43</td>
<td>-1.00</td>
<td>0.56</td>
<td>-13</td>
<td>-16.5</td>
<td>0.055</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>1.66</td>
<td>0.13</td>
<td>1.66</td>
<td>0.13</td>
<td>0.00</td>
<td>0.04</td>
<td>-0.0</td>
<td>-1.00</td>
<td>1.000</td>
</tr>
<tr>
<td>LDL Cholesterol\textsuperscript{1}</td>
<td>5.77</td>
<td>0.51</td>
<td>4.82</td>
<td>0.45</td>
<td>-0.95</td>
<td>0.54</td>
<td>-16</td>
<td>-16.5</td>
<td>0.051</td>
</tr>
<tr>
<td>Non-HDL Cholesterol</td>
<td>6.11</td>
<td>0.51</td>
<td>5.11</td>
<td>0.44</td>
<td>-1.00</td>
<td>0.55</td>
<td>-16</td>
<td>-17.5</td>
<td>0.035</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.70</td>
<td>0.17</td>
<td>1.43</td>
<td>0.21</td>
<td>-0.27</td>
<td>0.19</td>
<td>-16</td>
<td>-10.5</td>
<td>0.238</td>
</tr>
<tr>
<td>Total Cholesterol : HDL ratio</td>
<td>4.86</td>
<td>0.40</td>
<td>4.34</td>
<td>0.50</td>
<td>-0.52</td>
<td>0.30</td>
<td>-11</td>
<td>-14.5</td>
<td>0.098</td>
</tr>
<tr>
<td>LDL\textsuperscript{1} - HDL ratio</td>
<td>3.64</td>
<td>0.39</td>
<td>3.12</td>
<td>0.45</td>
<td>-0.52</td>
<td>0.28</td>
<td>-14</td>
<td>-15.5</td>
<td>0.074</td>
</tr>
<tr>
<td><strong>U-85-2, n=10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>6.83</td>
<td>0.23</td>
<td>6.64</td>
<td>0.26</td>
<td>-0.19</td>
<td>0.30</td>
<td>-2.8</td>
<td>-5.50</td>
<td>0.541</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>1.60</td>
<td>0.14</td>
<td>1.66</td>
<td>0.15</td>
<td>0.06</td>
<td>0.07</td>
<td>3.8</td>
<td>7.50</td>
<td>0.480</td>
</tr>
<tr>
<td>LDL Cholesterol\textsuperscript{1}</td>
<td>4.89</td>
<td>0.26</td>
<td>4.69</td>
<td>0.30</td>
<td>-0.20</td>
<td>0.29</td>
<td>-4.1</td>
<td>-6.50</td>
<td>0.539</td>
</tr>
<tr>
<td>Non-HDL Cholesterol</td>
<td>5.23</td>
<td>0.27</td>
<td>4.98</td>
<td>0.31</td>
<td>-0.25</td>
<td>0.29</td>
<td>-4.8</td>
<td>-8.00</td>
<td>0.443</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.70</td>
<td>0.28</td>
<td>1.45</td>
<td>0.24</td>
<td>-0.25</td>
<td>0.23</td>
<td>-15</td>
<td>-6.50</td>
<td>0.477</td>
</tr>
<tr>
<td>Total Cholesterol : HDL ratio</td>
<td>4.56</td>
<td>0.43</td>
<td>4.44</td>
<td>0.41</td>
<td>-0.12</td>
<td>0.20</td>
<td>-2.7</td>
<td>-6.00</td>
<td>0.574</td>
</tr>
<tr>
<td>LDL\textsuperscript{1} - HDL ratio</td>
<td>3.31</td>
<td>0.38</td>
<td>3.07</td>
<td>0.33</td>
<td>-0.24</td>
<td>0.18</td>
<td>-7</td>
<td>-6.50</td>
<td>0.557</td>
</tr>
</tbody>
</table>

Units are mmol/L.
1. LDL cholesterol calculated using the Friedwald equation: LDL = TC - HDL - TG/5.
2. Test used: Wilcoxon Signed Rank Test for paired differences on the difference from start to week 24.

cholersterol 10–16%\textsuperscript{7,26} In studies of dietary supplements, it is important that the subjects do not change their dietary patterns during the study period; therefore, we instructed the subjects in the present study to strictly maintain their dietary patterns and physical activity level.

U100-3 therapy led to a reduction in total cholesterol of 13% and in LDL cholesterol of 16%. This reduction corresponds to the levels obtained by guar gum\textsuperscript{11} and in the most optimistic studies, by garlic.\textsuperscript{15,16} However, guar gum has rarely been investigated in studies of similar long duration, which is relevant in evaluation of cholesterol-lowering food supplements and medications where a sustained effect is highly important. A comprehensive meta-analysis of studies investigating the cholesterol-lowering effect of garlic has shown that the studies found a reduction in total cholesterol at one and three months, but not at six months.\textsuperscript{17,18} It is possible that the body adjusts to a cholesterol-lowering therapy so that the effect may be transient.

This study is somewhat limited due to the small number of subjects; however, the results are statistically significant and of a clinically interesting magnitude.

It can be argued that the initial group differences in the total and LDL cholesterol values is of clinical importance, but these differences arise from one subject with high initial values in the U100-3 group and one subject with rather low initial values in the U85-2 group. As the distribution of subjects was due to chance because of the randomisation, it is not appropriate to exclude any of these subjects from the analysis and the use of a non-parametric test minimizes the impact of outliers.

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The adverse reaction profile of U100-3 was also positive, with only two subjects experiencing symptoms. One of them had known multiple allergies with general hyperreactivity, while the other developed abdominal pain that ceased at dose reduction. Side effects for other preparations used for the same purpose such as garlic preparations, would in all cases give a strong and characteristic odor, while colicky pain and flatulence could accompany guar gum and psyllium intake.

CONCLUSION

The study has shown that U100-3, a dietary supplement containing the spices hot paprika, vanillin, turmeric and gentian, has a beneficial and clinically relevant long-term effect in humans with hypercholesterolemia. U100-3 lowers cholesterol levels by affecting both total and LDL cholesterol, but not HDL cholesterol. It is a favorable aspect that the HDL cholesterol level is maintained, thereby improving the ratios between the atherosclerotic cholesterol forms (TC or LDL) and the protective HDL cholesterol. A larger controlled clinical trial is warranted.

ACKNOWLEDGEMENTS

The authors would like to thank the subjects enrolled for having invested their time and energy in this study, nutritionist Caritas Locher for having performed the dietary analysis, and statistician Martin Eeg for statistical supervision.

This study was supported by a grant from MB Pharmos A/S, Copenhagen, Denmark, which has the patent rights in U100-3. U100-3 is marketed under the brand name of Lippital.

CONTRIBUTIONS AND INTERESTS:

Eva Lydeking-Olsen designed the study, analysed the data and wrote the paper together with Janne Springborg Clewlow. Vita Damsoe and An-Mari Mey Hansen took care of data accrual and contributed to the writing of the paper. Institute for Optimum Nutrition, Holistic Therapy and Research is a private non-profit institute, independent of MB Pharmos, and none of the authors are affiliated with MB Pharmos. The sponsor and supplier of the study approved the study protocol, but had no role in data analysis, data interpretation, or writing of the report.

REFERENCES


ABSTRACT

Chronic deficiency of vitamins and other essential nutrients impairs cellular bio-energy production and can lead to disturbances in the generation and conduction of electrical impulses in the myocardium. We investigated the effect of long-term supplementation with a combination of vitamins and other essential nutrients on the number of clinically apparent episodes in patients with paroxysmal atrial arrhythmia. A randomized, double-blind, placebo-controlled, multi-center study in Germany was undertaken on 131 patients (ITT), aged 18 to 70 years, diagnosed with paroxysmal atrial arrhythmia, who were receiving anti-arrhythmic medication for at least three months, and who reported at least one paroxysmal cardiac episode per month. Study participants were advised to continue their prescribed medication and were treated with either an essential nutrient formulation or placebo during the 24-week study. Analysis of data demonstrated a significant decrease in the frequency of clinically apparent arrhythmic episodes with vitamin/essential nutrient supplementation (ITT analysis: \( p=0.0221 \); PP analysis: \( p=0.0160 \)) that improved with time (45.5% of the supplemented group experienced frequent arrhythmic episodes at three months, in contrast to only 27.3% at six months). By addressing the underlying cause of arrhythmia, a deficiency in nutrients that generate bio-energy in the heart muscle cells, a vitamin/essential nutrient supplementation program provides a safe and effective reduction of arrhythmic episodes.

Key Words: atrial paroxysmal arrhythmia, nutrient synergy, carnitine, coenzyme Q10, bioenergy, lysine, vitamin C, B vitamins

INTRODUCTION

Chronic deficiency of vitamins and other essential nutrients impairs cellular bio-energy production, and can lead to disturbances in the generation and conduction of electrical impulses in the myocardium. This disturbance of electrical impulses due to chronic vitamin and nutrient deficiency can be an underlying cause of the majority of arrhythmic episodes of unknown origins, according to the Rath concept.\(^1\)\(^2\) There is an accumulating body of evidence supporting the beneficial effects of optimal levels of vitamins and other essential nutrients on the metabolic, nutritional and functional status of the myocardium.\(^3\) Our previous study on the synergistic effect of a combined vita-
min/essential nutrient formula on progression of coronary artery calcification in patients with documented coronary artery disease, using Ultrafast CT, observed a decrease from a 44% calcification rate prior to intervention to 15% after the course of one year of nutritional supplementation.4

To generate electricity, the “electrical cells” of the heart need large amounts of bio-energy. Therefore, they need a constant supply of nutrients that facilitate the conversion of food into cellular energy. The most critical among them are: coenzyme Q10, carnitine, the B vitamins, lysine, and vitamin C, together with magnesium, calcium and potassium.2,4 McCarty suggests the use of meaningful doses of the mitochondrial megavitamins as protection from cardiovascular disease.5

Carnitine, an often deficient non-essential amino acid, is essential for cardiac energy production. It is produced from lysine, an essential amino acid, with the participation of vitamin C. Since both lysine and vitamin C are not produced in the human body, their deficiency is likely to impair endogenous carnitine levels. The carnitine molecule is necessary to translocate fatty acids through the outer mitochondrial membrane for conversion to ATP in order to sustain heart function. A hospital-based, double-blind clinical study of patients admitted after myocardial infarction demonstrated that intake of 2 grams of carnitine per day for four weeks cut the number of complications from arrhythmia, angina and heart failure in half.6

Like carnitine, coenzyme Q10 is essential for ATP production by the mitochondria. Low levels of coenzyme Q10 have been reported to be associated with increased severity of heart failure.7 Three months of adjunctive treatment of congestive heart patients with coenzyme Q10 resulted in reduction of arrhythmias in 62% of the treatment group in contrast to the placebo group.8 Furthermore, a one-month coenzyme Q10 treatment of patients with acute myocardial infarction, reduced angina pectoris and total arrhythmias, and improved ventricular function.9

In addition to carnitine and coenzyme Q10, other nutrients such as magnesium, the B vitamins, vitamin C and vitamin E help optimize the pumping performance of the heart.10,11 These nutrients optimize the function of the heart’s electrical cells, as well as the myocardial smooth muscle cells of the blood vessel walls, supporting regular heart contractions. A deficiency of these nutrients leads to an imbalance in cellular energy that can cause irregular heartbeat. Many studies have been done on the therapeutic effect of individual nutrients on cardiovascular health. However, for optimal biological effect, these nutrients must complement and support each other in synergy.

The objective of this Phase II clinical study was to investigate whether long-term administration of a combination of vitamins, amino acids and other essential nutrients, individually shown to be effective in improving cardiac health, in addition to conventional basic therapy, could lead to a reduction in the number of clinically apparent episodes in patients with paroxysmal atrial arrhythmia.

**MATERIALS AND METHODS**

A randomized, double-blind, placebo-controlled multicenter study was undertaken to evaluate the effect of vitamin/essential nutrient supplementation on arrhythmia. Internists and general practitioners at 35 clinics in Germany conducted this multi-center study on 131 patients diagnosed with paroxysmal atrial arrhythmia. Of these, 90 patients (44 in the supplemented group and 46 in the placebo group) strictly adhered to the study protocol and completed the six-month study. (See Figure 1–STARD diagram.) Although the number of patients in the ITT (n=131) and the PP (n=90) populations differed, the results from both groups shared the same trend.

Inclusion criteria for selection were: males/females from 18 to 70 years of age, and patients with paroxysmal atrial arrhythmia receiving anti-arrhythmic treatment for at least three months and reporting at least one paroxysmal cardiac episode per month. All participants met the entrance criteria and were diagnosed with long-lasting or chronic arrhythmia. There was no statistical difference in the distribution of males and females in each group, and the average age in the supplemented group was 58 years and in the placebo group, 56 years. (See Table 1 for baseline demographics and characteristics.)

The study was conducted according to the recommendations of the Declaration of Helsinki as amended in South Africa and Edinburgh, Scotland (1996 and 2000), and AMG, particularly sections 40 and 41 of the Tenth Amendment to the Drugs Act, the Principles of Proper Implementation of Clinical Studies and the ICH-GCP Note for Guidance.

**Table 1. Baseline Patient Demographics and Treatment Characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>Supplemented Group</th>
<th>Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>63 patients</td>
<td>68 patients</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>57.7 years</td>
<td>55.9 years</td>
</tr>
<tr>
<td>Females</td>
<td>57.7%</td>
<td>42.5%</td>
</tr>
<tr>
<td>Males</td>
<td>62.3%</td>
<td>57.7%</td>
</tr>
<tr>
<td>Race—Caucasian</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Body Weight</td>
<td>78 kg/171.83 lbs</td>
<td>76 kg/167.43 lbs</td>
</tr>
<tr>
<td>Body Height (cm)</td>
<td>169.4</td>
<td>168.8</td>
</tr>
<tr>
<td>Treated with Beta-blockers</td>
<td>64%</td>
<td>66%</td>
</tr>
<tr>
<td>Treated with Calcium channel blockers</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>Cardiac Therapy</td>
<td>20%</td>
<td>21%</td>
</tr>
</tbody>
</table>
Methods of determining efficacy

The primary target parameter was defined as the number of clinically symptomatic episodes in six months compared to the placebo group. Since the patient’s clinical benefit was the most important therapeutic aspect, participants were instructed to document, in a diary, all clinical arrhythmic symptoms (rapid heart rate, palpitations, chest pain, dyspnea, dizziness, weakness, tiredness, and perspiration outbreaks), time of occurrence, episode duration, and episode severity, and return for monthly follow-up. Since this study was designed as a first “proof of concept,” sophisticated technologies such as remote EKG telephonic transmission were not used.

Statistical Methods/Issues

For confirmatory analysis of the main target parameter, with $\alpha = 0.05$, the Mantel-Haenszel test was used. Patient classification for this purpose was: no episodes, 1-3, 4-6, 7-10, and over 10 episodes during the period. Analysis of the secondary parameters was done in an exploratory sense with $\alpha = 0.05$ without $\alpha$-adjustment. The secondary parameters, related to episode frequency, were analyzed analogously to the main target parameter, with class boundary adjustment. The time to first episode after the fifth week was statistically compared for the groups using Kaplan-Meier analysis and a log-rank test. No interim analysis was performed.

Intervention

All patients were advised to continue taking their prescribed conventional medications during the study period, and were provided conventional standard treatment for paroxysmal atrial arrhythmia. (Approximately 60% of the patients in both treatment groups were being treated with beta-blockers and 20% by calcium blockers.) In addition, patients allocated to the supplement group were each provided with blister packages containing vitamin/essential nutrients labeled with a lot # (to ensure the double-blind
character of the study) and the appropriate week. (See Table 3 for the nutrient composition.) The placebo group patients received identical blister packs of placebo tablets containing material of no medical significance such as cellulose, fructose, etc., but physically indistinguishable from the two types of nutrient tablets. Patients were instructed to take the prescribed nutrient/placebo tablets provided for 24 weeks.

**Efficacy Parameters**

Frequency of symptomatic episodes of arrhythmia was the primary target parameter for determining efficacy of treatment. Secondary parameters included: the number of clinically apparent episodes in each group during study months 1-3 and during months 4-6, time elapse before first occurrence of clinically apparent arrhythmic symptoms, pre-and post-study 24-hour Holter monitoring for assessment of arrhythmia-specific changes, and pre-and post-treatment scores on the SF–36 (Short Form 36 Healthy Survey,12 a standard Quality of Life Questionnaire) to evaluate how vitamin intake affected the patients’ perceived general well-being and quality of life. The questionnaire evaluated 36 parameters describing physical functions, role functions from the emotional perspective, social functionality, level of pain, psychological status, vitality, and perception of general health and other aspects.

**RESULTS**

For the primary efficacy parameter (clinically apparent arrhythmic episodes during months 1-6), a statistically significant effect of vitamin supplementation on the reduction of clinically apparent arrhythmic episodes was observed in both analysis sets (p=0.0221 for ITT analysis set, p=0.0160 for PP analysis set) (Figure 2). Only 47.8% of the supplemented patients reported seven or more arrhythmic episodes during the treatment study, in contrast to 73.9% reported in the placebo group (PP analysis). The number of patients with less than seven episodes in the supplemented group (52.7%) was almost twice that in the placebo group (26.1%). Furthermore, the number of patients with more than ten episodes was significantly less in the supplemented group (45.5%) than in the placebo group (69.6%). In addition, the elapse of time prior to the first arrhythmic episode was shorter in the placebo group than in the supplemented group (Log Rank Test: p=0.0332 for PP analysis set).

The data was also analysed to determine the effect of supplementation on arrhythmic episodes with time at three months vs. six months. At three months, 45% of the supplemented patients experienced seven or more arrhythmia attacks in contrast to 27.3% at six months (Figure 3). Approximately 22.7% of the supplemented patients reported no arrhythmic episodes at three months in contrast to 43.2% at six months (Figure 4).

For all dimensions of the SF-36, the differences between the post-and pre-study values of the supplemented group demonstrated a stronger perceived quality of life than those of the placebo group (Table 2). Baseline values were comparable between treatment groups. This questionnaire evaluates 36 different parameters that relate to the physical functioning of patients, including pain, emotional

<table>
<thead>
<tr>
<th>SF-36 Dimension</th>
<th>Mean - Supplemented Group</th>
<th>Mean - Placebo Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Health</td>
<td>+ 4.0</td>
<td>- 0.3</td>
</tr>
<tr>
<td>Vitality</td>
<td>+ 9.5</td>
<td>+ 2.8</td>
</tr>
<tr>
<td>Mental Health</td>
<td>+ 7.4</td>
<td>- 1.6</td>
</tr>
<tr>
<td>Physical Functioning</td>
<td>+ 5.9</td>
<td>+ 4.3</td>
</tr>
<tr>
<td>Bodily Pain</td>
<td>+ 11.4</td>
<td>+ 7.7</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>+ 10.4</td>
<td>+ 2.3</td>
</tr>
<tr>
<td>Role – Physical Functioning</td>
<td>+ 22.5</td>
<td>+ 18.0</td>
</tr>
<tr>
<td>Role – Emotional Functioning</td>
<td>+ 16.7</td>
<td>+ 13.5</td>
</tr>
<tr>
<td>Total Score</td>
<td>+ 90</td>
<td>+ 47</td>
</tr>
</tbody>
</table>

Two-sided Wilcoxon Rank Sum Test (Normal Approximation): p=0.0118
### Table 3. Nutrient Composition

#### Bottle 1A (Serving size - three tablets)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (from 7.5% Betatene (Henkel))</td>
<td>1665 IU</td>
</tr>
<tr>
<td>Vitamin C (as Ascorbic Acid, Ascorbyl Palmitate, Calcium Ascorbate)</td>
<td>600 mg</td>
</tr>
<tr>
<td>Vitamin D3 (as Cholecalciferol)</td>
<td>130 IU</td>
</tr>
<tr>
<td>Vitamin E (Mixed Covitol)</td>
<td>130 IU</td>
</tr>
<tr>
<td>Vitamin B1 (from Thiamine Mononitrate)</td>
<td>7 mg</td>
</tr>
<tr>
<td>Vitamin B2 (as Riboflavin)</td>
<td>7 mg</td>
</tr>
<tr>
<td>Niacin (as from Niacinamide)</td>
<td>45 mg</td>
</tr>
<tr>
<td>Vitamin B6 (from Pyridoxine HCl)</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>90 mcg</td>
</tr>
<tr>
<td>Vitamin B12 (as Cyanocobalamin)</td>
<td>20 mcg</td>
</tr>
<tr>
<td>Biotin</td>
<td>65 mcg</td>
</tr>
<tr>
<td>Pantothenic Acid (from D-Calcium Pantothenate)</td>
<td>40 mcg</td>
</tr>
<tr>
<td>Calcium (from Glycinate, Ascorbate)</td>
<td>35 mg</td>
</tr>
<tr>
<td>Phosphorus (from Dicalcium Phosphate)</td>
<td>15 mg</td>
</tr>
<tr>
<td>Magnesium (from Magnesium Glycinate, Magnesium Ascorbate)</td>
<td>40 mg</td>
</tr>
<tr>
<td>Zinc (from Zinc Glycinate)</td>
<td>7 mg</td>
</tr>
<tr>
<td>Selenium (from L-Selenomethionine)</td>
<td>20 mcg</td>
</tr>
<tr>
<td>Copper (from Copper Glycinate)</td>
<td>330 mcg</td>
</tr>
<tr>
<td>Manganese (from Amino Acid Chelate)</td>
<td>1.3 mg</td>
</tr>
<tr>
<td>Chromium (from Chromium Glycanate)</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Molybdenum (from Molybdenum Glycinate)</td>
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</tr>
<tr>
<td>Potassium (from Potassium Proteinate)</td>
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</tr>
<tr>
<td>L-Lysine (from L-Lysine HCl)</td>
<td>110 mg</td>
</tr>
<tr>
<td>L-Proline</td>
<td>110 mg</td>
</tr>
<tr>
<td>Citrus Fruit Peel Bioflavanoids</td>
<td>100 mg</td>
</tr>
<tr>
<td>L-Arginine (from L-Arginine HCl)</td>
<td>40 mg</td>
</tr>
<tr>
<td>L-Cysteine (from L-Cysteine Monohydrate HCl)</td>
<td>35 mg</td>
</tr>
<tr>
<td>Inositol</td>
<td>35 mg</td>
</tr>
<tr>
<td>L-Carnitine (from L-Carnitine Tartrate)</td>
<td>35 mg</td>
</tr>
<tr>
<td>CoEnzyme Q10</td>
<td>7 mg</td>
</tr>
<tr>
<td>Pycnogenol</td>
<td>7 mg</td>
</tr>
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</table>

#### Bottle 1B (Serving size - 2 tablets)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
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<tr>
<td>Vitamin C (from Calcium Ascorbate, Magnesium Ascorbate)</td>
<td>700 mg</td>
</tr>
<tr>
<td>Vitamin E (as d-Alpha Tocopheryl Succinate)</td>
<td>70 IU</td>
</tr>
<tr>
<td>Vitamin B1 (from Thiamine Mononitrate)</td>
<td>15 mg</td>
</tr>
<tr>
<td>Vitamin B2 (as Riboflavin)</td>
<td>15 mg</td>
</tr>
<tr>
<td>Niacin (as Niacinamide)</td>
<td>30 mg</td>
</tr>
<tr>
<td>Vitamin B6 (from Pyridoxine HCl)</td>
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</tr>
<tr>
<td>Vitamin B12 (as Cyanocobalamin)</td>
<td>7 mcg</td>
</tr>
<tr>
<td>Biotin</td>
<td>130 mcg</td>
</tr>
<tr>
<td>Pantothenic Acid (from Calcium D-Pantothenate)</td>
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<tr>
<td>Calcium (from Calcium Ascorbate)</td>
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<td>Taurine</td>
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<tr>
<td>L-Carnitine (from L-Carnitine Tartrate)</td>
<td>160 mg</td>
</tr>
<tr>
<td>CoEnzyme Q10</td>
<td>20 mg</td>
</tr>
</tbody>
</table>

### Figure 2. Arrhythmic episode frequency over six-month study of nutrient supplemented (n=44) and placebo (n=46) patients (PP analysis set) Exact Mantel-Haenszel Chi-Square Test: p=0.0160.

### Figure 3. Percentage of supplemented (n=44) patients experiencing frequent (>7) arrhythmic episodes during treatment intervals 1-3 months and 4-6 months.

### Figure 4. Percentage of supplemented (n=44) patients experiencing no arrhythmic episodes during treatment intervals 1-3 months and 4-6 months (PP analysis set).
status, vitality, general health perception, and other aspects of health. Evaluation of the “mental health” measure results, for example, showed an improvement of 7.4 in the supplemented group and a decrease of –1.6 in the placebo group (Two-sided Wilcoxon rank sum test was statistically significant for ITT analysis: p=0.0118). For PP analysis, statistical significance was almost achieved (two-sided Wilcoxon rank sum test p=0.0506).

DISCUSSION

Overall, the results of this randomized double-blind, placebo-controlled trial conclusively documented the effectiveness of supplementation using a combined vitamin/essential nutrient supplementation program in controlling arrhythmia, a condition for which conventional medicine does not provide a solution, and instead attempts to control symptomatically. Active treatment with the multivitamin/essential nutrient combination over a six-month period was significantly more effective in reducing clinically apparent symptoms of arrhythmia in patients than standard drug regimens alone. Furthermore, the reduction in arrhythmic episodes was more pronounced at six months than at three months of supplementation, suggesting increased benefits with a longer duration of supplementation. Thus, with the adjunctive use of the vitamin/essential nutrient supplement, the likelihood of being free from arrhythmia doubled (15.9% in the supplemented group vs. 6.5% in the placebo group).

These health benefits were achieved by addressing the underlying cause of arrhythmia, the deficiency of bio-energy-generating nutrients. The electrical cell cluster that triggers the heartbeat sends electrical impulses approximately once every 830 milliseconds. To generate electricity, these electrical cells of the heart need large amounts of bio-energy. Therefore, they need a constant supply of nutrients that facilitate the conversion of food into cellular energy. The most critical among them are coenzyme Q10, carnitine, the B vitamins, lysine and vitamin C, together with magnesium, calcium and potassium. For optimal biological effect, these nutrients must complement and support each other in synergy. These nutrients optimize the function of the heart’s electrical cells, as well as the cells building the heart muscle, blood vessels and other organs. When these nutrients are lacking, the heart cells fail to generate electrical energy and electrical impulses are sent in a chaotic manner.

This study sheds a new light on conventional medicine’s approach to arrhythmia, which has relied on mechanical regulation of the heart rhythm using catherization, or drugs such as beta-blockers and calcium channel blockers which can have severe side effects, the most important of these being the generation of even more irregular heartbeats and not infrequently, sudden cardiac death. Large clinical trials have revealed that anti-arrhythmic drugs, which are used by more than 1.5 million Americans and many more people in European and other countries, do not offer health benefits and increase the risk of serious complications, including death. In 1989, a study using anti-arrhythmic drugs in patients who had experienced heart attacks was prematurely stopped when preliminary results showed the risk of death was two-and-a-half times (2.5) greater in patients taking drugs.13 In 2002, two large studies, one conducted in Canada and the other in the Netherlands, provided similar evidence.14 The six-year study, conducted with more than 4,000 patients, showed higher death and hospitalization rates among patients on anti-arrhythmic drugs. These drugs included those that affect heart rate, such as dioxin, beta-blockers, and calcium channel blockers. The European study came to the same conclusion, and also found that women taking anti-arrhythmic medications faced a higher risk of heart failure, stroke and other medical events than men.

Of significance, the results of this study indicate that combining conventional drug treatment with a vitamin/nutrient supplement program to treat arrhythmia is an effective, safe, therapeutic approach that provides enhanced improvement with long-term use. Due to the synergistic effect of specific vitamin/essential nutrients, therapeutic effect is achieved with moderate levels of these nutrients in contrast to single nutrient megadose approaches. In addition to improvement in arrhythmic episode frequency, the vitamin/essential nutrient program used by the supplemented group significantly improved their perceived quality of life, especially in the area of mental health. This is an important benefit of supplementation, as arrhythmia patients not only suffer from depression and fear of experiencing heart dysfunction, but also from deteriorating health and a gradual diminishing of their quality of life. To a large extent, these adverse mental and emotional consequences are associated with drug side effects and the belief that a cure for paroxysmal atrial arrhythmia is not available.

SUMMARY

Although further clinical studies are warranted to better determine its effectiveness for treatment of arrhythmia, this nutrient combination offers great potential as an adjunctive treatment for arrhythmia patients and as a preventative measure for patients with a predisposition for developing arrhythmia.

ACKNOWLEDGMENTS

The study was conducted by internists in 35 health centers in Germany and supervised by Dr. Ute Engelmann and Frank Koch.

REFERENCES


ABSTRACT

Soy protein and dietary flaxseed, interventions rich in phytoestrogens, reduce renal injury in animal models. We studied the flax lignan, secoisolaricoresinol dyglycoside (SDG) and the soy isoflavone genistein to assess their contributions to this effect in animals with polycystic kidney disease (PKD). Male animals heterozygous for the Han:SPRD-cy gene and their healthy littermates were fed a diet with SDG, 20 mg/kg diet, or genistein, 75 mg/kg diet for 8 weeks from weaning. Renal histology was studied in animals with PKD. Renal function and fatty acid composition, which have been linked to dietary amelioration of renal injury in other studies, were then measured in both PKD animals and unaffected litter mates to differentiate physiologic and metabolic consequences of diet versus renal dysfunction. SDG reduced all indices of histologic injury. Genistein, however, only reduced macrophage infiltration and staining for oxidized LDL. There were weak dietary effects upon renal linoleic acid and docosahexanoic acid in healthy and PKD animals, but no difference in arachidonic acid proportion. SDG and genistein have measurable effects upon early pathology in rat PKD. They may prove to be a practical way of obtaining renal health benefit. They may not, however, reproduce all of the effects of a change in dietary practice.

Key Words: phytoestrogen, kidney, morphometry, renal failure, polycystic, soy, flaxseed, pathology.

INTRODUCTION

Flaxseed or flax oil ingestion has demonstrated possible benefits in the reduction of inflammation in rheumatic disease, reduction of atherosclerosis and modification of behavior of hormone-dependent tumors. Flaxseed is the richest natural source of mammalian lignans, present in the husk of the seed as an ester secoisolaricoresinol dyglycoside (SDG) which is hydrolysed in the intestinal lumen to enterodiol and enterolactone. SDG or its hydrolyzed products may influence health or disease through estrogenic pathways, an antioxidant effect, or through antagonism of platelet activating factor by specific receptor blockade. Flaxseed and SDG have been shown to reduce plasma levels of insulin-like growth factor 1 (IGF-1) in a rat mammary tumor model through an as yet unknown mechanism. Recent studies have identified potential benefits of flaxseed...
or flax oil in the 5/6 nephrectomy model and murine or human lupus erythematosus. Recently SDG has also been shown to ameliorate murine lupus. We have previously demonstrated that partial dietary substitution of ground flaxseed into standard rat chow or the use of flax oil as the lipid source in a synthetic diet modifies renal injury, particularly interstitial inflammation and fibrosis, in the Han:SPRD-cy rat. The Han:SPRD-cy is a model of polycystic kidney disease, characterized by autosomal dominant inheritance with marked sexual dimorphism in expression, and an excellent system in which to explore the modification of chronic renal injury. The disease is characterized by progressive dilatation of nephrons in young animals, and associated with marked interstitial inflammation and fibrosis with associated nephron loss in older animals.

We have previously demonstrated that soy protein feeding of the Han:SPRD-cy rat and pcy mouse reduced renal cyst formation, renal epithelial cell proliferation, and renal interstitial inflammation and fibrosis, and is associated with significant indirect effects on renal polyunsaturated fatty acid content, possibly through Δ6-desaturase inhibition. The soy saponin Bb has been linked to at least part of the benefit observed in the pcy mouse. Dietary soy protein is associated with reduced expression of IGF-1 in conjunction with reduced disease severity. Knight et al., using data from a large longitudinal study of health outcomes in nurses recruited at random in the New England area, have shown that protein intake from non-animal sources is associated with a slower decline in renal function in the subset of the study population that had mild renal insufficiency. Other than alteration in the availability of specific amino acids, soy protein may also contain other biologically active compounds such as saponins and phytoestrogens of which genistein is the most abundant. Genistein is known to have estrogenic effects in mammals and is also a potent inhibitor of tyrosine kinase, an enzyme intrinsic to many sub-cellular processes and induces the activity of antioxidant enzymes. The success of both soy and flaxseed based interventions in the amelioration of renal injury has led to speculation that dietary phytoestrogens may have a role to play in slowing progression of renal injury.

As major alteration in dietary practice is difficult to achieve at an individual patient level, we undertook studies to determine if genistein and SDG, both of which can be now be produced as concentrated micro-nutrients, contributed to the observed benefits of soy protein and flaxseed feeding in the Han:SPRD-cy rat.

**MATERIALS AND METHODS**

**Animals**

Han:SPRD-cy rats were obtained from our own breeding colony that is derived from animals that were kindly provided to us by Dr. Benjamin Cowley, University of Kansas Medical Center, Kansas City, Kansas. All animal procedures and care were examined by the University of Manitoba Committee on Animal Use and certified to be within the guidelines of the Canadian Council on Animal Care. Surviving male offspring of known Han:SPRD-cy heterozygotes were used in this study. Two thirds of these animals would be expected to be heterozygous, as homozygotes in our colony rarely survive beyond weaning.

Male animals were randomly assigned to treatment or control groups at weaning. Control animals in both experiments received a synthetic powdered AIN 76 diet using casein as the protein source and 7% corn oil as the lipid source. Experimental diets consisted of either supplementation with SDG at a level of 20 mg/kg diet (a gift of Dr. N. Westcott and Dr. A. Muir, Agriculture Canada, Saskatoon) or genistein 75 mg/kg (Toronto Research Chemical Inc., North York, Ont). Doses were based upon estimates of content of a soy and flaxseed based diet in previous studies and doses of SDG and genistein used in other studies in vascular, neoplastic and renal disease. Dietary phytoestrogens may have a role to amelioration of renal injury has led to permitting the exclusion of the amount of food eaten as a significant variable in the modification of disease. Animals were euthanized after 8 weeks on the diet, and kidney and liver tissue as well as serum were collected for analysis.

**Histology**

Tissue from the left kidney was used for histologic and immunohistochemical analysis. This tissue was fixed in 10% formalin for 120 minutes prior to embedding in paraffin and sectioning at 5 microns. Sections for measurement of cystic volume and qualitative study of renal histology were stained with hematoxylin and eosin. Sections for quantitative analysis of fibrosis were stained using aniline blue alone in adaptation of Masson’s trichrome stain. This adaptation demonstrates a perfect concordance of staining with an immunofluorescent detection of collagen type III. The aniline blue staining permits image analysis measurement using a standard incandescent microscope light source. Animals were classified as affected if a single longitudinal cross section of the kidney contained at least 10 areas of tubular dilatation with associated increase in extracellular matrix. Classification was confirmed by 2 experienced observers (NBC, MRO).

**Immunohistochemistry**

Cell proliferation was studied using immunohistochemical detection of proliferating cell nuclear antigen (PCNA) using a monoclonal anti-PCNA antibody (Dako M 0879, Dako A/S, Glostrup, Denmark) at a dilution of 1:50 for 90 minutes. Secondary detection was achieved with a Vectastain Elite rat adsorbed anti-mouse IgG kit (Vector Laboratories, Burlingame, CA) with peroxidase-diaminobenzidine color development.
Macrophages were identified using a monoclonal antibody against the rat equivalent of the human CD68 antigen (Chemicon MAB1435, Chemicon International Inc., Temecula, CA) at a dilution of 1:100 for 90 minutes. Secondary detection was achieved in the same way as for PCNA.

Oxidized LDL (ox-LDL) was identified using a rabbit antibody against copper-oxidized LDL (AB3230, Chemicon International, Inc, Temecula, CA) at a dilution of 1:100 incubated for 2 hours, with subsequent visualization using the DAKO Envision secondary detection system (DAKO Corporation, Carpinteria, CA).

**Image Analysis**

Image analysis of histologic and immunohistologic sections was performed on a minimum of 50 randomly selected digitally captured image fields. Macrophage numbers and PCNA positive cells were quantitated by counting, using module 2500 of the Imagemeasure software package. The counts were reported as a mean per high power image field (40X microscope objective) with at least 50 fields counted. Results were corrected for the extent of cystic change in each kidney and thus represent counts per solid tissue area.

**Chemistry**

Serum creatinine and cholesterol were determined in serum by spectrophotometric methods using Sigma kits (Sigma Chemical Co., St. Louis, MO) adapted to a 96-well plate reader.

**Gas Chromatography**

Lipids were extracted for gas chromatographic analysis using a modified Folch extraction procedure. Chromatographs were integrated using Varian Saturn software, version 5.51. Fatty acid methyl esters were identified by comparison to retention times of Supelco37 component FAME mix and expressed as percent total lipid.

**Statistical analysis**

Data were analyzed using a general linear model ANOVA to permit study of both disease and intervention effects using Minitab release 13 (Minitab Inc., PA) with the Tukey test applied for post hoc comparisons.

**RESULTS**

A total of 135 animals were studied, of which 87 had PKD and 48 were normal. The distribution of normal vs. PKD animals did not differ significantly between treatment groups (Chi-square test). Results from control-diet-fed animals in the two feeding trials were compared in all categories and then pooled to a single control group when that analysis revealed no effect due to experimental order. Animal weight was significantly reduced and serum creatinine increased by PKD compared to healthy animals (p<0.001, Table 1), but no dietary effect was observed. Post hoc comparisons confirmed significant differences between each group of normal animals and all groups of PKD animals. A very weak dietary effect upon cholesterol was detected (p=0.049), but no significant post hoc effects were noted (Table 1). No disease or dietary effect on serum triglyceride concentration was seen (Table 1).

Cystic change was significantly reduced by SDG (p<0.001 vs. control and p=0.006 vs. genistein; Figure 1).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Control</th>
<th>Genistein</th>
<th>Genistein</th>
<th>SDG</th>
<th>SDG</th>
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<tbody>
<tr>
<td>Disease status</td>
<td>normal</td>
<td>PKD</td>
<td>normal</td>
<td>PKD</td>
<td>normal</td>
<td>PKD</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>47</td>
<td>11</td>
<td>23</td>
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<tr>
<td>Weighta (gm)</td>
<td>374 (6)</td>
<td>348 (4)</td>
<td>381 (7)</td>
<td>356 (5)</td>
<td>374 (7)</td>
<td>334 (6)</td>
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<tr>
<td>creatinine (µmol/l)</td>
<td>75 (6.9)</td>
<td>115 (4.1)</td>
<td>76 (8.0)</td>
<td>125 (5.6)</td>
<td>78 (7.7)</td>
<td>128 (6.5)</td>
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<td>cholesterolb (mmol/l)</td>
<td>2.8 (0.5)</td>
<td>2.8 (0.3)</td>
<td>1.8 (0.5)</td>
<td>2.2 (0.4)</td>
<td>2.5 (0.5)</td>
<td>3.7 (0.4)</td>
</tr>
<tr>
<td>triglyceride (mmol/l)</td>
<td>0.52 (0.05)</td>
<td>0.55 (0.05)</td>
<td>0.50 (0.06)</td>
<td>0.53 (0.03)</td>
<td>0.41 (0.05)</td>
<td>0.57 (0.04)</td>
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</table>

Results are expressed as mean with SEM in parentheses.
a = significant disease effect, p<0.001, with significant pair-wise differences in post hoc testing between normal and PKD animals in each group, p<0.01.
b = significant dietary effect, p<0.05
SDG also significantly reduced interstitial fibrosis (p<0.001 vs. control and genistein; Figure 2) and epithelial proliferation (p<0.001 vs. control and genistein; Figure 3). Both genistein (p<0.001 vs. control) and SDG (p=0.002 vs. control) reduced macrophage infiltration (Figure 4), with no difference detected between genistein and SDG. Both genistein (p<0.001 vs. control) and SDG (p=0.002 vs. control) demonstrated reduced staining compared to control, with no difference detected between the 2 treatments.

GC analysis of renal tissue revealed modest dietary effects on linoleic acid (LA) in SDG treated animals, although pair-wise comparisons did not detect any significant differences (Table 2). The n-3 PUFAs, α-linolenic acid and eicosapentanoic acid were not consistently detected in most renal tissue samples and are not reported. This result is consistent with our previous studies using corn oil as a lipid source.20,22 There was a small but statistically significant effect of diet on the longer chain n-3 PUFAs, docosahexanoic acid, with genistein-treated animals demonstrating slightly higher levels compared to SDG-treated animals, although neither group differed from control in pair-wise comparisons. LA:arachidonic acid ratio did not differ between experimental groups, suggesting no diet or disease effect on Δ6-desaturase, as was demonstrated in our previous study with soy protein and flax oil.17

DISCUSSION

Our results indicate that dietary phytoestrogens may contribute to the effects of nutritional strategies on the evolution of renal injury in the Han:SPRD-cy rat. The results also suggest, however, that these compounds are unlikely to be responsible for all of the observed benefits seen in studies with relatively crude dietary substitutions in the form of whole flaxseed or soy protein concentrate.15,17,20-22 It also seems that SDG may have a broader range of action, influencing fibrosis and disregulated epithelial proliferation, in addition to sharing anti-inflammatory and anti-oxidative effects with genistein.

A number of previous studies with SDG suggest that it may have multiple actions, both through enterodiol and enterolactone that are produced by hydrolysis of the SDG, and through actions of the intact molecule.4,35,36 Following studies that had shown evidence of benefit of flaxseed feeding in murine and human lupus,12,13 Clark et al. demonstrated significant absorption of intact SDG in the MRL/lpr mouse model of lupus nephritis.9 A dose as low as 0.6 mg was sufficient to block the biological effects of a lethal dose

Table 2. Selected renal polyunsaturated fatty acid proportions in Han:SPRD-cy rats fed control, genistein-supplemented or SDG-supplemented diets.

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Control</th>
<th>Control</th>
<th>Genistein</th>
<th>Genistein</th>
<th>SDG</th>
<th>SDG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA a (18:2 ω6)</td>
<td>15.9 (1.2)</td>
<td>16.7 (0.7)</td>
<td>17.7 (1.3)</td>
<td>16.0 (0.9)</td>
<td>12.9 (1.2)</td>
<td>14.0 (1.1)</td>
</tr>
<tr>
<td>AA (20:4 ω6)</td>
<td>19.5 (2.0)</td>
<td>18.6 (1.2)</td>
<td>19.1 (2.4)</td>
<td>22.9 (1.6)</td>
<td>24.6 (2.0)</td>
<td>20.6 (1.9)</td>
</tr>
<tr>
<td>DHA b (22:6 ω3)</td>
<td>0.71 (0.08)</td>
<td>0.65 (0.05)</td>
<td>0.86 (0.09)</td>
<td>0.96 (0.06)</td>
<td>0.66 (0.08)</td>
<td>0.44 (0.08)</td>
</tr>
<tr>
<td>LA:AA ratio</td>
<td>1.33 (0.33)</td>
<td>1.57 (0.19)</td>
<td>1.13 (0.38)</td>
<td>0.89 (0.26)</td>
<td>0.67 (0.33)</td>
<td>0.85 (0.31)</td>
</tr>
</tbody>
</table>

Results are expressed as mean % total lipids with SEM in parentheses.

a = significant dietary effect, p=0.005, but no significance pair-wise differences in post hoc testing.

b = significant dietary effect, p<0.001, with genistein-fed animals having higher values than SDG-fed animals but not controls, p<0.01.
of platelet activating factor, and time and dose dependent benefits on renal function and proteinuria were observed. The extent to which the platelet activating factor receptor-blocking function of SDG can be generalized to other peptide growth or cell activation factors is not yet known. Rickard et al. have shown that SDG depresses plasma levels of IGF-1 through a mechanism that is yet to be identified.

Aukema et al. have recently demonstrated that IGF-1 levels correlate with disease expression and its modification by soy protein feeding and gender in the Han:SPRD-cy rat. IGF-1 is a known participant in regulation of renal epithelial proliferation and interstitial fibrosis and may be relevant to the anti-proliferative and anti-fibrotic actions of SDG.

SDG and its metabolites are known to be antioxidants, a finding supported by our results. Other studies have implicated oxidant injury in the evolution of Han:SPRD-cy rat renal disease. The use of α-tocopherol as antioxidant therapy in the Han:SPRD-cy rat was not associated with clinical or histologic improvement, although renal content of α-tocopherol was higher in female animals with lesser disease. Although SDG did have significant effects upon renal histology that may certainly warrant investigation with respect to modification of the final common pathway to renal failure, these effects were not associated with a change in decline of renal function, unlike our prior studies with both intact flaxseed and lignan-poor

**Figure 2.** Renal fibrosis in animals fed control, SDG or genistein supplemented diets.

**Figure 3.** Renal epithelial proliferation in animals fed control, SDG or genistein supplemented diets.

**Figure 4.** Renal macrophage infiltration in animals fed control, SDG or genistein supplemented diets.

**Figure 5.** Renal ox-LDL staining in animals fed control, SDG or genistein supplemented diets.
flax oil. It would seem that SDG might contribute to, but is not solely responsible for, the benefits of flaxseed.

The detection of an in vivo renal anti-inflammatory role for genistein is novel. An antioxidant effect of soy protein has been proposed as a basis for an experimental anti-atherogenic effect. Genistein is reported to upregulate the activity of antioxidant enzymes, thus reducing another potent stimulus for macrophage infiltration. A specific anti-inflammatory effect of genistein has been demonstrated in experimental ileitis, and has been correlated with inhibition of expression of inducible nitric oxide synthase. Our ox-LDL staining studies support a possible role for genistein as a renal antioxidant, an area where it demonstrated an effect similar to that of SDG.

Genistein is widely used in in vitro studies as an inhibitor of tyrosine kinases that are an important part of peptide-growth-factor mediated proliferative responses in renal epithelium, including an organ explant model of cystic disease. The concentration of genistein that produces this effect in vitro is higher than that likely to be achieved at a tissue level in in vivo studies. In this study, genistein alone failed to reproduce the cyst reduction and anti-proliferative effect of soy protein substitution containing genistein, suggesting that it is not the active factor in this part of the benefit of soy. The failure of genistein to change cyst formation or indices of epithelial proliferation both in this study and that by Tomobe et al. in the pcy mouse, in which inflammation and fibrosis are far less prominent than the Han:SPRD-cy rat, would suggest that genistein has no inhibitory effect on the proliferative growth factors that have been implicated in cyst formation, such as epidermal growth factor. It also suggests that there is no role for the observed inhibitory effect of genistein on DNA topoisomerase with subsequent interruption of the cell cycle, an effect also possibly limited to high concentrations.

The failure of genistein to reproduce the effects of soy protein does not exclude a micronutrient effect altogether. In addition to isoflavones, soy protein contains saponins that have been subject to recent interest. Philbrick et al. have recently reported treatment of pcy mice by supplementation with purified saponin preparation, supplementation with alcoholic extract of soy containing both saponins or isoflavones, or by replacement of dietary protein with an isolate containing saponins and isoflavones. Feeding of the alcoholic extract reduced kidney weights and water, and was associated with preservation of renal function. This finding was reproduced by soy protein isolate and purified soyasaponin Bb.

Another potential shared effect of SDG and genistein might be an estrogenic inhibition of macrophage infiltration. Disease expression in the Han:SPRD-cy and its modification by diet have been linked to alteration in expression of monocyte chemoattractant protein 1 (MCP-1) by our group and others. MCP-1 expression is reduced in the presence of estrogen or estrogen agonists and such reduction has been associated with anti-inflammatory and antifibrotic effects in experimental arthritis. SDG and genistein may, in part, reproduce the gender dimorphism that is characteristic of this model and has been proposed in human disease.

Our study indicates that these phytoestrogens have biologic activity that may be relevant to the management of the progression of chronic renal injury, but are unlikely to be responsible for the benefits observed with soy or flaxseed feeding alone. Both flaxseed and soy protein substitution are complex interventions and should not be overinterpreted as evidence of biologic effects of a single micronutrient. Although the possibility exists that pharmacologic dosing with these agents may be useful, recent enthusiasm for these agents as potential broad spectrum therapies for renal disease should be tempered until longer-term studies of efficacy and dose response relationship have been performed.

**ABBREVIATIONS USED**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor 1</td>
</tr>
<tr>
<td>LA1</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>ox-LDL</td>
<td>Oxidized low density lipoprotein</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemoattractant protein 1</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>PKD</td>
<td>Polycystic kidney disease</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>SDG</td>
<td>Secoisolariciresin diglycoside</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGMENTS**

This work was supported by operating grants from the Children’s Hospital Foundation of Manitoba, Inc., the Manitoba Health Research Council and the Flax Council of Canada. The work was performed in the laboratory facilities of the Manitoba Institute of Child Health in the John Buhler Research Centre, Winnipeg, Manitoba. Dr. Hope Weiler is the recipient of a New Investigator Award of the Canadian Institutes of Health Research.

**REFERENCES**


 IMMUNOSTIMULATING PROPERTIES OF TWO DIFFERENT β–GLUCANS ISOLATED FROM MAITAKE MUSHROOMS (Grifola frondosa) 

Vaclav Vetvicka, PhD,* Jana Vetvickova, MS 
University of Louisville, Department of Pathology 
Louisville, Kentucky

ABSTRACT 
β–glucans have been extensively studied for their immunological and pharmacological effects. The number of individual glucans is almost as great as the number of sources used for isolation. Not surprisingly, mushrooms are one of the prime sources of (1-3)-β-D-glucans, but their activities are not always described consistently. In addition, the most commonly used route of administration is injection, which is less convenient for clinical practice.

In this paper, we compared the immunostimulating properties of two different glucans from Maitake mushrooms with lentinan, a standard, well-researched mushroom-derived glucan. A number of major immunological parameters were tested – phagocytosis, NK cell activity, expression of surface markers, cytokine secretion and apoptosis. Our study showed not only have these glucans significantly increased all tested characteristics, but they also have similar, and in some tests even higher activity than lentinan, are active at lower doses and can be administered orally with no loss of activity. Therefore, this report represents evidence that Maitake-derived supplements taken orally can stimulate the defense systems.

Key Words: glucan, phagocytosis, cytokines, mushrooms.

INTRODUCTION 
Polysaccharides in general have a long history as immunomodulators, and interest in them rose particularly after experiments showed that zymosan stimulates macrophages via activation of the complement system.1 Various types of β-glucans can be isolated from numerous sources, the major ones being yeast, mushrooms and seaweed. Despite the fact that the number of individual β–glucans is almost as great as the number of sources used for its isolation, and despite the enormous numbers of studies performed all over the world, it is still impossible to say that only one particular glucan is the optimal immunomodulator.

As with all natural products, there are considerable variations not only among individual β–glucans, but also among individual batches. In view of the ever increasing popularity of glucans as immunomodulators, the functional comparison of new glucans is therefore more important than ever.

Thus far, the immunostimulating effects of β–glucans have been demonstrated in every single animal species tested, from earthworms2 to humans. This supports not only the conclusion that β–glucans are active over the broad spectrum of biological species, but also that they represent one of the first immunostimulants active across the evolutionary spectrum.

* Correspondence: 
Vaclav Vetvicka, PhD 
University of Louisville 
Department of Pathology and Laboratory Medicine 
511 S. Floyd St., MDR Bldg., Rm. 224 
Louisville, KY 40202 
Phone: 502-852-1612    FAX: 502-852-1177 
Email: vetvickavaclav@netscape.net
Soluble fungal β−glucans such as schizophyllan and lentinan have been used for tumor immunotherapy in Japan for the past 25 years. These polysaccharides promote natural host defense mechanisms and boost specific tumor immunity. For some time, investigators have failed to define the exact mechanisms of action. Only after identification of CR3 (CD11b/CD18) molecule as the principal receptor for β−glucans of leukocytes was it possible to determine the cellular basis for their action and to design more rational approaches for their potential use in immunotherapy. However, not all cellular and molecular mechanisms responsible for glucan effects on the immune system are completely understood.

In addition to the well-studied mushroom-derived glucans such as lentinan, numerous additional glucans have been isolated from various mushrooms. Some glucans, such as β−glucans from Phytophthora parasitica, were merely described without any biological tests. Many of them, however, have very interesting properties, including the strong antitumor activity of β−glucan from Glomereilla cingulata or potentiation of TNF by scleroglucan. As some of the published reports described rather confusing data, we decided to evaluate the immunostimulating effects of two mushroom-derived glucans and to compared them to the standard glucan, lentinan.

MATERIAL AND METHODS

Animals

Female, 6-to-10-week old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO2 asphyxiation.

Materials

RPMI 1640 medium, sodium citrate, dextran, Ficoll-Hypaque, antibiotics, sodium azide, bovine serum albumine (BSA), Wright stain, Limulus lysate test E-Toxate, and Concanavalin A were obtained from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT), and CytoTox 96 Non-Radioactive Cytotoxicity Assay from Promega (Promega, Madison, WI).

Antibodies

For fluorescence staining, the following antibodies have been employed: anti-mouse CD4, CD8, and CD19, conjugated with FITC were purchased from Biosource (Camarillo, CA).

Flow cytometry

Cells were stained with monoclonal antibodies on ice in 12x75 mm glass tubes using standard techniques. Pellets of 5x10⁶ cells were incubated with 10 μl of FITC-labeled antibodies (1 to 20 μg/ml in PBS) for 30 minutes on ice. After washing with cold PBS, the cells were resuspended in PBS containing 1% BSA and 10 mM sodium azide. Flow cytometry was performed with a FACSscan (Becton Dickinson, San Jose, CA) flow cytometer and the data from over 10,000 cell samples were analyzed.

Cell lines

Human cell lines ZR-75-1, PC-3, SW900 and K562 were purchased from ATCC. The cells were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, and antibiotics.

β -1,3 glucans

Two different soluble β−glucans were isolated from Maitake mushrooms (Grifola frondosa) according to manufacturer’s specification. Briefly, MaitakeGold 404 was produced under a patented method (US Patent 5,854,404). The product, a glucan/protein complex, is derived by thermally extracting the fruit body of Maitake with water under pressure at 100°C or more for 30 minutes to an hour. After that, alcohol is added to the extract at a final concentration of 20% to 60% by volume to remove floating material by filtration. The resulting extract is concentrated under heating to remove residual alcohol. The product, a hygroscopic powder in shades of brown is soluble in water, alkaline solutions, and dimethyl sulphoxide, and has a molecular weight around 1,000 kD.

Maitake Pro D Fraction is prepared as follows: Fruit bodies of Maitake are treated with hot water, and the water-soluble fraction is then saturated with ethyl alcohol. The resulting precipitate is then treated with acetic acid and alkali material. The extract is acid-insoluble, alkali-soluble, with a molecular weight around 1,000 kD.

MDF glucan is a highly-purified β−glucan, MTG glucan is a β−glucan-protein complex. MDF used in our experiments was Grifon-Pro Maitake D Fraction manufactured by Maitake Products, Inc. The MTG used in our experiments was MaitakeGold 404 purchased from Tradeworks Group, Inc. (Brattleboro, VT).

The soluble mushroom-derived β−glucan, lentinan (MW app. 1,000 kD), was obtained from the Developmental Therapeutic Program, Division of Cancer Treatment, Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD), and served as a control.

All media and buffers were tested for endotoxin contaminations and shown to contain >0.1 ng/ml of endotoxin using the Limulus lysate test (E-Toxate). The glucans were administered either by ip. injection or via intragastric tube (100 μg/day).

Phagocytosis

The technique employing phagocytosis of synthetic polymeric microspheres was described earlier. Briefly: peritoneal cells were incubated with 0.05 ml of 2-hydroxymethyl methacrylate particles (HEMA; 5x10⁶/ml). The test tubes were incubated at 37°C for 60 min with intermittent
smears were stained with Wright stain. The cells with three and more HEMA particles were considered positive. The same smears were also used for evaluation of cell types.

**In vitro cytotoxicity assay**

Spleen cells were isolated from the spleens of mice by standard methods. Cell suspension was generated by pressing minced spleen against the bottom of a petri dish containing PBS. After elimination of erythrocytes by 10-second incubation in distilled water and five washes in cold PBS, the cells were resuspended in PBS and counted. The viability was determined by trypan blue exclusion, and only cells with viability better than 95% were used in subsequent experiments. Splenocytes (10^6/ml; 0.1 ml/well) were incubated in an RPMI 1640 medium containing 5% FCS for 72 hr in a humidified incubator (37°C, 5% CO₂). At the endpoint of incubation, supernatants were collected, filtered through 0.45 µm filter and tested for the presence of IL-2.

**Cytokine evaluation**

BALB/c mice were intraperitoneally injected with various doses of tested glucans. Control mice obtained PBS only. After various time intervals, the mice were sacrificed and blood was collected in Eppendorf tubes. Subsequently, the serum was prepared, collected and stored at −80°C for no more than 1 week.

The levels of TNF-α and IL-1 in serum samples were evaluated using a commercial kit OptEIA Mouse TNF-α (Mono/Mono) and OptEIA Mouse IL-1 Sets (Pharmingen, San Diego, CA) according to the manufacturer’s instructions. The optical density was determined using an ELISA reader at 450 nm with a correction at 570 nm. Data were calculated from the standard curve prepared by the automated data reduction using linear regression analysis. A standard curve was run with each assay.

For evaluation of IL-2, we incubated purified spleen cells (2x10^6/ml in RPMI 1640 medium with 5% FCS) in wells of a 24-well tissue culture plate. After addition of 1 µg of Concanavalin A into positive-control wells, cells were incubated for 72 hr in a humidified incubator (37°C, 5% CO₂). At the endpoint of incubation, supernatants were collected, filtered through 0.45 µm filter and tested for the presence of IL-2.

**Apoptosis**

Apoptosis was evaluated using the APO-BRDU kit (BD Biosciences, San Diego, CA) according to the manufacturer’s instructions with the use of a flow cytometer equipped with a 488 nm laser.

**Statistics**

Student’s t-test was used to statistically analyze the data.

**RESULTS**

**Phagocytosis**

It is well established that β-glucans strongly influence the phagocytic activity of professional phagocytes. At the same time, however, it is always important to start with phagocytosis when a new glucan is being evaluated. First we compared the effects of MTG and MDF β-glucans on phagocytosis of synthetic HEMA microspheres by peripheral macrophages (Figure 1). When the glucans were administered intraperitoneally, both tested glucans stimulated the phagocytosis more than lentinan used as a control. On the
other hand, after oral administration (14 days of intragastric delivery of (100 µg of glucan/mouse) we found no differences between lentinan and our β-glucans, but all three types of glucan showed significant stimulation of phagocytosis.

In the next step we compared the effects of these glucans on phagocytosis by peripheral blood leukocytes. Results summarized in Figure 2 showed significant stimulation of monocytes by both MTG and MDF, whereas significant stimulation of neutrophils was achieved only after injection with lentinan (similar level of stimulation by MTG was not significant due to higher variation among samples). These experiments were followed up by evaluation of dose-dependence. Six different doses (from 1.8 to 250 µg/mouse) were used. Lentinan was active only in three higher doses, MTG caused increase in numbers of phagocytosing monocytes even at low 2.6 µg concentration, whereas MDF was effective only at the two higher concentrations (Figure 3).

**MEMBRANE MARKERS**

We then evaluated the effects of MTG and MDF on expression of several membrane markers. Twenty four hours after an ip. injection of either MTG, MDF, or lentinan, spleen cells were isolated and the surface expression of CD4, CD8, and CD19 was evaluated by flow cytometer. The results summarized in Figure 4 show that the application of both MTG and MDF significantly increase the number of CD4-positive T helper cells in the spleen.

**Cytokines**

Next, we compared the effects of a single intraperitoneal injection of MTG, MDF, or lentinan on systemic in

**Figure 2.** Effect of an ip. administration of different glucan (100 µg/mouse) samples on phagocytosis by peripheral blood granulocytes and monocytes. Each value represents the mean ± SD. *Represents significant differences between control (PBS) and glucan samples at P ≤0.05 level.

**Figure 3.** Potentiation of phagocytosis of synthetic microspheres by different doses of ip-injected glucans. Monocytes with three and more HEMA particles were considered positive. Each value represents the mean ± SD. *Represents significant differences between control (PBS) and glucan samples at P ≤0.05 level.

**Figure 4.** Effect of ip. injection of 100 µg of tested glucans on the expression of CD4, CD8, and CD19 markers by spleen cells. The cells from three donors at each time interval were examined and the results given represent the means ± SD. *Represents significant differences between control (PBS and samples) at P ≤ 0.05 level.

**Figure 5.** Stimulation of IL-1β secretion in peripheral blood by different types of glucan. Each value represents the mean ± SD. As the control values (PBS) were always zero, each value represents significant differences at P ≤0.05 level.
**vivo** release of two different cytokines, IL-1β and TNF-α. Peripheral blood was isolated at three different intervals after the injection and the serum obtained was stored at −80°C for no more than 1 week. The data summarized in Figure 5 shows that MTG and MDF caused significant elevation in levels of IL-1β in every tested interval. These elevated levels were demonstrated as early as 30 minutes and as late as 24 hours after application. Lentinan-caused increase could be observed only after 24 hrs. For both MTG and MDF glucans, the highest concentration of IL-1β was found 60 minutes after injection. There is a possibility that contamination of samples with LPS is causing the measured effects, but we also used LPS-free samples with virtually identical results.

A different situation was observed in the case of TNF-α, where only the MTG showed significant effects at the 60-minute interval. And again, LPS-free samples yielded similar data as normal samples (Figure 6). Several individual doses of all three glucans have been used, but the most pronounced effects were found with 100 µg doses.

Evidence of the immunomodulating activity was also demonstrated through effects on the production of IL-2 by spleen cells (Figure 7). The production of IL-2 was measured after a 72 hr *in vitro* incubation of spleen cells isolated from control and glucan-administered animals. Injection of lentinan and MDF resulted in strong stimulation of IL-2 production, which was even significantly higher than control stimulation by Concanavalin A. The effects of MTG were slightly lower than control Con-A-stimulated levels, but still almost 60 times higher than the level where only PBS was used (63 pg of IL2/ml).

**Cytotoxicity**

Next, for evaluation of the effects on NK cells, human K562 cells were incubated with mouse spleen cells stimulated by either by our glucan samples or lentinan (Figure 8). A short treatment of glucans was adequate to cause significant enhancement of cytotoxicity at the higher E:T ratio.

**Apoptosis**

We also measured the effects of our samples on induction of apoptosis. We decided to use three different and well-established human cancer cell lines: ZR-75-1 (breast cancer), PC-3 (prostate cancer), and SW900 (lung cancer). We used these lines to represent three different types of human cancer. However, using the APO/BRDU test, no apoptotic cells were observed (data not shown).

**DISCUSSION**

β-glucans, originally recognized as potent stimulants of the reticuloendothelial system, are commonly considered typical representatives of substances that achieve their resistance-enhancing effects by stimulation of macrophages. More recent discoveries of the interaction of immunocytes with β-glucans have demonstrated that complement recep-

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**Figure 6.** Stimulation of TNF-α secretion in peripheral blood by different types of glucan. Each value represents the mean ± SD. As the control values (PBS) were always zero, each value represents significant differences at P ≤0.05 level.

**Figure 7.** Stimulation of IL-2 secretion by splenocytes by different types of glucan. *Represents significant differences between control (PBS and samples) at P ≤ 0.05 level.

**Figure 8.** Effects of tested glucans on NK cell cytotoxicity of K562 cells. Different ratios of NK cells to target cells were tested for cytotoxicity in the presence or absence of β-glucans for 30 minutes at 37°C. The data points shown are mean values from three experiments. The differences were significant at P≤0.05 level at all three effector to target cell ratios.
tor type 2 (CR3; CD11b/CD18) is primarily responsible for both the binding and biological effects of β-glucans. Subsequent experiments helped to show that glucan used orally has similar biological effects to injected glucans, and that the mechanisms responsible for glucan-mediated killing of tumor targets is via interaction of anti-tumor antibodies with glucan-activated cells. The results of these experiments changed our view of glucans from merely nonspecific immunostimulators to natural agents specifically affecting immune reactions.

Despite our knowledge of the mechanisms of interactions between glucan and mammalian cells, and despite a vast number of individually described β-glucans, consensus on which glucan is best suited for stimulation of immunity remains lacking.

Most β-glucans are derived from fungi and have a backbone structure of linear β-1,3-linked D-glucose molecules (β1,3-D-glucan) with β-1,6-linked side chains of β1,3-D-glucan of varying sizes occurring at different intervals. However, contradictory data and conclusions exist on the effects of molecular weight, degree of branching, conformation and polymer charge on biological activities. Therefore, the quest for the biologically most active β-glucan continues.

Most of the β-glucans with high activity have been isolated from the Basidiomycetes, and not surprisingly, the two mushroom glucans most often studied, lentinan and schizophyllan, both came from the Basidiomycete family. The comparison of the biological and antitumor properties of these β-glucans can be found in Borchers et al.

Due to the significant differences in activities among various glucans isolated from numerous sources, it is imperative to evaluate the biological properties of any glucan before making suggestions for use in clinical practice. This investigation focused on the biological activities of two individual glucans, MTG and MDF, isolated from Maitake mushrooms (Grifola frondosa).

In the present study we demonstrated that the Maitake-derived glucans are functionally similar not only to lentinan, often considered a standard β-glucan, but also to other glucans. The evaluation of the biological activities started, as usual, by effects on phagocytosis. Using the synthetic microspheres as prey, we took advantage of the unique properties of these microspheres, which have an extremely low negative charge and thus no nonspecific adherence to the cell membrane. First we measured the phagocytosis of peripheral blood cells, and later the peritoneal cells. In both cases, we found a significant increase in the number of phagocytosis monocytes and macrophages after both MDF and MTG. When we compared the dose-dependence of the increased phagocytosis, the surprising result was that MTG was effective even in very small doses (2.6 and 3.7 μg/mouse). More importantly, the oral administration of our samples showed similar stimulation of phagocytosis.

The evaluation of the effects of Maitake glucans on expression of cell surface markers showed that both glucans caused a significant increase in the number of CD4-positive cells after the 24 hr interval. The rest, i.e., the numbers of CD8 and CD19 cells, were unchanged. The data on lentinan did not differ from PBS control, which is in agreement with previously described findings.

In addition to the direct stimulation of cells involved in immune reactions, the immunostimulating effects of natural immunomodulators such as glucans are indirectly caused by potentiation of synthesis and subsequent release of numerous cytokines. Individual glucans significantly differ in their effects upon cytokine synthesis. The only glucan found so far with either in vivo or in vitro stimulation of cytokines, is betafectin.

We found only minor effects of tested glucans on TNF-α production, with only MTG having significant effects. When IL-1β was tested, both glucans showed significant stimulation even 30 minutes after injection, and these effects lasted for the whole tested interval. On the other hand, all samples strongly stimulated the production of IL-2, which is comparable to the earlier finding using yeast-derived glucans. As cytokine production is extremely sensitive to the presence of LPS, contamination with LPS might mask or even overcome the real effects of any immunomodulator. Therefore we used LPS-free samples (depleted by addition of 10 μg/ml of polymixin B) in parallel to the regular glucan samples. In both cases, there were no differences between normal and LPS-free samples. Due to these results, we used only regular samples in all other experiments.

The sporadic data about glucan and apoptosis are confusing. While some authors found induction of apoptosis, others found no effects. Our data using breast, lung and prostate cancer cells showed absolutely no effects of tested samples (data not shown).

The evaluation of the effects of glucan injection on NK cell activity. Using a human K562 cell as a model, we found that in higher E:T ratios, the 4 hr incubation of spleen cells with glucan samples caused significant potentiation of natural killer cells. When compared to lentinan, MTG was effective at lower ratio.

The sporadic data about glucan and apoptosis are confusing. While some authors found induction of apoptosis, others found no effects. Our data using breast, lung and prostate cancer cells showed absolutely no effects of tested samples (data not shown).

Our current paper clearly demonstrates that Maitake-derived glucans act via the same mechanisms as yeast-derived glucans, and that they are, in many cases, even more biologically and immunologically active. When compared to lentinan, Maitake-derived glucans (MDF and MTG in particular) showed a higher stimulation of defense reactions. Another important conclusion is the fact that these glucans do not lose their biological activities when delivered orally. And finally, this report represents a proof that orally-taken Maitake-derived supplements can stimulate the defense systems.
REFERENCES


The Effects of Australian Tea Tree and Jojoba Essential Oils with Minerals for Treatment of Nail Fungus

Leigh Erin Connealy, MD*
South Coast Center for New Medicine
Tustin, California

OVERVIEW

Two case studies were performed over the course of ninety days to assess the effects and time of a natural anti-infective formula** on eliminating severe nail fungus of toes and fingers.

INTRODUCTION

Nail fungus is a common dermatologic disorder affecting more than 30 million Americans.5 It requires proper diagnosis and must be identified by microscopic examination and/or culture before being treated.1,4 Most cases are seen in men and women between the ages of 40 and 65,2,5 Distal subungal onychomycosis (DSO) is by far the most common pattern of the infection.1,3 Initiated by fungal invasion of the distal nail bed, the underside of the nail plate is subsequently infected. The infection spreads proximally and can involve the entire nail unit. An inflammatory response generally occurs within the nail bed, resulting in the accumulation of debris underneath the nail plate (subungal hyperkeratosis) which sometimes leads to the separation of the nail plate from the nail bed (onycholysis).5 Subungal hyperkeratosis and onycholysis are very common findings in DSO. As the nail plate is destroyed, it becomes progressively more discolored, thickened and brittle. Trichophyton rubrum is the most common cause of both DSO and proximal subungal onchomycosis (PSO). PSO has similar pathogenic mechanisms to DSO, except that PSO is initiated through assault at the proximal nail fold in patients with compromised immune systems.

Antimicrobial activities of one natural oil in the formula tested in this study, tea tree oil, obtained from Melaleuca alternifolia has been previously attributable to its hydrocarbon and terpine constituents, including terpinen-40l, a-terpineol and linalool.

TREATMENT

In the two case reports, a topically-used essential oil formulation was developed from a blend of Australian tea tree oil and jojoba essential oils with the minerals copper, chromium oxide green tea, and manganese dioxide. Patients were advised to soak their nails in warm water for five minutes to prepare the nail, then apply several drops of the liquid twice a day to the affected area. They were instructed to gently massage the liquid in, over and around the nail, nail bed and skin to maximize deep penetration. The subjects were observed over the course of ninety days.

* Correspondence:
Leigh Erin Connealy, MD
South Coast Medical Center for New Medicine
14642 Newport Avenue, Suite 200
Tustin, CA 92750
Phone: 714-669-4447    Fax: 714-669-4448
Email: connealymd@earthlink.net

** Formula comprised of Australian tea tree oil, jojoba essential oil, copper, chromium oxide green tea, and manganese dioxide.

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CLINICAL CASE REPORTS
CASE REPORT 1

A 55-year-old man came to the clinic to be treated for his left thumbnail, which was white in color around the nail. He complained of pain and dryness and there was a visible yellow cast to the surface. He explained that application of variousotions and creams had no positive effect. The patient was advised to use test essential oil formula twice per day on the affected areas. After thirty days of treatment, the patient showed significant improvement. In ninety days, the patient’s nail was pink with no abnormal discoloration, and there was no pain in the surrounding area.

**The test formula used in this study (Nature’s Oil for Nails) was acquired from Perfectly Healthy of Santa Ana, California. Leigh Erin Connealy, MD, has a personal financial interest in Perfectly Healthy.**

REFERENCES


CASE REPORT 2

A 47-year-old woman came to the clinic to be treated for nail fungus on her right foot. She complained of discoloration, thickening, brittleness and dryness. There was a visible yellow and brown coloration to her nails. Initial inspection revealed a grossly abnormal large nail on her right big toe. The patient was treated with the test essential oil formula, twice per day on all affected areas. After sixty days, there was marked improvement in pain reduction; after ninety days, the nails were significantly cleared and the fungal spread was arrested.
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