



PolyCell Technologies

Bioactive Solutions for Health and Nutrition

January 31, 2013

Food and Drug Administration
Office of Nutritional Products, Labeling and Dietary Supplements (HFS 800)
5100 Paint Branch Parkway
College Park, MD 20740

Dear Sirs:

DKSH Italia Srl. and PolyCell Technologies LLC submit this health claim petition pursuant to section 403(r)(4) of the Food and Cosmetic Act relative to the relationship between soluble fibers from certain foods and the reduced risk of coronary heart disease. This petition requests that the “Soluble Fiber from Certain Foods and Risk of Coronary Heart Disease Health Claim” (21 CFR 101.81) be expanded to include glucagel barley beta-glucan fiber (GlucagelTM) among the certain foods eligible to bear the claim.

The petition enclosed contains extensive documentation concerning this barley beta-glucan fiber, its characteristics, safety, food applications, scientific documentation of its efficacy and prospective effect upon the availability of soluble fibers to consumers. The petition demonstrates our fulfillment of the health claim requirements set forth in 21 CFR 101.14 to permit a health claim for the relationship between glucagel barley beta-glucan fiber and CHD.

Should the Food and Drug Administration (FDA) grant preliminary approval of this health claim petition, DKSH Italia and PolyCell Technologies also request that FDA grant an “Interim Final Rule” by which products containing GlucagelTM could carry the barley soluble fiber and CHD health claim during the period after FDA preliminary approval and prior to the publication of a Final Rule.

We, Thomas Jorgens President, PolyCell Technologies LLC and Dr. Natale Capri, Managing Director, DKSH Italia Srl do hereby certify that to the best of our knowledge this petition is a representative and balanced submission that includes unfavorable, as well as favorable information that is known by the petitioners to be pertinent to the evaluation of the proposed health claim.

Please do not hesitate to contact PolyCell Technologies or DKSH Italia if further information and/or discussion is required. On behalf of the petitioners we have agreed that PolyCell Technologies will serve as primary correspondent with FDA regarding this petition. PolyCell can be contacted at the address and phone shown below.



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Glucagel Barley Beta-glucan Fiber Heart Disease Health Claim

Petition to Expand the of Barley Soluble Fiber and Coronary Heart Disease Health Claim

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January 31, 2013

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1. Introduction

A. Overview of Proposal

DKSH Italia Srl and PolyCell Technologies LLC submit this health claim petition pursuant to Section 403 (r)(4) of the Food, Drug and Cosmetic Act relative to the relationship between consumption of soluble fibers from certain foods and the reduced risk of coronary heart disease. This petition requests that “Soluble Fiber from Certain Foods and Risk of Coronary Heart Disease Health Claim” (21 CFR 101.81) be expanded to include glucagel barley beta-glucan fiber (Glucagel™), as an additional substance eligible for this health claim based upon significant scientific agreement. This proposal contains human and animal evidence that Glucagel™ barley beta-glucan fiber has demonstrated efficacy in lowering serum cholesterol, and other risk factors for cardiovascular disease.

According to the National Center for Health Statistics, one out of six adult Americans has high blood cholesterol levels. Changes recommended for individuals in this group include reduced intake of saturated fat and cholesterol in the diet, moderate physical activity, and diet enriched with foods containing at least 5 grams to 10 grams of viscous soluble fiber daily, according to the National Cholesterol Education Program – Adult Treatment Panel. Intakes of 10 grams to 25 grams of soluble fiber daily may of additional benefit, according to this Panel. (NCEP, 2002) Glucagel™ barley beta-glucan fiber is a source of soluble fiber.

Soluble fiber is part of overall dietary fiber intake, which averages considerably below recommended levels in the American populace. The National Academies’ Institute of Medicine (IOM) recommends daily intake of 30-38 grams for adult males and 21-25 grams for adult females (IOM 2005). Actual intake of dietary fiber, among adult Americans, ranges from 14 to 19 grams daily (USDA, 1997). Including glucagel barley beta-glucan fiber in foods could contribute to an increase in dietary fiber consumption, as has been recommended by the USDA/DHHS Dietary Guidelines for Americans (2005).

Barley is widely recognized as healthy cereal, however in the U.S. food consumption of barley as food peaked in 1947 at 6.7 pounds per capita annually, and is currently less than 1 pound per capita. (ERS, 2002) Overall much more barley is being consumed in the form of beer, ale and other alcoholic beverages, made from malting barley.

An increasing emphasis on healthy eating, whole grains, and the Barley Health Claim may be changing that trend and may show up as data sets come to reflect current patterns. Glucagel™ barley beta-glucan fiber provides a high purity (75%) form of barley beta-glucan that lends itself to inclusion in a wide array of foods and beverages at rates of 1 gram or more per serving. As such it can contribute to wider use of beneficial barley ingredients in consumer ready applications.

This petition contains evidence that Glucagel™ barley beta-glucan fiber is efficacious in lowering serum cholesterol and related risk factors for developing Cardiovascular Heart

Disease. Evidence is also presented that shows that Glucagel™ is effective in modulating post-prandial blood glucose levels, promoting satiety and helping in weight management. Barley beta-glucan soluble fibers have demonstrated cholesterol efficacy in a number of human studies using cooked, baked or otherwise prepared foods, using high purity isolates (>50% beta-glucan), concentrates (20-30% beta-glucan), and barley flours (<19% beta-glucan).

The petitioners believe that we have fulfilled the health claim requirements set forth in 21 CFR 101.14 to permit a health claim between glucagel barley beta-glucan fiber and CHD. We propose that 3 grams of beta-glucan soluble fiber per day be required as the minimum amount needed to make a significant impact on serum lipid levels, with Glucagel™ providing at least 0.75 grams of beta-glucan soluble fiber per serving in foods or beverages, (along with a diet low in saturated fat and cholesterol) that are eligible to bear the claim.

B. Background

The Food and Drug Administration approved a Health Claim in 1997 on the association of soluble fiber from whole oats and certain oat materials and reducing the risks of CHD. (FDA, 21 CFR 101.81, 1997). Two primary findings were: 1) that a dose response relationship was evident between the level of beta-glucan consumed and reducing blood cholesterol (TC) and LDL cholesterol. 2) That intake of 3 or more grams daily of beta-glucan was effective in lowering serum lipids.

FDA acknowledged at that time that there is evidence that consumption of beta-glucan soluble fiber from a variety of food sources may help to lower serum cholesterol levels. However, it decided to limit eligibility to bear a claim to rolled oats, oat bran, and whole oat flour for which it had specifically reviewed the evidence. FDA adopted an interim final rule that was structured so that it could be amended to include claims for other sources and types of soluble fibers and the risk of CHD. Amendments have been considered based on the evidence and several additions have been made.

A petition from the Kellogg Company in 1998 sought authorization for including psyllium seed husk, and an amendment (to 21 CFR 101.81) to include psyllium was Approved. In 2002, the Quaker Oats Company and Rhodia, Inc. submitted a petition for seeking an amendment for a category of "Oatrim", described by the manufacturer as an acid base and enzymatic hydrolysis produced beta-glucan concentrate. An amendment for this category of Oatrim was granted to 21 CFR 101.81 (FDA, 21 CFR 101.81, 2002).

The National Barley Foods Council submitted a petition to include barley beta-glucan soluble fiber as a source associated with reduced risk pf CHD. In late 2005, FDA published an interim final rule to add barley as an additional source of soluble beta-glucan fiber. (FDA, 21 CFR 101.81(c)(2)(ii)(A)(5), 2005). This amendment included both whole grain and dry milled barley products as sources of soluble fiber. The FDA concurred that the totality of scientific evidence, in addition to oat products, these barley products are appropriate sources of beta-glucan soluble fiber for the CHD health claim.

In 2006, a petition was filed by Cargill, Inc. seeking the inclusion of barley betafiber, a purified material, as an eligible source of barley beta-glucan fiber under the health claim. Barley betafiber is described as an ethanol precipitated soluble fraction of cellulose and enzyme hydrolyzed high purity barley beta-glucan concentrate, derived from whole grain barley. Barley betafiber has a content of at least 70% beta-glucan soluble fiber by dry weight basis. 21 CFR 101.81 was amended in 2008 to include barley betafiber as a source of beta-glucan soluble fiber under the CHD health claim (FDA, 21 CFR 101.81(c)(2)(ii)(A), 2008).

Health claims have also been issued for barley beta-glucan in the European Union under European Community (EC) Regulation 1924/2006, specifically under Articles 13.1 and 14.1 under the jurisdiction of the European Food Safety Authority (EFSA). An Article 13.1 Health Claim for barley beta-glucan for maintaining healthy LDL Cholesterol was reviewed and approved by an EFSA Scientific Panel in 2009 and later adopted by the European Commission. An Article 14 Health Claim for the reduction of blood cholesterol and reduced risk of coronary heart disease was reviewed and approved by an EFSA Scientific Panel in 2011 and formally adopted by the European Commission in 2012 (See Appendix C). Requirements include at least 3 grams of barley beta-glucan daily and at least 1 gram of barley beta-glucan per serving. Glucagel™ barley beta-glucan soluble fiber is covered under both of the EFSA Health Claims.

2. Meeting the Preliminary Requirements

A. Glucagel™ Barley β -glucan Fiber is Safe and Lawful - 21 CFR 101.14(b)(3)ii

Glucagel™ barley β -glucan soluble fiber is the food ingredient that is the object of this health claim petition. An independent panel of experts qualified in evaluating the safety of food and food ingredients concluded that glucagel barley β -glucan soluble fiber is Generally Recognized as Safe (GRAS), for use at levels of up to 3 grams of β -glucan per serving. (see Appendix A). In reaching this conclusion the expert panel reviewed evidence from toxicology studies, animal studies, and human clinical studies, history of safe use, safety of manufacturing and raw materials and other relevant evidence. Toxicology studies have been conducted in animals with a variety of barley beta-glucan soluble fiber containing materials, including preparations with both high and low molecular weight profiles, including Glucagel™ barley beta-glucan soluble fiber and very similar materials. Tests with beta-glucan constituting up to 10% of the diet (8g/kg bw) was the No-Observed-Adverse-Effect-Level.

Uses of Glucagel™ barley β -glucan soluble fiber are as a fiber supplement, a nutritional supplement, a humectant, a texturizing agent, and/or a fat replacer, consistent with current Good Manufacturing Practice (cGMP). (GRAS document – see Appendix A)

Glucagel™ barley β -glucan soluble fiber is made from whole grain barley, a GRAS substance. Barley produced in North America, typically of a waxy, hulless variety, is used for the production of Glucagel. Barley is milled and then dry concentrated prior to being separated in a process that uses water, along with thermal and mechanical forces to concentrate the β -glucan soluble fiber. Once separated and purified to 75% or more β -glucan soluble fiber it is dried and milled prior to packaging for shipment.

Barley is a traditional food with a very long history of safe use. β -glucan soluble fiber found in both wild and domesticated varieties of barley has been widely consumed by humans, in the form of foods and beverages, for more than 10,000 years (Newman and Newman, 2008). Barley was the major staple in the diet of Greek civilization (about 800 BCE). And later, barley provided the staple food of the Roman gladiators, who were known as *hordearii*, because of the belief that their high barley (*hordeum*) diet was the source of their physical strength.

In Northern Europe, Rieska, unleavened barley bread, was the earliest bread in Finland. Bread made from barley, and ‘small beer’, a low-alcohol product from fermented malt, formed the staple diet of the common people in medieval England, and as late as the beginning of the 20th century it was the main food in rural Denmark (Munck 1981). However, since the 18th century higher yielding barley has been progressively replaced by wheat as a food staple in Western cultures. This is due to wheat’s elastic proteins,

superior baking and milling characteristics, taste and tradition, as well as its decreasing cost due to increasing yields through improved breeding (MacGregor, 1993).

Today barley is used for food in greatest quantities in geographic areas where other cereals grow poorly due to altitude, latitude, low rainfall or soil salinity (Nilan & Ullrich 1993). It is a common food source in semi-arid regions of North Africa, the Near and Middle East, Russia, the former Soviet Republics in Central Asia, Mongolia, China, Korea, India and Afghanistan. Not surprisingly, the greatest per capita consumption today is found in those regions. According to FAO reports, the world's highest contemporary per capita annual barley consumption for food was recorded in Morocco (1988) (FAO STATS 1990) at 68.2 kg (150 pounds) per person, and in Estonia at 74.3 kg (163 pounds) per person in 2000 (FAO STATS 2002).

Estimated beta-glucan consumption levels for historical and contemporary consumers based upon available data for barley (as well as oat and wheat) consumption is shown in Table 1. This suggests that even levels up to 10 times consumption levels associated with current suggested intake levels for health benefits have a history of safe use.

Table 1 - Per capita historic and current levels of β-glucan consumption

Estimated from available historical and contemporary data as follows:

Location and Time	β-glucan Source Cereal grain	Total grams Consumed per day 300 days per year*	β-glucan grams consumed daily per capita
Greek and Roman Soldiers BC (Macgregor)	Barley	550-770 g	25 – 35g
Denmark – 1900 (Munck)	Barley	274 g	15 g
Morocco – 1988 (FAO)	Barley	222 g	10 g
Estonia– 2000 (FAO)	Barley	248 g	11 g
Algeria– 1988 (FAO)	Barley	51 g	2 g
USA – 1947*** Cereals with (USDA) β-glucan	Barley Oats Wheat	42 g 15 g 220 g	2 g 1 g 2 g
USA – 2000*** Cereals with (USDA) β-glucan	Barley Oats Wheat	10 g 11 g 227 g	.5 g .5 g 2 g

* Total consumption in grams by β-glucan content of 4.5% in barley, 4% in oats and 1% in wheat

*** Current exposure at the 95th percentile is calculated from the USDA consumption data in Table 6. For children maximum consumption at the 95th percentile is 260% of the mean consumption, and for adults maximum consumption is 208% of the mean. This suggests that US exposure at the 95th percentile of β-glucan for 2000 would occur in a range from 6.2 to 7.8 grams per day. For 1947, it is estimated that 95th percentile β-glucan consumption may have been 10.4 to 13 grams per day.

B Glucagel™ Barley β-glucan Fiber is a Food Ingredient that Provides Nutritive Value and Technical Functionality – 21 CFR 101.14(b)(3)(i)

Glucagel™ barley β-glucan fiber is a highly concentrated source of beta-glucan soluble fiber. It adds nutritive value, healthy benefits and technical function to American diets that are typically underrepresented in fiber content, and particularly soluble fiber content. In addition to fiber, it adds small amounts of protein and other carbohydrates that contribute to a modest caloric content.

Glucagel™ barley β-glucan fiber is 75% dietary fiber as measured by the Official AOAC Method for Dietary Fiber - AOAC 991.43.

Functional benefits of this material are as a fat replacement (e.g. dairy products, dressings), hydrocolloid (e.g. baked goods), thickening agent (e.g. puddings, soups), texturizing agent, and as a humectant.

Nutrient content of Glucagel™ Barley β-glucan Fiber is shown in Table 2 below.

Table 2. Macronutrient Content of Glucagel™ Barley β-glucan Fiber

Macronutrients	In 100 grams of Glucagel™*
Total carbohydrate	89.65
Non-β-glucan carbohydrate	10.50
Beta-glucan	75.03
Protein	4.55
Lipid	1.34
Ash	1.61
Moisture	2.69

* From an average of analysis from six production runs.

C Glucagel™ Barley β-glucan Fiber is Associated with Reduced Risk of Coronary Heart Disease – 21 CFR 101.14(b)(1)

The leading cause of death and disability in the U.S. population is coronary heart disease, taking the lives of 2,200 Americans each day. The American Heart Association (AHA) indicates that 750,000 people have a first heart attack annually, another 470,000 have a subsequent attack and 795,000 people have a stroke annually.

Elevated total cholesterol levels in excess of 200 mg/dL are used by physicians as key biomarker indicating an increased risk of developing heart disease. AHA Statistics further show that 41.8%, 37.0% and 50.1% of white, black and hispanic males, and 47.0%, 41.2% and 46.5% of white, black and hispanic females respectively have Total Cholesterol levels above 200 mg/dL¹⁸. Those statistics underscore that a substantial portion of the U.S. population is at risk for future heart disease and strokes.

The National Cholesterol Education Program recommends several methods of intervention to reduce excessive cholesterol levels in individuals. The first stage includes reducing saturated fat intake, dietary changes and moderate physical exercise. The second stage includes increased consumption of soluble fibers (up to 10 grams daily), such as beta-glucan from oat or barley, which can lower LDL cholesterol by 5% or more. (NCEP, 2002).

Glucagel™ barley β-glucan fiber is a source of barley β-glucan fiber, which has shown efficacy in lowering cholesterol and other risk factors. At least 3 grams of beta-glucan must be consumed daily to achieve beneficial effect. Consumption of this substance is associated with reducing risks of developing Coronary Heart Disease, a serious health concern in the American population.

3. Summary of Scientific Evidence

A. Overview of the Scientific Data

A large and growing body of scientific evidence has substantiated the clinical efficacy of barley beta-glucan soluble fiber in modifying serum blood lipids and reducing risk factors for heart disease. A scientific consensus underscores Heart Health Claims for barley in the United States, the European Union, Canada and a number of other countries. In addition, there is significant evidence supporting the efficacy of barley beta-glucan on lowering blood pressure, modulating blood glucose, on satiety, digestive health and other human health parameters.

A review of the scientific literature on barley beta-glucan was conducted with particular emphasis on studies following on the Soluble Fiber Health Claim Petition by the National Barley Foods Council (approved by FDA in 2005), and on the Health Claim Petition by Cargill, Inc (approved by FDA in 2008). Two Meta-Analyses have recently been published by AbuMWeiss et.al (2010), and Talati et.al. (2009) focusing on barley beta-glucan studies for cholesterol reduction and glucose modulation. Both research groups included studies using barley beta-glucan in both background and extracted forms, and delivered in both minimally processed and prepared food items, (cooked or baked).

In a 2010 European Journal of Clinical Nutrition article - β -glucan from barley and its lipid lowering capacity: a meta-analysis of randomized controlled trials, SS. AbuMweiss, S. Jew and N.P. Ames (2010) identified a total of 266 studies of barley beta-glucan (as of July of 2008) of which they selected 11 studies meeting a series of quality parameters for inclusion in the meta-analysis.

The 11 studies were selected based on trial design, control group, subject inclusion criteria, food exposure, power, duration, health outcomes measures, and randomization. In all, the 11 studies showed a decrease in mean TC (total cholesterol) by 0.30 mmol/ (95% Confidence Interval) (CI) and in mean LDL (low density cholesterol) by 0.27 mmol/l (95% CI) when compared to control. (AbuMweiss, 2010). All studies showed beneficial effects on total cholesterol and LDL cholesterol, although not all studies reached the threshold of significant CI. HDL and Triglyceride effects were not significant.

Barley beta-glucan intake levels used in these studies varied from 3 grams to as much as 12.9 grams per day. Studies with higher doses of beta-glucan (>7 grams per day) and those with lower doses (3-5 grams per day) tended to perform about equally well – 0.25 mmol/l and – 0.28 mmol/l reductions in TC respectively. The best results were evident with medium doses (ranging from 5-7 grams per day) with a mean reduction in TC of – 0.45 mmol/l. A similar pattern was shown with LDL cholesterol – reductions of -0.22 mmol/l (3-5 grams daily), -0.33 mmol/l (5-7 grams daily) and – 0.24 mmol/l (>7 grams

daily). (AbuMweiss, 2010). This suggests that higher doses may be subject to diminishing returns in further effect on blood lipids.

Cumulatively these 11 studies involved 333 adult male (4 all male studies) and female subjects (1 was an all female study) and 6 mixed gender. The preponderance of studies were focused on older adults with mean ages above 40 years (8), with 6 having mean ages over 45 years. 1 had a mean age of 38.8 years, including some subjects in their 20's, and was conducted with college age females (mean of 20.4 years) and one of the selected studies did not report mean age. (AbuMweiss, 2010).

TABLE 3
Meta-Analyses of Barley Soluble Fiber Studies
Summary of Mean Results on Lipid Parameters

Author Journal	Studies No. Subjects No.	Total Chol	LDL Chol	HDL Chol	Triglycerides
AbuMweiss EJCN 2010	11 studies 333 subjects	- 0.30 mmol/l	- 0.27 mmol/l	0.00 mmol/l	- 0.05 mmol/l
Talati Annals FM 2009	8 studies 391 subjects	- 13.38 mg/dl	- 10.2 mg/dl	+ 0.99 mg/dl	- 11.83 mg/dl

Sources: AbuMweiss, 2010 and Talati, 2009

Another Meta-analysis appeared in 2009 in The Annals of Family Medicine, entitled, *The effects of barley derived soluble fiber on serum lipids*. Talati, (2009) conducted an analysis of 8 trials using barley beta-glucan rich soluble fibers. The selected trials were randomized controlled trials of 4 to 12 weeks in duration involving a total of 391 subjects. Daily dose rates ranged from a low of 3 grams to a high of 10 grams of barley beta-glucan.

In aggregate they reported that barley soluble fiber lowered total cholesterol (TC) by 13.38 mg/dl (95% CI), lowered LDL cholesterol by 10.02 mg/dl, and triglycerides by 11.83 mg/dl (95% CI), while showing no significant effect on high density cholesterol. (Talati, 2009)

The findings of these two most recent analyses are consistent with and supportive of evidence presented by the National Barley Foods Council (NBFC, 2003) and by Cargill, Inc. (Cargill, 2006) in their respective Health Claim Petitions.

B. Animal Studies

Barley beta-glucan studies assessing the effects of these soluble fibers on blood lipids and other parameters have been conducted using an array of animal trials over the past 4 decades (35 studies identified). Animals that have been used in trials have included chickens, geese, mice, rats, hamsters and pigs. Both background (23 studies) and extracted (12 studies) beta-glucan materials have been trialed in relation to effects on Total, LDL, and HDL Cholesterol and Triglycerides. Beta-glucan delivery been accomplished with prepared rations in most instances and in background form in some instances, with controls being absent beta-glucan. Controls using cellulose, corn, or wheat bran have been sources of fibers in many studies that included an insoluble fiber source.

Two animal studies have been conducted using water extracted barley beta-glucan fiber produced by the glucagel procedure (aqueous - thermo-mechanical) along with ten others that using highly concentrated barley beta-glucan made using other extraction methods.

The first study was conducted by Jonker, et. al. (2010) of TNO Netherlands using 6 week old specific pathogen free, mixed sex Wistar rats (no. 40) in a 28 day feeding trial. Rats were randomly assigned to 4 groups of 5 males and 4 groups of 5 females each using a control with 0% (potato starch) and test groups consuming rations containing 1%, 5% or 10% of barley beta-glucan fiber. Parameters measured included cholesterol, triglycerides, and phospholipids as well as an array of other blood, physiological and toxicological measurers. Significant decreases in total cholesterol were reported overall with 1% and 10% diets barley beta-glucan fiber, but not with the 5% diet. High dose 10% diet) males showed the most significant reduction in total cholesterol (-12%) compared to the control. Triglycerides were also reduced in male rats consuming 5% (-7%) and 10% (-7%) diets, and in female rats consuming the 10% (-30%) diet, and were lowered overall at the 10% diet level by 19%. No supplemental source of lipids (oil) was used. Phospholipids were lowered overall with the 10% (-6.8%) diet and in males on the 1% (-7%), and the 10% (-12%), as well as females on the 5% (-8%) and 10% (-13%) diets respectively. (Jonker, et. al. 2010, pg 426)

The second study was by de Gueverra, et. al (2000) using 36 – 4 week old Sprague Dawley rats allocated to a 2 x 2 factorial arrangement of treatments. A control diet based on cornstarch and casein was used. Flax oil or coconut oil was used as an oil source and paired with or without beta-glucan added at 10% of the diet. Total blood cholesterol levels were reduced by 10.3% (1.58 mmol/L) compared to control (1.76 mmol/L). No correlation was found between the type of oil added to the diet and barley beta-glucan (BG). Triglyceride (TG) levels were significantly influenced by the type of oil in the diet. BG in combination with coconut oil decreased TG levels by 40% in the trial, while BG in combination with flax oil decreased TG levels by 13%. (de Gueverra, et al, 2000) The authors suggest that the presence of gelling beta-glucan in the digestive tract may delay and/or decrease absorption of lipids.

A study by Delaney, et.al. (2003a) was conducted with barley betafiber (Cargill, Inc) with a comparable protocol to the previously referenced Jonker (2010). Betafiber is a barley beta-glucan extract with 64% beta-glucan. (Delaney, page 478). The study was a 28 day feeding study with 6 week old Wistar rats divided into 4 groups of 5 rats per sex and placed on diets containing 0%, 0.7%. 3.5% and 7% β -glucan. No supplemental source of lipids (oil) was included in the diet. Parameters measured included cholesterol, triglycerides, and phospholipids as well as an array of other blood, physiological and toxicological measurers. Total cholesterol levels were not significantly lowered compared to control, except for males at the 0.7% BG diet level (13%) lower. TG levels were reduced for both males and females at the 0.7% and 7% BG diet level, and phospholipids were lowered for males and females at the 0.7% BG and 7% BG diet levels. Delaney 2003a. (p. 485).

Eleven comparable studies of blood lipid parameters using various barley β -glucan extracts are shown in Table 4. Most of these studies were conducted using rats (9) and many include supplementation of lipids in the diet in order to accentuate cholesterol effects. Two studies used hamsters, which are often considered to be a more ideal animal model for lipid studies. While these studies vary considerably in dosage levels of β -glucan, they show a common pattern of total cholesterol (range of -7.5 to -36%), LDL cholesterol (range of -20% to -49%) and other blood lipid responses to barley beta-glucan fiber as compared to controls. The exception is HDL where no consistent pattern is evident in those instances where HDL was measured. Triglyceride (TG) reductions (range of -13% to -58%) were noted in the 3 of the 4 studies where that parameter was reported. Three of the studies in Table 4 also contain comparatives to oat bran extracts and show consistent patterns between oat and barley materials in lowering blood lipids.

A further group of fifteen comparable studies of blood lipid parameters using various barley products with background or enhanced levels of β -glucan (not extracts) are shown in Table 5. Again most of these studies were conducted with rats (9), the most readily available animal laboratory model. Five hamster studies are included in this group and one study used pigs. Three of these studies included oat or other cereal products as comparatives. Most of these 15 studies reported reductions in total cholesterol (range of - 13.9% to 55%), and/or in LDL cholesterol (range of -22% to -80%). A comparable pattern in cholesterol reductions was apparent in studies that contained oat and psyllium in addition to barley. There was no consistent pattern of effects across the 6 studies that reported HDL measures. The five studies reporting TG results all showed significant reductions in TG with barley β -glucan in the diet.

Comparing the studies shown in Table 4 (barley β -glucan extracts) with the studies in Table 5 (barley products with β -glucan), there does not appear to be any significant differences between results based on whether the β -glucan containing test material used was an extract or a background material. Also tests conducted using barley β -glucan fiber produced by aqueous extraction were consistent with results from trials using barley beta-glucan fibers produced by other methods, as well as with typical barley products containing β -glucan.

Table 4: Animal Response on Lipids when Consuming Barley Beta-glucan Fiber Extracts

Reference	Animal Type	Source of β -glucan	Number	Days	Diets Used	Control	β -glucan	Total Cholesterol	LDL	HDL	TG	Phospholipids	Lipid added	Notes
Jonker, et.al. 2010	rat	barley β -glucan extract	40	28	barley BG fiber (GL) potato starch	0.75%, 3.75%, 7.5% ns, ns, - 7.5%, *	n.a	n.a	ns, ns, -18.5%	ns, ns, -12.5%	none	*males	TC -12% (7.5%)	
Wilson, et.al., 2004	hamster	betafiber - barley extract	39	42	betafiber LMW barley fiber HMW	cellulose	8% 8%	-36% -32%	-49% -43%	ns ns	-58% -38%	n.a. n.a.	0.15% 0.15%	
Delaney, et. al. 2003b	hamster	betafiber - barley extract	70	63	oat extract barley betafiber	cellulose	2%, 4%, 8% 2%, 4%, 8%	ns, -17%, -26% ns, -14%, -27%	ns, 26%, -41% ns, -20%, -30%	+22%, ns, -15% ns, ns, -19%	ns, ns, ns ns, ns, ns	n.a. n.a.	0.15% chol coconut oil	
Delaney, et.al, 2003a	rat	betafiber - barley extract	40	28	barley betafiber	potato starch	0.7%, 3.5%, 7%	-13%, ns, ns	n.a	n.a.	-16%, ns, -20%	-13%, ns, ns	none	
Yang, et. al. 2002	rat	barley β -glucan extract	42	14	barley BG fiber	no fiber	2.50%	- significant	- significant	n.a.	n.a.	n.a.	0.50%	
de Gueverra, et. al, 2000	rat	barley β -glucan extract	36	28	barley BG fiber	cornstarch	8%	-10%	n.a	n.a	- 40% ^A , -13% ^B	n.a.	coconut, flax oil ^A coconut oil ^B flax oil	
Hecker, et. al. 1998	rat	barley β -glucan extract	20	25	barley BG fiber	cellulose	7.8% SDF	ns	-40%	ns	n.a.	n.a.	1%	
Oda, et. al. 1993	rat	barley β -glucan extract oat β -glucan extract	28	9	barley BG fiber oat extract	cellulose 1.9% SDF	2.8% SDF ns	ns -27%	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	1% 1%	
Oakenfell, et. al. 1991	rat	barley β -glucan extract	40	21	barley BG fiber	cellulose	1%, 2%, 3%, 4%	n.a.	ns, ns, -20%, -20%	n.a.	n.a.	n.a.	1%	
Thomas, et. al., 1990	rat	barley NDF extract	24	75	barley BG fiber	no fiber	30% NDF	-26%	-42%	n.a.	n.a.	n.a.	2%	
Klopfenstein, et.al, 1987	rat	barley β -glucan extract	60	35	barley extract oat bran extract oat bran extract	white bread	4.80% 4.80% 8.90%	-18% ns -15%	n.a. n.a. n.a.	19% ns ns	n.a.	n.a.	5%	

Table 5: Animal Response on Lipids when Consuming Barley Beta-glucan Fiber

Reference	Animal Type	Source of β-glucan	Number	Days	Diets Used	Control	β-glucan	Total Cholesterol	LDL	HDL	TG	Phospholipids	Lipid added	Notes
Son, et.al, 2008	rat	barley β-glucan extract	40	28	waxy barley	rice	9.6% DF	-18%	-41%	32%	n.a.	n.a.	1%	
Sohn, et.al., 2007	rat	glutinous barley	30	60	barley flour	rice	8% BG	-22%	n.a.	ns	n.a.	n.a.	0% chol	w/w fat diet
Bird, et. al.	pig	Himalaya barley	21	21	wheat bran		8% BG	ns	ns	n.a.	-15, ns, -15	-15**, ns, ns	none	
		hulless barley wholemeal					8% BG	-16%	-22%	ns	n.a.	n.a.	none	
Yang, et. al. 2003	rat	refined barley BG	21	14	no fiber		2.50%	-14%	ns	ns	n.a.	n.a.	0.50%	
		waxy barley			no fiber			-19%	-24%	ns	n.a.	n.a.		
Li, 2003	rat	barley	40	9m	barley vs rice	no fiber	0.7 g per day	-17%	n.a	n.a	n.a.	n.a.	n.a.	
					barley vs cornstarch			-29%	n.a	n.a	n.a.	n.a.	n.a.	
Shao, et.al, 2002	hamster	barley	20	25	barley + HF	cellulose+HF	7.70%	-55%	-80%	ns	n.a.	n.a.	butter	
		psyllium			psyllium +HF		7.7% TDF	-55%	-80%	-47%	n.a.	n.a.		
		HPMC			HPMC + HF		7.7% TDF	ns	ns	n.a.	n.a.	n.a.		
Knuckles, et. al., 2000	hamster	seived barley	55	21	stanuwax hulless	cellulose	3.40%	ns	-57%	ns	n.a.	n.a.	0.5%	
					cholestyramine .05%			-42%	-69%	ns	n.a.	n.a.	2%	
								-49%	-60%	ns	n.a.	n.a.	0.50%	
								-19%	-20%	ns	n.a.	n.a.	2%	
Kalra, et. al, 2000	rat	barley	24	40	hulless barley DM	cellulose	4.50%	-39	-61	34%	n.a.	n.a.	none	
					hulless barley DL		3.40%	-24%	ns	ns	n.a.	n.a.		
					hulled barley BM		1.70%	ns	NS	ns	n.a.	n.a.		
Ranhotra, et. al., 1998	hamster	barley CDC Candle	40	28	barley	no fiber	2.0%	-16%	ns	-18%	n.a.	n.a.	0%	
							3.9%	-20%	-24%	-21%	n.a.	n.a.		
							5.9%	-21%	ns	-21%	n.a.	n.a.		

Table 5 Animal Studies Continued

Reference	Animal Type	Source of β-glucan	Number	Days	Diets Used	Control	β-glucan	Total Cholesterol	LDL	HDL	TG	Phospholipids	Lipid added	Notes
Danielson, et.al., 1997	rat	barley β-glucan extract	40	21	barley shorts	cellulose	2.30% 4.60% 6.90%	ns ns ns	-49% -54% -69%	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a n.a	0.5%	
German, et. al., 1996	hamster	seived barley	32	21	barley + olive oil barley + fish oil	cellulose	4.9% -24%	ns -22%	16% -22%	-12% -18%	n.a. n.a.	n.a. n.a.	0% chol	
Jackson, et.al., 1994	rat	oat bran barley malted barley	24	14	wheat bran	cellulose	2.70% 2.10% 0.30%	-36% -32% -30%	-36% -36% -49%	-38% -27% ns	n.a. n.a. n.a.	n.a. n.a. n.a.	0.50%	
Oda, et. al. 1994	rat	barley gum	40	14	oat gum barley gun psyllium ain76	cellulose	1.30% 1.30% 2% 2%	-17% -20% -48% ns	n.a. n.a. n.a. n.a.	n.a. n.a. n.a. n	n.a. n.a. n.a. n.a.	cocunut oil		
Kahlon et. al. 1993	hamster	whole barley	70	21	oat bran whole barley rice bran ge barley ge barley ge barley	cellulose	5% 3.30% 0.80% 3.30% 4.30% 6.00%	-13.9% ns ns ns ns -14.6%	-30.30% ns ns ns ns -23.70%	-10% n.a. n.a. ns -13% -15%	n.a. n.a. n.a. n.a. n.a. n.a.	n.a. n.a. n.a. n.a. n.a. n.a.		
McIntosh, et.al. 1993	rat	pearled barley	30	180	50% pearl barley 5% pearl barley	cellulose	2% SDF 0.3% SDF	ns ns	n.a. n.a.	n.a. n.a.	n.a. n.a.	n.a. n.a.	Lard 10%	

C. Human Studies

1. Lipids:

Barley beta-glucan studies assessing the effects of these soluble fibers on blood lipids and other parameters have been conducted with human subjects over the past 40 years. It is now well established that soluble β -glucan fibers in barley and oats can have a beneficial effect on reducing key risk factors for coronary heart disease. In addition, growing clinical evidence supporting beneficial effects on blood glucose metabolism, satiety and weight control, and digestive health has been accumulated.

A summary of 14 studies assessing effects of barley β -glucan on blood lipid parameters is presented in Table 6. Each of these studies was conducted using adult subjects (range 10-155 subjects) running from 4 weeks to 17 weeks in length. Barley β -glucan used in these trials ranged from basic forms (eg. flour or flakes), to concentrated forms with elevated β -glucan, and more highly concentrated extracts. A broad spectrum of delivery methods (foods, beverages), dosage levels and potentially various molecular weight profiles occur across the selected studies.

Of the 14 studies in table 6, five are most directly correlated to the barley beta-glucan fibers in GlucagelTM. Four of these trials were conducted with glucagel or the source barley material used to produce it. And one, the Keenan Study (Keenan 2007) was conducted using a nearly identical extracted barley betafiber that has been recognized by FDA with a Health Claim.

A study at the University of Minnesota (Smith, 2008, Smith 2006) involved 90 adult subjects using two different molecular weights of barley beta-glucan (62 kD to 139 kD) consumed as beverages. The cardiovascular metrics were similar for the two molecular weights but differences were seen between older and younger cohorts. Trial subjects consuming the higher molecular weight (HMW) project lost weight during the study, and reported increased satiety. Cholesterol reductions were reported at 6 weeks for an older cohort of subjects (51 years and older) with a -.20 mmol/l reduction in total cholesterol and at -.27 mmol/l drop in LDL consuming the low molecular weight (LMW) beta-glucan diet. This was not shown in that group for the HMW diet. In contrast, the younger cohort of subjects (under 51 years) showed a decrease in total (-.21 mmol/l) and in LDL cholesterol (-.28 mmol/l) at 3 weeks while consuming the HMW beta-glucan diet. Weight loss and increased satiety was statistically significant in the HMW group.

A study at Auckland University (NZ) with live-in group of 18 men was conducted using an early prototype of barley beta-glucan fiber made by the glucagel process (Keogh, et.al., 2003). This trial was designed and carried out in a live-in metabolic ward in an Auckland hospital and required subjects to commit to a 12 week live-in stay with meals provided, which presented recruitment issues and narrowed the field of prospective subjects. An outside review by Dr. Paul Nestel, a very experienced Australian research scientist, had recommended that the subject pool be: over age 45, with BMI over 25, and LDL

cholesterol greater than 4 mmol/l. The selected subjects were younger, of lower BMI and of lower LDL on average than the recommended subject profile.

Keogh (2003) studied 18 male volunteers in a metabolic ward trial. The study was designed to be a randomized crossover study on cardiovascular risk factors in mildly hypercholesterolemic men with Glucagel™. All foods were prepared and the caloric intake was 38% fat, with beta-glucan in the test diet at a high level of 10%. No subjects withdrew or were excluded for non compliance after the start, but the recruitment goal was reduced from 23 to 18, minimum age was reduced, as were cholesterol levels and BMIs compared to those considered to be ideal in a trial designed to demonstrate the full power of the fiber as a hypocholesterolemic agent.

A significant divergence was found in the effects of beta-glucan fiber in this trial by age, as well as BMI and base cholesterol levels. A significant effect was found in subject cohort over the age of 31 years with an 11% reduction in total cholesterol, while the overall reduction was non-significant due to the adverse selection of younger, less hypercholesterolemic, and lower BMI subjects (this sub group experienced a 14% rise in total cholesterol) admitted to complete recruitment. Despite this the entire group showed an 8% reduction in TC and a 9% reduction in LDL levels with the beta-glucan diet at 3 weeks, which did not carry through to the Week 4 conclusion when LDL cholesterol was reduced by 3.8%. There was also an unexplained rise in the cholesterol of the placebo group proportional to subject age that was not repeated in the Glucagel™ group suggesting for this group of individuals, the metabolic ward structure and the placebo diet, may have contributed to this rise, while the diet of the individuals in the Glucagel™ arm may have protected against this rise. Overall despite its difficulties, this trial does provide evidence of beneficial effects particularly on the subject cohort that has been most widely recognized by experienced researchers as the most appropriate set of test subjects.

A trial by (Rondanelli 2011) was conducted by a very experienced team of researchers from the Universities of Pavia and Milan (Italy) Medical School involving 24 men used Barley Balance® a barley beta-glucan concentrate that is the raw material for making Glucagel™. The subjects consumed pasta, soups, rice cakes etc. with or without barley beta-glucan fibers (up to 1.7 g/100g). Overall a 5% reduction occurred in total cholesterol and an 8.9% reduction occurred in LDL cholesterol. A 7% reduction also was noted in the LDL/HDL ratio. In this study a particularly significant effect on satiety was also observed in this group of subjects whose mean age was 50 years.

A Japanese study by Shimizu (2008) with 44 men aged from 30 to 60 years used pearled Fibar barley in prepared foods with rice controls (7 g/day). Fibar barley is the resource material used to make the barley beta-glucan fiber Glucagel™. The study showed significant effects on total and LDL cholesterol, as well as on reducing visceral body fat. Total cholesterol was reduced by 8% and LDL cholesterol was reduced by 7% in this trial. Visceral body fat and waist circumference were also significantly reduced further supporting the findings of other studies, including Smith (2008) and Pins (2000), who have reported significant effects on weight as well as satiety.

Keenan (2007) present the results of a study of 155 subjects (mean age 55 yrs) in a study of two molecular weights of beta-glucan dosed at 5g/day with a 15% reduction in LDL cholesterol after 6 weeks, the low molecular weight produced a 13% reduction in the same study. The subject groups consumed 3 g / day had similar results – so both the high and low (1,000 kD down to 50-400 kD) molecular weights produced lower LDL and lower total cholesterol. The differences between these two groups were not statistically significant. Significant reductions were also shown for triglycerides and for the LDL/HDL ratio when using this material.

This lower molecular weight beta-glucan used by Keenan was prepared by an ethanol/water extract for a dry milled barley beta-glucan. In contrast, Glucagel™ is prepared by water extraction of dry milled barley beta-glucan and is essentially the same molecular weight as the low molecular weight product used in the study. While the results for the two molecular weights were not identical, they were similar, with similar effects of serum cholesterol. The low molecular weight form performed like the high molecular weight form.

Another pair of studies of special note were conducted by a well known USDA researcher Kay Behall and colleagues (2004a, 2004b). Behall's study of males showed a 10.8% reduction in total cholesterol (TC), and a 14.6% reduction in LDL cholesterol at 3 grams of beta-glucan daily, and a 16.6% reduction in TC and 23.8% reduction in LDL cholesterol with a diet of 6 grams/day of β-glucan. A second study with a group of males, pre-menopausal females and post-menopausal females indicated similar results for males and post-menopausal females (-11% TC, -14% LDL), and somewhat lesser effects with pre-menopausal females (-7% TC, -14% LDL).

Comparing the results of Keogh, Behall, and Keenan, the one study that did not produce as strong a physiological effect as expected – the Keogh study - was different primarily in the age, weight, and BMI of the test subjects. For example, mean age in the Behall studies were almost 10 years older than the mean age of Keogh subjects, while the average age of subjects in the Keenan study was nearly 20 years older than Keogh's group. Keogh also had a small sample size, which may magnify anomalies. In addition, the Keogh study used a relatively high β-glucan dosage level at 10 grams per day, compared to 3g and 6g for Behall, and 3g and 5 g for Keenan. Dr. Behall said she chose 3 grams/day and 6 grams/day as the most effective range from her long clinical experience, suggested that a point of diminishing returns on lipid parameters is likely at higher rates (personal comm.).

The two extracted products are physically very similar, and should produce very comparable results. The primary difference being that the one product has added enzymes, and is extracted with an organic solvent (food grade ethanol), and the other with water and a thermo-mechanical freeze-thaw process. Given the chemical similarities and the similarities of the processes, both of these products might be termed “beta-fiber” as they

are the same size, made from barley beta-glucan through similar processes, and behave the same way.

Additional studies included in Table 6 provide further evidence of the efficacy of barley beta-glucan fibers on blood lipids.

It is reasonable to conclude that the beta-glucan fibers inherent in glucagel and its constituent material are consistent with the patterns of efficacy on lipid parameters shown across the range of these studies.

Table 6: Summary of Human Clinical Trials with Dietary Barley β -glucan to Lower Risk Factors for CHD

Reference	Subjects	Source of β -glucan	Trial Days	Diets Used	Delivery	β -glucan	Total Cholesterol	LDL	HDL	TG	LDL/HDL	Glucose	Notes
Rondanelli et. al., 2011	24 males, mean age 50, barley β -glucan con- BMI 24.9, mildly hyper- centrate (Barley Balance [®]) cholesterolemic randomized DB	barley β -glucan con- Rice Bran 30% BG	98	Barley BG Rice Bran	Rice cakes, soups, pasta,sauces, and bread	0.9-1.7 grams/ 100 grams,	- 0.34 mmol/l -5.00%	- 0.33 mmol/l -8.90%	+0.05 mmol/l + 3% ns	- 0.05 mmol/l -3% ns	-0.18 mmol/l -7%	-0.15 mmol/l -3% ns	Barley Balance (from Fibar) is concentrated glucagel
Shimizu, et. al., 2008	39 males, ages 30-60 hypercholesterolemic BMI > 22 randomized DB	pearled CDC Fibar barley	84	barley/rice rice	foods & beverage	3.5% of diet 7 g per day	- 19.4 mg/dL -8%	- 11.7 mg/dL -7%	ns	ns	ns	*	*lowered visceral fat Fibar is used to make glucagel
Smith, et. al. 2008	90 adult males/females hypercholesterolemic BMI 26	barley β -glucan fiber (glucagel) 1) LMW 2) HMW	42	normal diet with Bg or taken twice daily control bev	β -glucan was taken twice daily in a beverage	6 grams day high MW Bg low MW Bg	-2.7 mmol/l LMW 0.3 mmol/l HMW	-5.4 mmol/l LMW 1.6 mmol/l HMW	0.8 mmol/l LMW 1.0 mmol/l HMW	12 mmol/l LMW 1 mmol/l HMW	-10.0 LMW ns	-.21 HMW ns	LDL & CRP ↓ LMW .08 LMW Weight ↓ in HMW ns Hunger ↓ in HMW
Sunberg et. al. 2008	48 adult males/females hypercholesterolemic randomized DB	barley flakes	84	barley wheat/cellul	cereal flakes beverage	3 gram Bg Control <1g Bg	-0.25 mmol/l	- 0.20 mmol/l	n.a.	n.a.	n.a.	n.a.	
Keenan, et. al. 2007	155 adult male/female, ages 25-73, BMI <40	barley betafiber HMW, LMW Bg	70	low transfat & saturated fat diet	cereal + beverage	3 gram Bg 5 gram Bg	-29.2 mg/dL 5g HMW -26.4 mg/dL 5g LMW -19.1 mg/dL 3g HMW -17.1 mg/dL 3g LMW	-22.5 mg/dL 5g HMW -20.3 mg/dL 5g LMW -19.1 mg/dL 3g HMW -13.4 mg/dL 3g LMW	ns	-25.4 mg/dL 5g HMW -20.3 mg/dL 5g LMW -21.0 mg/dL 3g HMW -12.7 mg/dL 3g LMW	-15% 5g HMW -10% 5g LMW -9% 3g HMW ns	n.a. n.a. n.a. n.a.	
Bjorklund, et. al 2005	89 adults males/females hypercholesterolemic mean age 55 yrs random single blind	concentrated barley Bg 36% (MW 40) concentrated oat Bg 18% (MW 200)	56	beverage diet concentrated oat Bg	usual diet beverage 10 g barley Bg 10 g oat Bg	5 g barley Bg 5 g oat Bg -0.20 mmol/l 5g -0.49 mmol/l 5g -0.27 mmol/l 10g -0.29 mmol/l 10g	-0.08 mmol/l 5g -0.29 mmol/l 5g -0.17 mmol/l 10g -0.16 mmol/l 10g	-0.04 mmol/l 5 g -0.08 mmol/l 5g -0.05 mmol/l 10g -0.01 mmol/l 10g	n.a. n.a. n.a. n.a.			TC, LDL and HDL measures for 5 7 10 g barley and oat were not significant	
Behall, et. al. 2004	25 adults, female/male hypercholesterolemic 9 F postm mean BMI 34, 9 F prem mean BMI 30 7 M mean BMI 26 Crossover Latin Square	barley pearls, flakes and seived barley meal/flour	17 wks	prepared diets w Bg vs Step 1 diet (NCEP) brown rice & whole wheat control	Bg and control included in prepared diets no sig differences among groups	0 gram Bg 3 gram Bg 6 gram Bg	-0.21 mmol/l ns -0.48 mmol/l (-7%) -0.53 mmol/l (-11%)	-0.11 mmol/l ns -0.36 mmol/l (-14%) -0.43 mmol/l (-17%)	ns ns ns	+ 0.1 mg/dL ns -0.02 mg/dL ns +0.11 mg/dL ns	-5% -10% -10%	9 preM F - m age 47 9 post F - m age 50 7 males - m age 43	

Table 6 - Continued

Reference	Subjects	Source of β-glucan	Trial Days	Diets Used	Delivery	β-glucan	Total Cholesterol	LDL	HDL	TG	LDL/HDL	Glucose	Notes
Behall, et. al. 2004 b	18 adult males hypercholesterolemic BMI 28.5, Age 45.6 yrs Crossover Latin Square	barley pearls, flakes and seived barley meal/flour	17 wks vs Step 1 diet (NCEP) brown rice & whole wheat control	prepared diets w Bg prepared diets	Bg and control included in Step 1	0 g Bg 3 g Bg 6 g Bg	-22.0 mg/dL -24.2 mg/dL (-10.8%) -37.1 mg/dL (-16.6%)	-23.1 mg/dL -23.1 mg/dL (-14.6%) -33.8 mg/dL (-23.8%)	+3.3 mg/dL +1.4 mg/dL ns +2.4 mg/dL (+7.3%)	-5.8 mg/dL -12.5 mg/dL 23 mg/dL	-1.0 mg/dL -0.8 mg/dL -1.2 mg/dL	n.a. n.a. n.a.	
Keogh, et. al. 2003	18 adult males, ages 26- 61 years, BMI 22-39, Randomized, single blind crossover subjects varied widely	barley β-glucan fiber prototype of glucagel	12 wks process	Prepared meals in controlled unit live-in unit in hospital live in for 12 wks	prepared meals in meals in subjects were required to agree to live in for 12 wks	0 g Bg 9.9 g Bg* 9.9 g Bg** 9.9 g Bg*** 9.9 g Bg#	n.s. -0.9 mmol/l -11% + 14.3% -8%	n.s. -0.21 mmol/l (3.8%) -9%	n.s. n.s. n.s. n.s.	n.s. n.s. n.s. n.s.	Protocol Age, BMI * all subjects ** Over 31 years *** Under 31 years # results at 21 days		
Li, et.al. 2003	10 adult females, age 20.4 yrs, healthy, randomized crossover	whole grain barley	12 wks	barley diet with rice, rice diet	prescribed diet meals supplied	3.9g SDF day 8.9g SDF day	138 mg/dL 118 mg/dL (-15%)	52.8 mg/dL 41.7 mg/dL (-21%)	-4.1% ns	87.9 mg/dL 75.6 mg/dL (-14%)			
Pins, et. al. 2000	60 adult males hypercholesterolemic Randomized parallel	barley endosperm barley bran	10 wks	Muffins w barley Bg wheat bran	Bran muffins with barley Bg, barley bran or wheat bran	10 g/day Bg 1 g/day Bg 0 g/day Bg	-7.80% n.s.	-8.22% n.s.	n.s.		+ satiety + satiety weight gain	.6 lb weight loss/wk	
Ikegami, et. al. 1996	32 adults MF, aged over 35 years, hypercholesterolemic & normolipidemic	pearled barley	6 wks	barley diet rice diet	prepared diets	2 g/day Bg 6.1 g/day TDF	5 males (mean age 38) 20 males (mean age 41) 7 females (mean age 56)	-5.4%, n.s. -9.90% -9.80%	n.s. n.s. n.s.	n.a. n.a. n.a.	n.a. n.a. n.a.		
McIntosh, et. al. 1991	21 hypercholesterolemic males, ages 30-59, BMI 25.6, TC 6.26 \pm 64 random crossover	concentrated barley	12 wks	prepared with barley or wheat diets	bread meusli pasta biscuits	8 g Bg/day 1.5g Bg/day both diets had 38.4 g TDF	-0.39 mmol/l (-6%) -0.33 mmol/l (-7%) -12.30%	+0.02 mmol/l (n.s.) -0.01 mmol/l (n.s.) -14%	-0.01 mmol/l (n.s.)	-0.05 mmol/l (n.s.)			
Newman, et. al. 1989	14 males, healthy randomized parallel	waxy barley flour, high BG wheat flour and bran	4 wks	test foods made with 1) barley 2) wheat	subjects had normal diet, plus 3 test foods daily	12 g Bg/day	- 24.3 mg/dL - 12.30%	- 18.9 mg/dL - 14%	- 9.4 mg/dL ns	+ 19.7 mg/dL ns			

2. Glucose Metabolism and Satiety

Clinical studies investigating the beneficial effect of barley beta-glucan fibers on lowering post-pyramidal glucose metabolism and increasing satiety have provided significant evidence for such effects. Modulating glucose levels and reducing food intake levels has been associated with multiple health benefits including reducing the risks of developing diabetes and obesity. Lowering insulin levels and controlling body weight are also associated with lessening risks of heart disease and other chronic conditions.

Modes of action suggested for these effects are that barley beta-glucan fibers form non-digestible triple helix matrices in the digestive tract that help to entrap some of the simple sugars in digesta, and slow the rate of absorption of remaining sugars so that uptake occurs over a longer period. These actions help to extend glycemic cycles and reduce the area under the curve (AUC) in the postprandial rise of blood sugars. Fermentation of barley beta-glucan fibers in the upper colon produces short chain fatty acids that trigger the release of satiety hormones by endothelial cells, signaling the brain to discontinue further food consumption.

Lumaga (2012) conducted a trial with barley beta-glucan fiber (glucagel) in beverages with 14 healthy volunteers (8 males/6 females) with mean age 27.8 and normal BMI. Test diets included a fruit based beverage, sweetened with sucrose and glucose, a fruit flavored barley beta-glucan beverage (3 grams) and a control beverage. The beta-glucan beverage diet at breakfast significant reduced energy intakes at the following lunch period by 18% and by 40% over the entire day. This beverage also reduced ghrelin levels by 8.1% and increased PP levels by 34.6%. Glycemia, but not insulin levels, was reduced at 60 and 120 minutes compared to control on the beta-glucan containing beverage diet.

Bays (2011) conducted a 12 week trial with barley beta-fiber using 50 mixed gender (68% female) subjects with a mean age of 56 years, BMI of 32, and baseline fasting glucose of 102 mg/dl. The test diets included a beta-glucan beverage (3 g/day), a beta-glucan beverage (6 g/day), and a control beverage. 3 grams of barley betafiber reduced glucose AUC and 6 grams significantly reduced insulin (-7.8%) and insulin resistance. Subjects had no change significant change in body weight during the trial.

Chillo (2011) conducted a trail with 9 healthy volunteers (18-60 years), BMI > 30, and fasting glucose over 6.1 mmol/L. The test diets and control came in the form of pasta (spaghetti) with 0, 2, 4, 6, 8 and 10% barley beta-glucan. Two forms of barley beta-glucan were included in the trial, a concentrated barley beta-glucan and a high purity extract. The lower molecular weight barley beta-glucan extract (glucagel) decreased AUC compared to control significantly at 2%, 4% and 6%, but not at 8% or 10%, and most significantly at 6%. Glucagel™ reduced the glucose AUC by 8.6% at 2%, 4.6 % at 4%, and 32.6% at 6% compared to spaghetti without beta-glucan. The higher molecular weight barley beta-glucan fiber (barley balance) showed significant AUC glucose reductions at 2%, 4%, 6%, 8% and 10%. Reductions in AUC compared to spaghetti

without beta-glucan were 19.3% lower with 2%, 25.7% lower with 4%, 31.9% lower with 6%, 42.6% lower with 8% and 51.5% lower with 10% Barley Balance® added to the spaghetti.

Thondre (2011) conducted a randomized crossover trial with glucagel in chapattis using 10 healthy adult subjects. Beta-glucan was added in the test material at 0%, 4% and 8% on separate occasions. There was no significant difference in the amount of glucose released or in glycemic response to beta-glucan over 120 minutes after consumption.

Vitaglione (2010) conducted a trial with glucagel in a snack format using 20 healthy adult subjects (mean age 18, BMI 23.2) using appetite and postprandial food intake as key parameters. Beta-glucan (5.2%) in biscuits lowered AUC on appetite, and increased AUC for satiety. Food intake at a subsequent meal was reduced only in female subjects

Thondre (2009) conducted a trial with 8 healthy adults aged 26-60 years with BMI over 30. Her protocol used chapattis (Indian flat bread) with 0%, 2%, 4%, 6% and 8% barley beta-glucan (barley balance concentrate). Postprandial glucose was significantly reduced compared to control with 4% and 8% beta-glucan added to the diet. GI values were very significantly reduced by 43% (4 g) and 47% (8 g) of added beta-glucan.

Vitaglione (2009) conducted a trial with 14 healthy adult subjects assigned to have isocaloric breakfasts with a 3% beta-glucan enriched bread (glucagel) or a control bread. A significant reduction in hunger, increased fullness and satiety occurred in the beta-glucan group. A 195 reduction in energy intake at a postprandial lunch was recorded in the beta-glucan group. The AUC of ghrelin was reduced by 23% and AUC of PYY was increased by 16% following beta-glucan intake.

Casaraghi (2007) conducted a trial with 10 healthy adults (mean age 25.4, BMI 22.6) using whole wheat crackers and cookies, and barley beta-glucan crackers and cookies to look for glycemic effects. She concluded that barley beta-glucan enriched cookies lowered Glycemic Index compared to Whole Wheat versions (GI = 78. and 81) to G=34 for the barley version.

Behall (2004) conducted a study with 10 females of mean age 50 and BMI 30 in a latin square design with barley and oats (as barley flakes, oat flour, oatmeal, barley flour) to compare effects on glucose, as well as glucagons and leptin. AUC for glucose was reduced by 29-36% by oats and 59-65% by barley postprandial. Insulin AUCs compared to glucose AUC were significantly reduced by barley beta-glucan by 44-56% ($p<0.005$).

3. Updated Literature Search

An updated literature search was conducted for recent clinical and scientific studies with barley beta-glucan published in 2012 and 2013. No relevant recent studies, not already considered in this petition, were found in the updated literature search.

D. Other Potential Health Effects

1. No Significant Adverse Effects on the Gastrointestinal System and on Mineral and Vitamin Availability

Tolerance of Barley β -glucan Fiber (GlucagelTM)

A large study at the University of Minnesota (Smith 2008) involving 90 subjects included extensive monitoring of symptoms that might indicate a lack of tolerance for the barley beta-glucan and control diets. At each visit, subjects were asked to assess tolerance of the treatment products and completed symptoms forms at each visit. Each participant filled out symptom questionnaires comparing any changes in their gastrointestinal activity to their normal baseline behavior. Questions covered stool frequency, stool consistency, degree of intestinal bloating and flatulence. Stool frequency was simply a count per day. Measures of stool consistency were ranked on a scale between 1 and 10, 1 = diarrhea, 10 = hard stool/constipation. Scores were based on subjective rating scales. When measuring degree of bloating or flatulence, a subject would rank this symptom between 1 and 10, 1 = “minimal” and 10 = “excessive.” Gastrointestinal side effects during this study were minimal and are shown in Table 2-4. There were no major complaints of gastrointestinal distress. There were no significant changes in stool frequency, stool consistency, degree of flatulence or the degree of bloating.

Similar findings have been reported by other studies with glucagel and are consistent with the findings of studies with other barley beta-glucan materials. In all cases minor symptoms associated with increased dietary fiber dissipated within the first several days of beginning the trial. In nearly every case symptoms were noted on both test and control diets, and were not statistically different.

Tolerance of Other Highly Concentrated Barley β -glucan Fibers

A large study by Keenan (2008) with 155 adult subjects who consumed food products containing 3g and 5g barley betafiber over the course of 6 weeks. Keenan reported:

“The treatment was well tolerated by most subjects, with excellent compliance (average treatment compliance by group: control, 96 %; 5 g HMW, 95 %; 5 g LMW, 97 %; 3 g HMW, 94 %; 3 g LMW, 97 %). The fact that there were no study dropouts further indicates the tolerability of the study treatments. Moreover, adverse events were monitored at all study visits and none were reported. Treatment-related side effects were also assessed at each study visit. There were no differences in the frequency of side-effects at baseline between any of the study treatment groups or the control group. Additionally, there was no change in the frequency of side-effects from baseline to the mid-study visit or to them final study visit in any of the treatment groups when compared to the control group except for the frequency of intestinal gas. In all groups except the control group the frequency of intestinal gas increased over the first 3 weeks of the study

and persisted over the final 3 weeks of treatment. However, the change in frequency of intestinal gas only reached statistical significance in the 5 g HMW group (at week 3 and week 6 of treatment) when all treatment groups were compared to the control group (P<0.05)." (Keenan, et. al., 2007, BJN, Page 1166.)

Table 7

Smith (2006) – Table 2-4, Gastrointestinal Symptoms Expressed as Change From Baseline^{1,2}

	3 Weeks	6 Weeks
Stool Frequency, Change (No Age Interaction)		
Low-MW	0.16 ± 0.10	0.09 ± 0.11
High-MW	0.22 ± 0.11	0.27 ± 0.11
P-value	0.70	0.25
Stool Consistency ³ , Change (No Age Interaction)		
Low-MW	0.06 ± 0.29	0.29 ± 0.32
High-MW	0.54 ± 0.26	0.27 ± 0.23
P-value	0.13	0.95
Bloating ⁴ , Change (No Age Interaction)		
Low-MW	0.69 ± 0.29	1.2 ± 0.30
High-MW	0.83 ± 0.34	0.78 ± 0.37
P-value	0.77	0.42
Flatulence ⁴ , Change (No Age Interaction)		
Low-MW	1.1 ± 0.27	1.1 ± 0.32
High-MW	1.2 ± 0.34	1.3 ± 0.35
P-value	0.71	0.71

¹Mean ± SEM

² No significant differences found in gastrointestinal symptoms

³Stool Consistency measured subjectively; 1 = diarrhea, 10 = hard (constipation)

⁴Bloating and Flatulence measured subjectively; 1 = minimal, 10 = excessive

* Source, Kristen Smith, 2006, Effects of Highly Concentrated Barley β-Glucan on Biomarkers for Cardiovascular Disease, U of MN Doctoral Dissertation, Chapter 2, page 33.

No Adverse Effects on Mineral Balance or Vitamin Availability

The consumption of barley beta-glucan does not significantly affect the availability of vitamins and minerals in the digestive tract for uptake by the body. No studies have reported problems with uptake. Prior Health Claim Petitions to FDA from the National Barley Foods Council, Cargill, Quaker Oats, and Quaker-Rhodia all state that no evidence of serious side effects are associated with consumption of beta-glucans from oats or barley.

The Cargill Health Claim Petition (2006) contains an extensive analysis of evidence on mineral balance and on vitamin availability (pp 48-52).. They conclude that neither mineral balance or vitamin availability are adversely affected by the consumption of barley beta-glucan fibers. We have reviewed that information and find that it accurately reflects the current evidence and could find no newer evidence that would contradict those conclusions.

No Adverse Effects from an Estimated Increase in Soluble Fiber

Glucagel barley beta-glucan fiber is primarily an ingredient for use in foods, beverages and nutraceuticals. In these applications it is most common to see manufacturers seeking to provide the minimum of 0.75 grams of beta-glucan or slight more in order to meet the FDA per serving requirement established for barley and oat products containing beta-glucans. And consumers typically seeking to consume at least 3 grams of beta-glucan on daily basis from all sources in order to gain desired health benefits.

The Food and Nutrition Board of the Institute of Medicine at the National Academies of Science makes periodic recommendations for daily fiber (and other nutrients) consumption in the diet. Their recommended level of fiber consumption has increased significantly in recent years as more evidence has come forth about the important benefits associated with dietary fiber. A summary of their recommendation is contained below:

“Dietary Fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. *Functional Fiber* consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. *Total Fiber* is the sum of *Dietary Fiber* and *Functional Fiber*. Fibers have different properties that result in different physiological effects. For example, viscous fibers may delay the gastric emptying of ingested foods into the small intestine, resulting in a sensation of fullness, which may contribute to weight control. Delayed gastric emptying may also reduce postprandial blood glucose concentrations and potentially have a beneficial effect on insulin sensitivity. Viscous fibers can interfere with the absorption of dietary fat and cholesterol, as well as with the enterohepatic recirculation of cholesterol and bile acids, which may result in reduced blood cholesterol concentrations. Consumption of *Dietary* and certain *Functional Fibers*, particularly those that are poorly fermented, is

known to improve fecal bulk and laxation and ameliorate constipation. The relationship of fiber intake to colon cancer is the subject of ongoing investigation and is currently unresolved. An Adequate Intake (AI) for *Total Fiber* in foods is set at 38 and 25 g/d for young men and women, respectively, based on the intake level observed to protect against coronary heart disease. Median intakes of *Dietary Fiber* ranged from 16.5 to 17.9 g/d for men and 12.1 to 13.8 g/d for women. There was insufficient evidence to set a Tolerable Upper Intake Level (UL) for *Dietary Fiber* or *Functional Fiber*.”

This recommendation includes β-glucans.

Food and Nutrition Board (FNB) National Academy of Science, Institute of Medicine: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) (2005)

Table 8 – Estimated US Fiber Consumption for Children and Adults 2005-2008

Fiber Consumption (gram)

	Food At Home		Away		From Fast Food	Home	
	Total	Home	Total	Restaurant	School	Other	
Total population	15.23	11.19	4.05	1.22	1.53	0.26	1.03
Children age 2-19	12.84	8.99	3.86	0.62	1.46	1.01	0.77
Adults age 20 and older	16.05	11.94	4.11	1.43	1.56	N/A	1.12

Source: 2005-08 NHANES, two-day averages for individuals age 2 and older who are not pregnant or lactating.

National data as shown in Table 7 indicates that both children and adults on average fall far short of the recommended levels of fiber (combination of soluble and insoluble fiber) on a daily basis. It establishes that there is more than ample room in the typical American diet to accommodate the addition of glucagel fiber.

Consumption of 4 grams of Glucagel™ barley beta-glucan fiber can provide 11 to 16% of the Food and Nutrition Board recommended daily requirement of fiber for men and women respectively.

Barley, and therefore the soluble beta-glucan fibers therein, has been in the diet on a substantial basis for at least 10,000 years. Barley was the major staple in the diet of Greek civilization (about 800 BCE). And later, barley provided the staple food of the Roman gladiators, who were known as *hordearii (barleymen)* because of the belief that their rich barley diet was the source of their physical strength. Each Roman Soldier was allocated 1 khonix (about 1.5 liters) of barley flour daily as part of their ration. On the manors of Cathedral Priory in Norfolk, England about 1300 AD, peasant worker diets were comprised mainly of barley bread and oatmeal pottage, along with small amounts of herring, salted cod, cheese and bacon. The ration was calculated – for every 2 pounds of barley bread, workers received 2 ounces of cheese, 1 ounce of meat and 4.5 ounces of fish. It was estimated that 76% of their diet came from bread and porridge. (Dyer, 1994)

In Northern Europe, Rieska, unleavened barley bread, was the earliest bread in Finland. Bread made from barley, and ‘small beer’, a low-alcohol product from fermented malt, formed the staple diet of the common people in medieval England, and as late as the beginning of the 20th century it was the main food in rural Denmark (Munck 1981). However, since the 18th century higher yielding barley has been progressively replaced by wheat as a food staple in Western cultures. This is due to wheat’s elastic proteins, superior baking and milling characteristics, taste and tradition, as well as its decreasing cost due to progressively increasing yields through improved breeding (MacGregor, 1993).

Today barley is used for food in greatest quantities in geographic areas where other cereals grow poorly due to altitude, latitude, low rainfall or soil salinity (Nilan & Ullrich 1993). It is a common food source in semi-arid regions of North Africa, the Near and Middle East, Russia, former Soviet Republics in Central Asia, Mongolia, China, Korea, India and Afghanistan. Not surprisingly, the greatest per capita consumption today is found in those regions. According to FAO reports for 2002, the world’s highest contemporary per capita levels of annual barley consumption for food were recorded in Morocco (35.6 kgs), Moldova (19.5 kgs), Latvia (19.5 kgs), Lithuania (17.8 kgs), Algeria (15.4 kgs), Estonia (13.4 kgs) and Ethiopia (12.9 kgs). (FAO Stat data, 2005).

In addition to these countries, barley is widely grown as a food and animal feed crop in the highlands of Tibet, Nepal, Mongolia, China, Ethiopia and the Andes, as well as in regions with short seasons and very long summer daylight periods, including the State of Alaska, Canada, Norway, Sweden, Russia, Denmark, Poland, Finland and the Baltic states. (Newman, 2008)

Barley consumption for food has been declining in nearly all modern developed countries as urbanization, rising incomes and an expanding array of food choices have altered demand in the era since WWII through 1990. Since then increasing knowledge of barley as a healthy food has begun to boost demand back onto a slowly increasing trend per capita in Europe. Per capita consumption there has risen from less than 1 kg in 1991 to 1.6 kg in 2002. (FAO Stat Data 2005).

U.S. data indicates per capita consumption is holding stable at about 1.1 pounds through the most recent data up to 2009. This is down from a recorded high of 10.7 pounds per capita in 1947 according to the available data. (USDA ERS Food Availability Data Sets, 2011)

4. Chemical and Physical Nature of the Substance

A. MANUFACTURING OF GLUCAGEL™ BARLEY β -GLUCAN FIBER

1. The Raw Material

Glucagel™ barley beta-glucan fiber is manufactured using high quality barley grain produced in Canada. Barley varieties used have high beta-glucan levels and have been bred for food applications, as opposed to common barley varieties bred for malting or feed purposes where low beta-glucan levels are desired.

Grain is produced under contract by Canadian growers using proprietary seed of our selected variety or varieties and is grown under specified conditions to optimize quality. Allowed pesticides are those registered with Health Canada and labeled for use on barley. When harvested, contracted grain is analyzed for mycotoxins and pesticide residues and must meet acceptance standards in order to be used for barley beta-glucan concentrate production and ultimately for Glucagel production.

Contracted grain is typically stored in on-farm storage by the grower until it is required to be delivered to the production plant to be made into concentrate. Prior to processing, it is stored in silos at the plant. Since all grain comes directly from the growers it is identity preserved.

2. Manufacturing Process

The manufacturing process for Glucagel has two major steps, a dry milling and separation process that produces a concentrate (sieved barley meal), and a wet extraction process that produces high purity barley beta-glucan fiber.

Dry Concentrate Production

Barley produced as described above and meeting acceptance criteria is trucked to the production plant in Saskatchewan and placed in storage bins. The barley is cleaned to remove any extraneous material (chaff, stems, other seeds, etc). It is then debranned to remove outer bran layers, and then milled to a fine flour. The flour is then sent through a dry mechanical separation process that produces a highly beta-glucan enriched (circa 25%) sieved barley meal or concentrate. This concentrate is packaged in totes for shipment to the Glucagel plant and is the raw material used for manufacture of Glucagel™ barley beta-glucan fiber.

The barley beta-glucan concentrate is made in Saskatchewan and is manufactured with GMP and HACCP certification, is recognized as “sieved barley meal” (A

GRAS substance) for the 2005 barley amendment to the Soluble Fiber Health Claim. A similar concentrate is also commercially marketed as Barley Balance[®] (*PolyCell Technologies, USA*) and widely used in foods and beverages.

Ocean going containers are carefully inspected to meet acceptance standards and then are loaded with totes containing barley beta-glucan concentrate and sealed for shipment to the Glucagel factory.

Glucagel Barley Beta-glucan Fiber Production

Glucagel is manufactured in a pharmaceutical production facility in Mandva, India operated by Alkem Laboratories, under license and toll manufacturing agreement with DKSH. Glucagel manufacturing takes place in a high quality, state of the art facility that is certified ISO 22000 2005 and HACCP. The manufacturing process uses only potable water, barley beta-glucan concentrate and a small amount of the bran fraction removed from the same barley early in the concentration process. No chemicals, solvents, flocculants or other exogenous materials are used.

Production begins as the dry concentrate is gradually incorporated into a hot water (80° C) solution in large steeping tanks under agitation. The slurry is held in the steeping tanks for a sufficient time to facilitate separation of much of the starch fraction. Following removal of starch, the remaining supernatant (a slurry that consists mainly of fibers, proteins and residual carbohydrates) is ready for the next stage of concentration. This supernatant includes the majority of the beta-glucan as well associated grain polymers (e.g. pentosans) that with beta-glucan are integral to the endosperm cell wall structure of all cereals.

The second stage of processing involves mixing a small amount of the bran that was originally removed in the debranning step to the starch depleted slurry. The bran fraction contains natural barley enzymes that facilitate release of the cellwall components into the slurry solution. The slurry (55° C) is then ready for centrifugation which further removes solids and further concentrates the β-glucan component.

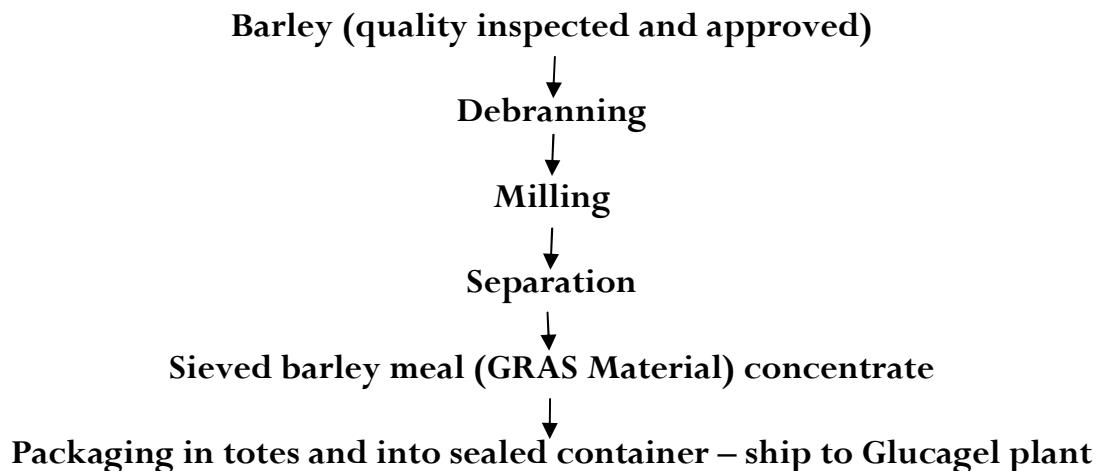
The third stage begins with controlled cooling of the supernatant to 4° C followed by freezing and then thawing – a thermo-mechanical process commonly used to concentrate plant polymers destined for food use, which induces the beta-glucans to re-associate and subsequently form gels of enriched beta-glucan. Gels are then washed to remove extraneous material. Then the glucan rich gels are heated to 85° C and residual protein precipitates and is removed.

The beta-glucan rich gels are then dried on a roller dryer which removes moisture and sterilizes the product. Then the dried Glucagel is milled and packaged for shipment.

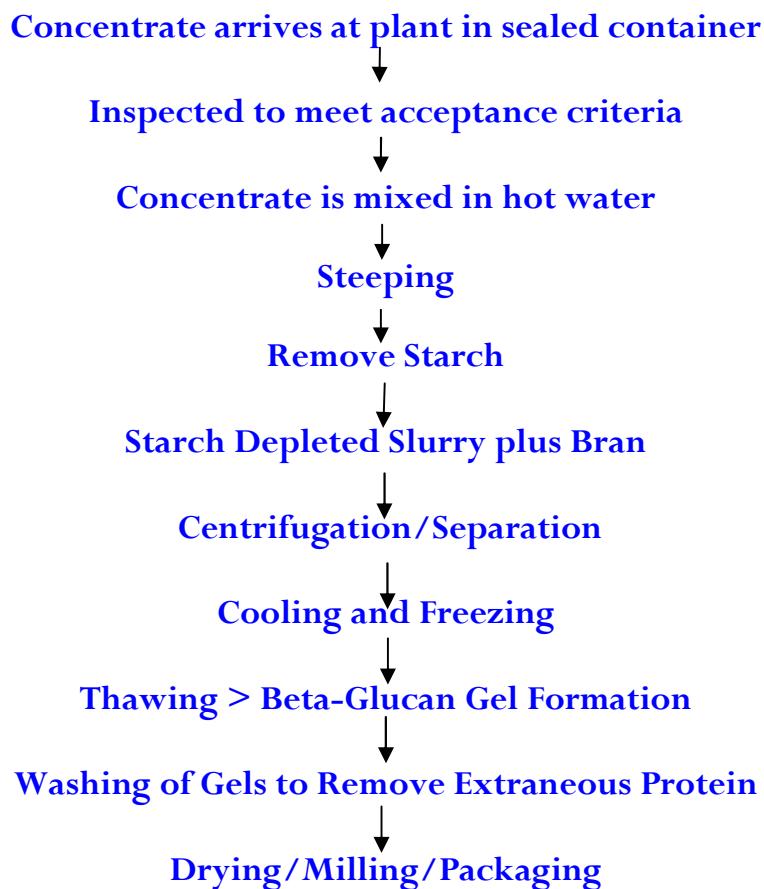
A diagram of these manufacturing processes is shown on the following page:

Figure 1 - FLOW DIAGRAM FOR MANUFACTURING

Concentrate – Starter Material



Glucagel Production



3. Safety of Raw Materials and Chemicals Used in the Process

1. The raw materials consist of two food materials, sieved barley meal (a dry milled concentrate) that is enriched in beta-glucan (circa 25%), and barley bran (a source of natural barley enzymes). Both materials are components of whole grain barley (GRAS) and have been a continuous part of the human diet over many millennia and are widely consumed in modern foods. Raw materials used in Glucagel™ barley beta-glucan fiber are regularly tested for heavy metals, and toxins, and must meet applicable standards before use.
2. The sole chemical solvent used in Glucagel isolation is water. The water used in this production is potable drinking water, which is tested regularly to ensure that it is safe.
3. The manufacturing of Concentrate and Glucagel™ barley beta-glucan fiber is conducted under the provisions of approved HACCP Plans. At each stage of production raw materials are inspected and must meet quality criteria before being approved to enter the manufacturing process. Critical Control Points in both the dry and wet processes have been identified and protocols are in place to control any potential safety hazards throughout production. These plans are regularly audited and by independent auditors for compliance.

B. Chemical and Physical Characteristics

1. Chemical

Common Name:	barley beta-glucan fiber, glucagel
Chemical Name	(1-3)(1-4) β -d-glucan
Synonyms	barley fiber, barley beta-glucan
Trade Name	Glucagel™
CAS Registry	9041-22-9 (all β -glucan) 55965-23-6 (mixed linkage cereal β -glucan)
Empirical Formula	$(C_6H_{10}O_5)_n$
Molecular Weight	Glucagel™ barley beta-glucan fiber consists of beta-glucans in the molecular weight range of less than 100 kD to over 350 kD, and average molecular weight of about 175 kD. Barley beta-glucan has been cited in various studies to be a mixed linkage polymer ranging in size from approximately as small as 80 kD to as large as in excess of 3,000 kD. (Beer et al., 1997 ¹ , Wood et al., 1991) Processing, as in food preparations, as well as extraction, has been widely correlated with reduced molecular weights, as compared to the unprocessed form. (Kerkhoffs et.al, 2003 ³)
Chemical Structure	Barley beta-glucan is a linear, unbranched polysaccharide comprised of cellobetaosyl units, along with cellobetaosyl units. This is commonly reported by the DP3:DP4 ratio, which is 2.8-3.3 for barley beta-glucan, in comparison to oat beta-glucan with a lower reported ratio of 2.1-2.4. (Wood, et. al. 1994 ⁴ , Izydorczyk et. al. 1998 ⁵).

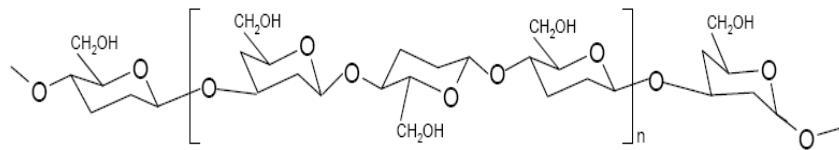


Figure 3: barley (1-3)(1-4) β -D-glucan

2. Physical

Solubility	Glucagel™ barley beta-glucan fiber is water soluble.
Viscosity	Barley beta-glucans are known to produce high levels of viscosity in aqueous solution. Viscosity is related to chemical structure and polymerization (DP), as well as to pH, concentration, and temperature. Glucagel™ barley beta-glucan fiber exhibits lower viscosity than less processed barley beta-glucan.
Gelling Hydrocolloid	Glucagel™ barley beta-glucan fiber is a very active hydrocolloid in solution and forms reversible, thermoplastic gels readily.

3. Other Properties

Chemical Stability	Glucagel™ barley beta-glucan fiber is stable when stored at normal room temperature conditions. It is stable in water solutions with pH levels ranging from 3.0 to 10.0.
Temperature	Glucagel™ barley beta-glucan fiber is stable at temperatures used in heating and freezing conditions encountered in typical food processing applications.

4. Specifications of the Product

Specifications		
Tests	Methods	Standards
Total Carbohydrate	By Difference	--
Non β-glucan Carbohydrate	AOAC 996.11	<15%
β-glucan	AOAC 995.16	>75%
Moisture	AOAC 950.46	<10%
Protein (other nitrogenous)	AOAC 981.10	<7%
Lipids	AOAC 960.39	<2%
Ash	AOAC 942.05	<2%
Foreign Matter	Visual Screen	ND
Tapped Density	USP616	> 0.25 g/ml
Lead	AA-ICP	<0.2ppm
Cadmium	AA-ICP	<0.1ppm
SPC CFU per gram	USP	<1,000
Salmonella per 25 grams	USP	ND
Vomitoxin ppm	USP	<0.25

C. Comparison to Other Highly Concentrated Barley β -glucan Fibers

Table 1. Macronutrient Composition of Barley Betafiber^a

Macronutrients	per 100g of Product
Calories	368
Total Fat (g)	0.07 (trace)
Total Carbohydrate (CHO) (g)	90
Dietary Fiber (g)	82.2
Soluble Fiber (g)	81.6
Beta-glucan (g)	73.2
Sugars (g)	0.8
Protein (g)	2.5
Moisture (g)	4.6
Ash (g)	2.7

^a Average based on analytical data of 5 samples

Source

Cargill Inc, Petition for Health Claim: Barley Betafiber and CHD, 2006, page 12
Molecular weight of barley beta-fiber used in UM trial – 150 kD average. Source Cargill Inc, Petition for Health Claim: Barley Betafiber and CHD, 2006. page 23.
Molecular weight range cited by manufacturer as 150 kD \pm 20%, Source: Cargill Inc, Petition for Health Claim: Barley Betafiber and CHD, 2006. page 57.

Glucagel

Macronutrients	In 100 grams of Glucagel™*
Total carbohydrate	89.65
Lipid	1.34
Dietary Fiber	78.40
Beta-glucan	75.03
Soluble Fiber	78.30
Sugars	<1.00
Protein	4.55
Ash	1.61
Moisture	2.69
Molecular Weight	242 kD*

Molecular weight of Glucagel in Smith (UM) trial #2 – 130 kD average

*Molecular weight mean of four Glucagel production lots (2012) – 242 kD

5. Foods Eligible to Bear the Claim

A. Qualifying Definition of Glucagel β -glucan Fiber

DKSH Italia Srl and PolyCell Technologies LLC request that FDA amend 21 CFR 101.81 to include glucagel barley beta-glucan fiber as a qualifying source of beta-glucan soluble fiber processed by the method shown in Figure 2. The qualifying definition for glucagel beta-glucan fiber is that it produced from cleaned, debranned and dry concentrated barley flour, further processed by extraction in water solution along with thermo mechanical processing. This provides a beta-glucan soluble fiber that is 75% concentrated beta-glucan with an average molecular weight of approximately 150 kD or greater. Beta-glucan levels are determined by AOAC Method 995.16 for mixed linkage (1-3)(1-4) β -d-glucan.

This definition of glucagel barley beta-glucan fiber is inclusive of the product, as well as the barley beta-glucans in the source material, that have efficacy in lowering cholesterol and related parameters in animals and humans.

B. Qualifying Level of Glucagel β -glucan Fiber

FDA established in 21 CFR 101.81 (2005) that 3 grams of beta-glucan is the minimum effective daily consumption of beta-glucan to reduce cholesterol. The interim final rule issued by FDA in 2005 for whole grain oats and dry milled barley sources concluded that the cholesterol lowering effect of both sources appear equivalent. It was further stated that the minimum effective daily intake from dry milled barley beta-glucan is the same as that from whole oats at 3 grams per day.

21 CFR 101.81 established the reference amount for barley beta-glucan to be a minimum of 0.75 grams per serving as the threshold for foods containing barley beta-glucan to qualify for the health claim. Such foods are also required to be low in cholesterol, fat and saturated fats in order to make a claim.

DKSH Italia and PolyCell Technologies propose that the qualifying level for glucagel β -glucan fiber be consistent with the minimum of 3 grams per day of beta-glucan intake, as provided in 4 servings daily of a minimum of 0.75 grams per serving, as previously specified for oat bran, rolled oats, oat flour, specified oatrimms, whole grain barley, certain dry milled barley products, and barley betafiber.

C. Representative Foods that May Bear the Claim

DKSH Italia Srl proposes that all eligible foods and beverages containing a minimum of 0.75 grams of glucagel barley β -glucan fiber per reference amount customarily consumed, and low in cholesterol, fat and saturated fat, should be eligible for the health claim.

Table 9 – Examples of Foods and Beverages that May Include Glucagel β -glucan Fiber

Representative Foods and Beverages	Typical Serving Size	B-glucan from Glucagel™ (per serving)
Bars	40 g	0.75 g – 3g
Fruit Beverages	240 ml	0.75 g – 3g
Bread	50 g	0.75 g – 3g
Breakfast Cereal	30 g	0.75 g – 3g
Cookies, Biscuits	30 g	0.75 g – 3g
Dairy Beverages	240 ml	0.75 g – 3g
Dry Beverage Mix	240 ml	0.75 g – 3g
Muffins (bran, low fat)	50 g	0.75 g – 3g
Pasta	50 g	0.75 g – 3g
Soup	245 g	0.75 g – 3g
Tortillas	30 g	0.75 g – 3g
Yogurt (low fat)	150 g	0.75 g – 3g

D. Projected Effect on Food Consumption

Glucagel barley β -glucan fiber will be included in the diet first and foremost as a source of soluble dietary fiber. It is expected that inclusion rates in foods and beverages will be at levels of 1 gram or more in individual servings, providing minimum beta-glucan per RACC of 0.75 grams. Even at consumption levels at the 95% percentile (shown in Table 10 on the following page) increases in soluble fiber consumption would be 5-7 grams or less daily, and remain well within the current fiber consumption recommendations issued by the Institute of Medicine (IOM 2005).

It is expected that the inclusion of glucagel barley beta-glucan fiber will have no significant effect on the food intake of Americans, excepting the possibility of a slight increase in soluble fiber intake.

Table 10: Estimates of Potential Consumption of Glucagel in Children and Adults

Food Categories*		For Children Ages 2-19 in grams/day			
	Mean Food consumed (grams)	<u>50th percentile</u> Grams consumed	<u>95th Percentile</u> Grams consumed	<u>Consumption of Glucagel at 50th percentile (g)</u>	<u>Consumption of Glucagel at 95th percentile (g)</u>
Whole grain bread	52	39-55	73-115	1.6-2.2	2.9-4.6
Quickbreads and muffins	76.7	54-111	112-291	1.4-3.0	3.0-7.2
Crackers	27	13-32	35-89	0.3-0.6	0.7-1.8
Biscuits	49.3	30-59	77-118	0.8-1.5	1.9-3.0
Cooked cereal	244.5	215-246	353-493	1.7-2.0	2.8-3.9
Ready-to-eat cereal	48.5	30-62	61-125	1.0-2.1	2.1-4.2
Pasta	121.6	70-155	189-398	0.7-1.6	1.9-4.0
Soups	339.8	240-479	480-722	1.0-1.9	1.9-2.9
Fruit drinks	411.3	249-480	613-1124	1.0-2.0	2.0-4.7
Pourable salad dressing	27.4	14-39	32-79	0.5-1.3	1.1-2.6
Ice cream	143.1	66-198	175-385	0.4-1.3	1.2-2.6

Food Categories*		For Adults Aged 20-59 in g/day			
	Mean Food consumed (grams)	<u>50th percentile</u> Grams consumed	<u>95th percentile</u> Grams consumed	<u>Consumption of Glucagel at 50th percentile (g)</u>	<u>Consumption of Glucagel at 95th percentile (g)</u>
Whole grain bread	64.3	50-56	91-122	2.0-2.2	3.6-4.9
Quickbreads and muffins	89.7	112-129	114-171	2.9-3.4	3.1-4.7
Crackers	31.1	18-28	53-83	0.4-0.6	1.1-1.7
Biscuits	47.7	50-67	97-153	1.2-1.7	2.4-3.8
Cooked cereal	272.5	204-245	361-498	1.6-2.0	2.9-4.0
Ready-to-eat cereal	65.2	49-65	93-140	1.6-2.2	3.0-4.7
Pasta	134.4	105-147	277-418	1.1-1.5	2.8-4.2
Soups	419.9	360-467	569-962	1.4-1.9	2.3-3.8
Fruit Drinks	487.7	251-494	751-1081	1.1-2.1	3.1-4.5
Pourable salad dressing	41.2	31	87-113	1.0-1.0	2.9-3.8
Ice cream	167.5	132-172	251-398	0.9-1.1	1.7-2.7

* Selected food categories where beta-glucan may be added – serving sizes are from Table 4. These are the daily consumptions of these foods listed in a 2-day consumer dietary study. The amounts consumed are the actual amounts of ready to eat foods in grams. The 50th percentile and 95th percentile of consumption are taken from the USDA ARS Survey.

These foods were selected because they are made of ingredients that may commonly contain significant amounts of beta-glucans, or they are potential product application areas for products that may have Glucagel added (e.g. salad dressings, cereals, baked goods, dairy, soups, beverages).

Smiciklas-Wright, H., Mitchell D.C., Mickle S.J., Cook A.J., and Goldman, J.D. 2002. Foods Commonly Eaten in the United States: Quantities Consumed Per Eating Occasion and in a Day. 1994-96, **USDA ARS**

6. Proposed Example Health Claims

Consistent with 21 CFR 101.81 (c)(2)(e), DKSH Italia is proposing the use of the following health claims to be used in food labelling:

- 1) Soluble fiber from Glucagel™ barley beta-glucan, as a part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of _____ (food name) provides _____ grams of beta-glucan soluble fiber. Three grams of beta-glucan soluble fiber are necessary each day to get this effect.**

- 2) Diets low in saturated fat and cholesterol that include 3 grams of barley beta-glucan soluble fiber from Glucagel™ may reduce the risk of heart disease. One serving of _____ (food name) provides _____ grams of this soluble fiber.**

7. Environmental Impact

DKSH Italia claims a categorical exclusion under 21 CFR 25.32 (p) for an environmental assessment and environmental impact statement. Under the environmental impact consideration regulations, actions involving the issuance of a health claim petition do not individually or cumulatively have a significant effect on the human environment and therefore do not require the preparation of an environmental assessment and environmental impact statement.

8. Conclusion

The information presented in this petition provides scientific evidence that barley beta-glucan fiber from glucagel can reduce serum lipids, and help in reducing the risk of CHD in the U.S. population. The totality of the scientific evidence presented in this petition demonstrates significant scientific agreement to authorize an amendment to 21 CFR 101.18 allowing a health claim for glucagel barley beta-glucan fiber. Approval of barley beta-glucan fiber in glucagel as an eligible source of soluble fiber will provide American consumers with additional food choices providing beta-glucan soluble fiber.

Expanded consumption of barley beta-glucan soluble fiber is in accord with NCEP ATP III guidelines for reducing serum cholesterol. NCEP ATP III recommends that a cholesterol lowering diet be enriched with foods that provide at least 5-10 grams per day of viscous soluble fiber (NCEP 2002). Reduction of serum cholesterol and CHD is a national health policy objective to address CHD as a major national health concern.

The evidence presented in this petition demonstrates that the barley beta-glucan soluble fiber in glucagel is consistent with cholesterol lowering effects shown in oat and barley beta-glucans that have been previously reviewed by FDA and bear health claims. Key highlights are as follows:

- a) Human clinical trials presented in this petition clearly show that soluble barley beta-glucan fibers derived from whole grains and from more concentrated sources, including glucagel, can reduce serum cholesterol.
- b) Animal trials presented in this petition clearly show that soluble barley beta-glucan fibers derived from whole grains and from more concentrated sources, including glucagel, can reduce serum cholesterol.
- c) The chemical and physical properties of the barley beta-glucan soluble fibers in glucagel are very similar to a previously health claim approved for a barley betafiber (FDA, Federal register Final Rule 73 FR 47828, August 15, 2008).
- d) The minimum consumption levels of 3 grams of barley beta-glucan daily and 0.75 grams per serving recommended are safe, effective and consistent with prior FDA rules.

We request FDA to issue an interim final rule effective on publication of the glucagel barley beta-glucan health claim. We believe this is in keeping with the statutory criteria cited in Section 403(R)(7) of the Federal Food, Drug and Cosmetic Act.

9. Proposed Regulation Amendment

DKSH Italia and PolyCell Technologies requests that 21 CFR 101.81 be changed to include glucagel barley beta-glucan fiber as a source of beta-glucan soluble fiber eligible to bear the health claim.

We request that 21 CFR 101.1(c)(2)(i)(G)(1) be revised as follows:

- (1) 3g or more of beta-glucan soluble fiber from whole oats, barley, barley betafiber or glucagel.

We request that 21 CFR 101.81 (c)(2)(ii)(A) be revised as follows:

- (A) Beta-glucan soluble fiber from whole oat, barley, barley betafiber and glucagel sources.

We request that 21 CFR 101.81 (c)(2)(ii)(A) be revised by adding a new subparagraph (6) as follows:

Glucagel: The concentrated soluble fraction of whole grain barley. Glucagel is produced from hulless or dehulled whole grain barley as defined in 21 CFR 101.81 (c)(2)(ii)(A)(5), by an aqueous process using thermal and mechanical separation steps. Glucagel shall have a minimum beta-glucan soluble fiber content of 75%.

We request that 21 CFR 101.81 (c)(2)(iii)(A)(1) be revised as follows:

- (A)(1) One or more of the whole oat, barley, barley betafiber, or glucagel from paragraphs (c)(2)(ii)(A)(1)(2)(3)(5) and (6) of this section and whole oat, barley, barley betafiber, and glucagel foods shall contain at least 0.75 gram (g) soluble fiber per reference amount customarily consumed of the food product;

10. Certification

Attached herewith are copies of the scientific studies and other information referenced in and constituting the basis for this petition. To the best of the petitioner's knowledge, the clinical trials included in this petition were conducted in compliance with the requirements for informed consent set forth in CFR Part 50. To the best of the petitioner's knowledge, all clinical investigations were either conducted in compliance with the requirements of institutional review set forth in 21 CFR Part 56, or were not subject to such requirements in accordance with 21 CFR 56.104 or 56.105. To the best of the petitioner's knowledge, all non-clinical studies included in this petition were conducted with FDA good laboratory practices regulations (21 CFR Par 58).

On behalf of the petitioners, Thomas P. Jorgens (President, PolyCell Technologies LLC) certifies that, to the best of our knowledge, this petition is representative and balanced submission that includes unfavorable information as well as favorable information, known to us to be pertinent to the evaluation of the proposed health claim.

Respectfully submitted,

PolyCell Technologies, LLC



Thomas P. Jorgens

President

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Appendices

To

Glucagel Barley Beta-glucan Fiber Heart Disease Health
ClaimPetition to Expand the of Barley Soluble Fiber and
Coronary Heart Disease Health Claim

January 31. 2013

Appendix A

GRAS Status



Notification of GRAS Status of Glucagel™ Barley Beta-glucan Fiber

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July 2012

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1) INTRODUCTION

A. Declaration of GRAS Status

Glucagel™ is a high purity barley beta-glucan extract (concentrate) containing a (1-3) (1-4) β-d-glucan soluble fiber content of $\geq 75\%$. It is produced from barley using only water, with thermal and mechanical extraction steps. In addition to beta-glucan, it contains small amounts of starch, protein, lipids, ash, other common cereal polysaccharides, along with less than 8% moisture. Glucagel™ barley beta-glucan fiber will be used in foods and beverages as a source of soluble dietary fiber. It may also be used to replace fat, to improve texture, increase viscosity or to improve mouthfeel in certain food applications.

Glucagel™ is produced by DKSH Italia s.r.l. (DKSH) and manufactured at Alkem Laboratories Ltd., (pharmaceutical manufacturer) located in Mandva, India. The production facility for Glucagel™ is ISO 22000 2005 certified, HACCP and GMP certified, with defined GMP's for the components of the process. (Appendix E),

DKSH is notifying FDA of the safety status of Glucagel™ barley beta-glucan fiber. An extensive analysis of this material has been conducted by a panel of qualified experts (Expert Panel). The Expert Panel has determined that Glucagel™ barley beta-glucan fiber is GRAS (Generally Recognized as Safe) for application to all foods, excluding infant formula, and meat products.

The Expert Panel is comprised of individuals qualified by their scientific training and extensive professional experience to assess food safety. The Panel reviewed the science and the data for this notification.

B. GRAS Notification Regulations

DKSH is submitting this Notification regarding the safety status of Glucagel™ barley beta-glucan fiber for use in all foods, except infant formula and meat products. This GRAS determination is supported by an analysis from a panel of independent experts (Expert Panel), qualified by their scientific training and extensive professional experience to assess food and food ingredient safety. A comprehensive search of the current scientific literature

was completed and covers safety, toxicology and other related topics for Glucagel™ and barley beta-glucans. This was included in the report to the Expert Panel.

The Expert Panel independently assessed the material submitted in addition to any other material they found relevant. From their critical assessment the Expert Panel concluded that the intended uses of Glucagel™ barley beta-glucan fiber are appropriate and Generally Recognized as Safe (GRAS) based on scientific procedures. The signed report of the Expert Panel is included in Appendix A.

This notification contains scientific data and information summarized from a thorough review of literature contained in books, articles and reviews covering Glucagel™ barley beta-glucan fiber and beta-glucans from other cereal sources. This GRAS Notification provides the information required by proposed 21 CFR §170.36(c)(2),(3) and (4) to support the evaluation of qualified experts in fulfillment of the requirements of 21 CFR § 170.36(c)(4)(1)(c) in the body of the document and appendices.

C. Summary Basis for GRAS Status

GRAS determination for general food use of Glucagel™ barley beta-glucan fiber for all foods except infant formula and meats, at levels consistent with cGMP is based on the evidence provided in the comprehensive report of the Expert Panel. A summary basis for the determination of GRAS Status is provided below and is further discussed throughout this document.

1. Glucagel™ barley beta-glucan fiber is derived from food barley by hydrolysis and liquid extraction, by decanting and clarification, by freezing, and by thawing to produce beta-glucan gel. The gel is washed to remove proteins and then dried, milled and packaged. The end product consists exclusively of barley components. No chemical solvents other than water are used in the process.
2. Beta-glucans found in barley, oats and other cereals are (1-3) (1-4) β -d-glucans and are mixed length linear polymers. Other types of beta-glucans are found in fungi and yeast, each type has unique characteristics and differences that affect properties and biological function.
3. Glucagel™ barley beta-glucan fiber is of the so-called mixed linkage (1-3) (1-4) linkage type common to cereals, and is very similar to oat beta-glucan. Because they are common components of nearly all cereal grains, this type of beta-glucan has been an important part of human and animal diets at least since cereals were domesticated over 5,000 years ago. Barley is a traditional food (the world's 4th largest cereal crop) that was the staple of the Roman Army diet and was a core staple of European, American and Asian diets well into the 20th Century. It remains the core staple for a large part of the Middle East and North

Africa, as well as the Asian highlands from Korea to India. It is especially important to the survival of Nepalese and Tibetan populations from which the barley germplasm was derived for the current varieties used to make Glucagel™. In cooking, beta-glucans that are released from barley (and oat) cell walls are nearly identical in size and function to those in Glucagel™.

4. Glucagel™ barley beta-glucan fiber is non-digestible in the human upper gastrointestinal tract (GI) and forms a viscous matrix when hydrated. It is not absorbed in the small intestine and is not known to be associated with any adverse effects. As it passes into the lower GI tract this soluble fiber is largely fermented.

5. Toxicology trials with animals have been conducted using Glucagel™ barley beta glucan at levels up to 10% of the diet without observed significant adverse effects (Jonker et al 2010). These are intake levels substantially higher than any anticipated human consumption.

6. At least 10 clinical trials using Glucagel™ barley beta-glucan fiber and constituent material have been conducted with human subjects to assess effects on cholesterol and other blood lipid parameters, blood glucose, and satiety and weight control. In these trials, involving more than 200 individuals, no significant adverse effects relative to control were reported and this material was well tolerated by test subjects.

7. The utility and efficacy of cereal mixed-linkage beta-glucans have been recognized for several years by the FDA and other agencies in the United States and elsewhere as a safe and useful products for lowering cholesterol..Food products containing beta-glucan enriched oat bran, barley fiber, and other similar cereal products are manufactured and distributed internationally. The demand for sources of cereal beta glucan (especially those of oats and barley) has steadily increased in the past 15-20 years.

8. A detailed review of the literature reveals that many studies using human and animal subjects with barley and other sources of beta-glucan, including Glucagel™, have focused on blood lipids, blood glucose, digestive function, satiety, weight control, and other end points. Nearly all studies report beneficial effects on one or more test parameters, and no reports of significant safety issues are in evidence.

2. PROPERTIES OF GLUCAGEL™ BARLEY β -GLUCAN FIBER

A. Chemical

Common Name:	barley beta-glucan fiber, Glucagel™
Chemical Name	(1-3) (1-4) β -d-glucan
Synonyms	barley fiber, barley beta-glucan
Trade Name	Glucagel™
CAS Registry	9041-22-9 (all β -glucan) 55965-23-6 (mixed linkage cereal β -glucan)
Empirical Formula	$(C_6H_{10}O_5)_n$
Molecular Weight	Glucagel™ barley beta-glucan fiber consists of beta-glucans in the molecular weight range of less than 100 kD to over 350 kD, and average molecular weight of about 175 kD. Barley beta-glucan has been cited in various studies to be a mixed linkage polymer ranging in size from approximately as small as 80 kD to as large as in excess of 3,000 kD. (Beer et al., 1997 ¹ , Wood et al., 1991) Processing, as in food preparations, as well as extraction, has been widely correlated with reduced molecular weights, as compared to the unprocessed form. (Kerkhoffs et.al, 2003 ³)
Chemical Structure	Barley beta-glucan is a linear, unbranched polysaccharide comprised of cellobiosyl units, along with cellobiosyl units. This is commonly reported by the DP3:DP4 ratio which is 2.8-3.3 for barley beta-glucan, in comparison to oat beta-glucan with a lower reported ratio of 2.1-2.4. (Wood, et.al. 1994 ⁴ , Izydorczyk et. al. 1998 ⁵).

See Figure 1

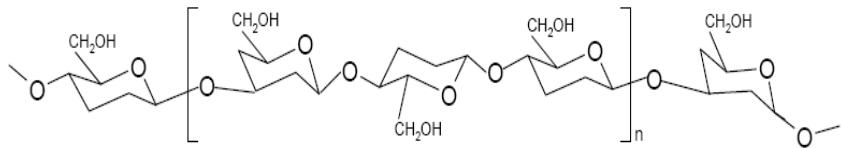


Figure 1: barley (1-3) (1-4) β -D-glucan

B. Physical

Solubility	Glucagel™ barley beta-glucan fiber is water soluble.
Viscosity	Barley beta-glucans are known to produce high levels of viscosity in aqueous solution. Viscosity is related to chemical structure and polymerization (DP), as well as to pH, concentration, and temperature. Glucagel™ barley beta-glucan fiber exhibits lower viscosity than less processed barley beta-glucan.
Gelling Hydrocolloid	Glucagel™ barley beta-glucan fiber is a very dynamic hydrocolloid in solution and forms reversible, thermoplastic gels readily.

C. Other Properties

Chemical Stability	Glucagel™ barley beta-glucan fiber is stable when stored at normal room temperature conditions. It is stable in water solutions with pH levels ranging from 3.0 to 10.0.
Temperature	Glucagel™ barley beta-glucan fiber is stable at temperatures encountered in typical food processing applications from heating to freezing.

3. SPECIFICATIONS OF GLUCAGEL™ BARLEY β -GLUCAN FIBER

A. Specifications of the Product

Table 1
Specifications

Tests	Methods	Standards
Total Carbohydrate	By Difference	--
Non β -glucan Carbohydrate	AOAC 996.11	<15%
β -glucan	AOAC 995.16	>72%
Moisture	AOAC 950.46	<10%
Protein (other nitrogenous)	AOAC 981.10	<7%
Lipids	AOAC 960.39	<2%
Ash	AOAC 942.05	<2%
Foreign Matter	Visual Screen	ND
Tapped Density	USP616	> 0.25 g/ml
Lead	AA-ICP	<0.2ppm
Cadmium	AA-ICP	<0.1ppm
SPC CFU per gram	USP	<1,000
Salmonella per 25 grams	USP	ND
Vomitoxin ppm	USP	<0.25

Table 2

Analytical data from three Glucagel™ production runs.

Analytical

Tests	Methods	Lot GLC1203047	Lot GLCS03091	Lot GCL1203046
Total Carbohydrate	<i>By Difference</i>	90.93%	87%	90.85%
Non β -glucan Carbohydrate	AOAC 996.11	10.73%	12.2%	13.03%
β -glucan	AOAC 995.16	76.60%	75.52%	73.92%
Moisture	AOAC 950.46	2.06%	3.79%	2.01%
Protein+other nitrogenous	AOAC 981.10	4.13%	5.54%	4.38%
Lipids	AOAC 960.39	1.13%	1.82%	1.02%
Ash	AOAC 942.05	1.75%	1.91%	1.74%
Foreign Matter	Visual Screen	ND	ND	ND
Tapped Density	USP616	0.39g/ml	0.50g/ml	0.42g/ml
Lead	AA-ICP	<0.2ppm	<0.2ppm	<0.2ppm
Cadmium	AA-ICP	<0.1ppm	<0.1ppm	<0.1ppm
SPC CFU/gram	USP	<1000	<1000	<1000
Salmonella per 25g	USP	ND	ND	ND
E coli	USP	ND	ND	ND
Vomitoxin	USP	<0.25 ppm	<.25 ppm	<.25 ppm
Zearalenon	ppb	ND	ND	ND
Ochratoxin A	ppb	ND	ND	ND
Aflatoxin B1	ppb	ND	ND	ND
Sum of Aflotoxin B1, B2, G1,G2	ppb	ND	ND	ND

B. Potential Impurities

1. From Raw Materials, or Chemicals used in Manufacture

Glucagel™ barley beta-glucan fiber is made from selected high quality barley (e.g. waxy hulless) grown and produced contract growers (e.g. in Saskatchewan). Grain is carefully inspected and analyzed to ensure that it meets quality standards and for possible contaminants, including pesticide residues and mycotoxins. The methods of analysis are those specified by the Canadian Grain Commission. Barley meeting the acceptance criteria can be selected for processing.

2. Impurities from Processing

The manufacturing process for Glucagel™ barley beta-glucan fiber consists entirely of dry milling and separation to produce a beta-glucan concentrate, which in turn goes into water solution for isolation of beta-glucan. The wet process phase includes only water (see Appendix B), with extraction facilitated by thermal and mechanical forces. Water used in the process is routinely tested to ensure that it exceeds drinking water purity standards. No other chemicals are used in this process so there are no other additions to the process that could be a source of potential contaminants.

3. Impurities from Microbial Activity

During the wet processing and drying phase of manufacture, microbes that may accompany the raw material are subjected to high levels of heat in both the extraction and drying stages of the process. Heat is effective in eliminating nearly all microbial activity, as confirmed by testing the finished product for salmonella, staphylococci, coliforms, and SPC shown in Table 2.

4. MANUFACTURING OF GLUCAGEL™ BARLEY β-GLUCAN FIBER

A. The Raw Material

Glucagel™ barley beta-glucan fiber is manufactured using high quality barley grain produced in Canada. Barley varieties used have high beta-glucan levels and have been bred for food applications, as opposed to common barley varieties bred for malting or feed purposes where low beta-glucan levels are desired. It is very similar to food barleys that have formed the basis of diets in other countries for centuries.

Grain is produced under contract by Canadian growers using proprietary seed of our selected variety or varieties and is grown under specified conditions to optimize quality. Allowed pesticides are those registered with Health Canada and labeled for use on barley. When harvested, contracted grain is analyzed for mycotoxins and pesticide residues and must meet acceptance standards in order to be used for barley beta-glucan concentrate production and ultimately for Glucagel™ production. Contracted grain is typically stored in on-farm storage by the grower until it is required to be delivered to the production plant to be made into concentrate. Prior to processing, it is stored in silos at the plant. Since all grain comes directly from the growers it is identity preserved and therefore readily traceable.

B. Manufacturing Process

The manufacturing process for Glucagel™ has two major steps, a dry milling and separation process that produces a concentrate (sieved barley meal), and a wet extraction process that produces high purity barley beta-glucan fiber.

Dry Concentrate Production

Barley produced as described above and meeting acceptance criteria is trucked to the production plant in Saskatchewan and placed in storage bins. The barley is cleaned to remove any extraneous material (chaff, stems, other seeds, etc). It is then debranned to remove outer bran layers, and then milled to fine flour. The flour is then sent through a dry mechanical separation process that produces a highly beta-glucan enriched (circa 25%) sieved barley meal or concentrate. This concentrate is packaged in totes for shipment to the Glucagel™ plant and is the raw material used for manufacture of Glucagel™ barley beta-glucan fiber.

The barley beta-glucan concentrate is made in Saskatchewan and is manufactured with GMP and HACCP certification, is recognized as “sieved barley meal” (a GRAS substance) for the 2005 barley amendment to the Soluble Fiber Health Claim.

A similar concentrate is also commercially marketed as Barley Balance[®] (*PolyCell Technologies, USA*) and widely used in foods and beverages.

Ocean-going containers are carefully inspected to meet acceptance standards and then are loaded with totes containing barley beta-glucan concentrate and sealed for shipment to the GlucagelTM factory.

GlucagelTM Barley Beta-glucan Fiber Production

GlucagelTM is manufactured in a pharmaceutical production facility in Mandva, India operated by Alkem Laboratories, under license and toll manufacturing agreement with DKSH. GlucagelTM manufacturing takes place in a high quality, state of the art facility that is certified ISO 22000 2005 and HACCP. The manufacturing process uses only potable water, barley beta-glucan concentrate and a small amount of the bran fraction removed from the same barley early in the concentration process. No chemicals, solvents, flocculants or other exogenous materials are used.

Production begins as the dry concentrate is gradually incorporated into a hot water (80° C) solution in large steeping tanks under agitation. The slurry is held in the steeping tanks for a sufficient time to facilitate separation of much of the starch fraction. Following removal of starch, the remaining supernatant (a slurry that consists mainly of fibers, proteins and residual carbohydrates) is ready for the next stage of concentration. This supernatant includes the majority of the beta-glucan as well associated grain polymers (e.g. pentosans) that with beta-glucan are integral to the endosperm cell wall structure of all cereals.

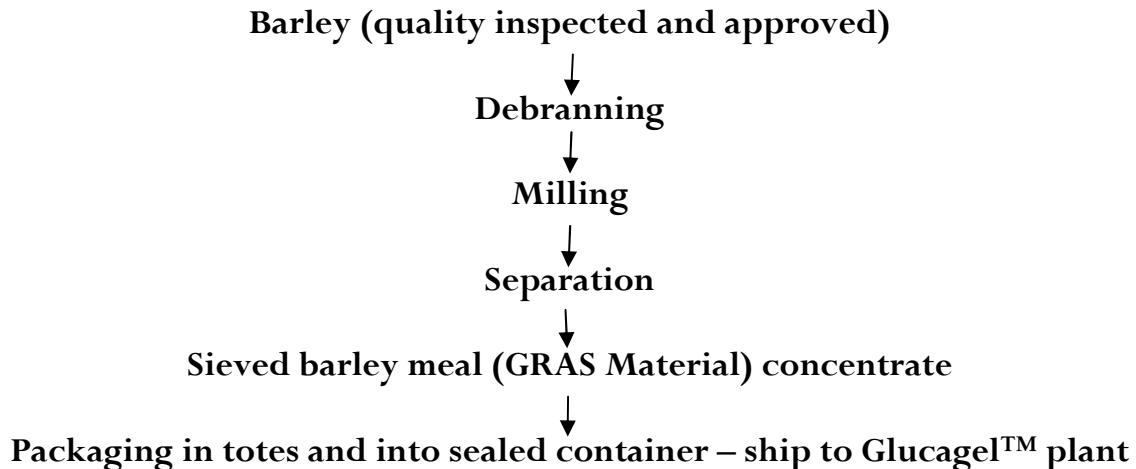
The second stage of processing involves mixing a small amount of the bran that was originally removed in the debranning step to the starch depleted slurry. The bran fraction contains naturally occurring barley enzymes that facilitate release of the cell wall components into the slurry solution. The slurry (55° C) is then ready for centrifugation which further removes solids and further concentrates the β-glucan component.

The third stage begins with controlled cooling of the supernatant to 4° C followed by freezing and then thawing – a thermo-mechanical process commonly used to concentrate plant polymers destined for food use, which induces the beta-glucans to re-associate and subsequently form gels of enriched beta-glucan. Gels are then washed to remove extraneous material. Then the glucan rich gels are heated to 85° C and residual protein precipitates and is removed.

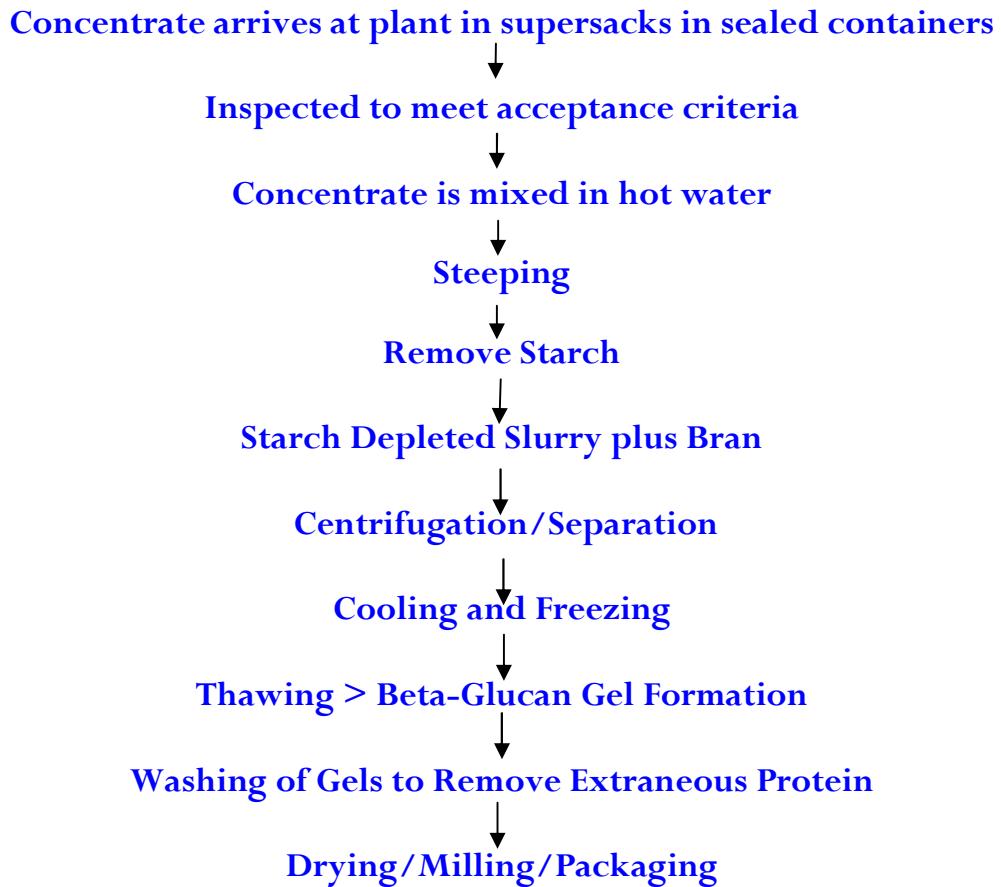
The beta-glucan rich gels are then dried on a roller dryer which removes moisture and sterilizes the product. Then the dried GlucagelTM is milled and packaged for shipment. A diagram of these manufacturing processes is shown on the following page:

Figure 2 - FLOW DIAGRAM FOR MANUFACTURING

Concentrate – Starter Material



Glucagel Production



C. Safety of Raw Materials and Chemicals Used in the Process

1. The raw materials consist of two food materials, sieved barley meal (a dry milled concentrate) that is enriched in beta-glucan (circa 25%), and barley bran (a source of natural barley enzymes). Both materials are components of whole grain barley (GRAS) that has been a continuous part of the human diet over many millennia and are widely consumed in modern foods. Raw materials used in Glucagel™ barley beta-glucan fiber are regularly tested for heavy metals, and toxins, and must meet applicable standards before use.
2. The sole chemical solvent used in Glucagel™ isolation is water. The water used in this production is potable drinking water, which is tested regularly to ensure that it is safe.
3. The manufacturing of concentrate and Glucagel™ barley beta-glucan fiber is conducted under the provisions of approved HACCP Plans. At each stage of production raw materials are inspected and must meet quality criteria before being approved to enter the manufacturing process. Critical Control Points in both the dry and wet processes have been identified and protocols are in place to control any potential safety hazards throughout production. These plans are regularly audited and by independent auditors for compliance.

D. Analytical data of Glucagel™ made in this plant by this process

Glucagel™ characterization is in Table 2 on page 9

5. INTENDED USES IN FOODS AND BEVERAGES

DKSH Italia is pursuing GRAS Status for Glucagel™ barley beta-glucan fiber for general use in all foods, excepting infant formula, and meat products. GRAS is proposed for use in food consistent with the manufacturing of the particular products.

A. Dietary Fiber

Glucagel™ barley beta-glucan fiber provides an excellent source of very concentrated soluble dietary fiber – beta-glucan (>72% barley beta-glucan). Barley beta-glucan is a dietary fiber recognized by the AOAC method for fiber analysis (AOAC 991.43). It is further defined as “soluble fiber” which according to ADA guidelines should be 1/4th to 1/3rd of daily fiber consumption (Marlett, ADA 2002⁶).

Under the Food and Drug Modernization Act of 1997, a different approach to the creation of a Health Claim for foods and Dietary Supplements was authorized. Under the act the government authorized the use of “current published authoritative statements from a scientific body of the United States with official responsibility for public health protection or research directly related to human nutrition . . . or the National Academy of Sciences (NAS) or any of its subdivisions.”

As cited by FDA in guidance documents this has been clarified:

“The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) are federal government agencies specifically identified as scientific bodies by FDAMA.

FDA believes that other federal agencies may also qualify as appropriate sources for such authoritative statements. Along with NAS (or any of its subdivisions), the agency currently considers that the following federal scientific bodies may be sources of authoritative statements: the CDC, the NIH, and the Surgeon General within Department of Health and Human Services; and the Food and Nutrition Service, the Food Safety and Inspection Service, and the Agricultural Research Service within the Department of Agriculture.”

FDA was very helpful by putting together criteria for such claims and publishing them in guidelines “FDA also believes it is necessary to clarify what constitutes an authoritative statement under FDAMA. FDAMA itself states that an authoritative statement: (1) is “about the relationship between a nutrient and a disease or health-related condition” for a health claim, or “identifies the nutrient level to which the claim refers” for a nutrient content claim, (2) is “published by the scientific body” (as identified above), (3) is “currently in effect,” and (4) “shall not include a statement of an employee of the scientific body made in the individual capacity of the employee.

In addition, given the legislative history of sections 303 and 304 of FDAMA, FDA currently believes authoritative statements also should: (5) reflect a consensus within the

identified scientific body if published by a subdivision of one of the Federal scientific bodies, and (6) be based on a deliberative review by the scientific body of the scientific evidence.”

The Food and Nutrition Board of the Institute of Medicine at the National Academies of Science makes periodic recommendations for daily fiber (and other nutrients) consumption in the diet. Their recommended level of consumption has increased significantly in recent years as more evidence has come forth about the important benefits associated with dietary fiber. A summary of their recommendation is contained below:

“*Dietary Fiber* consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. *Functional Fiber* consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. *Total Fiber* is the sum of *Dietary Fiber* and *Functional Fiber*. Fibers have different properties that result in different physiological effects. For example, viscous fibers may delay the gastric emptying of ingested foods into the small intestine, resulting in a sensation of fullness, which may contribute to weight control. Delayed gastric emptying may also reduce postprandial blood glucose concentrations and potentially have a beneficial effect on insulin sensitivity. Viscous fibers can interfere with the absorption of dietary fat and cholesterol, as well as with the enterohepatic recirculation of cholesterol and bile acids, which may result in reduced blood cholesterol concentrations. Consumption of *Dietary* and certain *Functional Fibers*, particularly those that are poorly fermented, is known to improve fecal bulk and laxation and ameliorate constipation. The relationship of fiber intake to colon cancer is the subject of ongoing investigation and is currently unresolved. An Adequate Intake (AI) for *Total Fiber* in foods is set at 38 and 25 g/d for young men and women, respectively, based on the intake level observed to protect against coronary heart disease. Median intakes of *Dietary Fiber* ranged from 16.5 to 17.9 g/d for men and 12.1 to 13.8 g/d for women. There was insufficient evidence to set a Tolerable Upper Intake Level (UL) for *Dietary Fiber* or *Functional Fiber*.”

This recommendation includes β-glucans.

⁷Food and Nutrition Board (FNB) National Academy of Science, Institute of Medicine: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients) (2005)

Table 3 - US Fiber Consumption for Children and Adults 2005-2008

Fiber Consumption (gram)

	Food At		Away		From	Home	
	Total	Home	Total	Restaurant	Fast Food	School	Other
Total population	15.23	11.19	4.05	1.22	1.53	0.26	1.03
Children age 2-19	12.84	8.99	3.86	0.62	1.46	1.01	0.77
Adults age 20 and older	16.05	11.94	4.11	1.43	1.56	N/A	1.12

Source: 2005-08 NHANES, two-day averages for individuals age 2 and older who are not pregnant or lactating.

Barley beta-glucan soluble fiber was authorized for a Health Claim by U.S. FDA in 2005 for reducing risks of developing coronary heart disease. Foods containing at least 0.75 grams of beta-glucan derived from whole grain barley and fractions thereof, may be eligible to carry this health claim. This is based on evidence that barley beta-glucan soluble fiber in the diet can contribute to lowering LDL cholesterol, a known risk factor for coronary heart disease. The FDA Claim (21CFR § 101.54(c)) is based on consumer intake of at least 3 grams of pure beta-glucan daily

Consumption of 4 grams of Glucagel™ barley beta-glucan fiber can provide 11 to 16% of the Food and Nutrition Board recommended daily requirement of fiber for men and women respectively.

In addition to health benefits associated with Glucagel™ barley beta-glucan fiber, it may also provide technical benefits in the form of fat replacement, viscosity, and thickening properties in certain food applications.

The health effects of fiber led to a recommendation that the consumption of whole grains is one way to increase fiber. This statement resulted in a FDAMA health claim for whole grains. The claim was authorized to be used as of July 8, 1999.

While Glucagel™ is not a whole grain product; its inclusion in the diet makes more fiber available for consumption and addition to healthful substances in the food stream. There is a cardiovascular health claim for barley β-glucan but it applies to glucans that were prepared by a totally dry process and those any that have been specifically added by amendment (e.g. Barliv™) at present

B. Self Limiting Factors in the Use of Glucagel™ Barley Beta-glucan Fiber

It is expected that Glucagel™ barley beta-glucan fiber will be used in foods and in beverages at or near levels associated with functionality of other barley β-glucans. The minimum beta-glucan levels recognized in the FDA Health Claim are 0.75 grams per serving (equivalent to 1 gram of Glucagel™) and 3 grams daily in 4 servings (equivalent to 4 grams of Glucagel™) of one or more food or beverage products containing beta-glucan in the required amount.

This level of use is affected by the following self-limiting factors:

1. It is consistent with the FDA Health Claim for approved barley β-glucans. Food and beverage manufacturers are seeking to meet FDA thresholds for health claims with most new products.
2. Substantial clinical evidence exists in the scientific literature to support efficacy at the

3 grams of beta-glucan consumption level daily. Since clinical studies at higher levels generally do not show proportional improvements in efficacy, it is unlikely that many products would exceed this level.

3. Beta-glucans can be readily incorporated into foods and beverages at low levels, but often present technical challenges at higher inclusion rates. These include Increases in viscosity, thickening, texture changes, increased water uptake, taste or mouthfeel changes, color changes, changes in performance in baking, cooking, culturing, blending, extrusion, etc., and others. Manufacturers have developed a number of methods for handling these challenges in many new and traditional products.
4. Beta-glucan is usually more expensive than many other ingredients used in foods and beverages, so economics also becomes an important factor in determining optimum and/or maximum levels of use as well.

6. BARLEY CONSUMPTION IN THE U.S. & OTHER COUNTRIES

A. Historic and Contemporary Use

Barley has been widely cultivated as a food source for centuries: it was one of the earliest crops to be domesticated, and fragments of grains and husks from archaeological excavations in the near and Middle East have been carbon dated to periods of 5000 to 17,000 years BCE (Newman & McGuire 1985⁷).

Its use as a food in early civilizations is documented in a large body of published literature (summarized in MacGregor et al. 1993⁸; Rasmussen 1985⁹). About 2500 BCE, barley was introduced from Northern Mesopotamia to the Indus Valley Civilization (largely in present day Pakistan), and from there it spread to other parts of the Indian subcontinent.

Barley was the major staple in the diet of Greek civilization (about 800 BCE). Later, barley provided the staple food of the Roman gladiators, who were known as *hordearii* (*barleymen*) because of the belief that their rich barley diet was the source of their physical strength. In the manors of Cathedral Priory in Norfolk, England about 1300 AD, peasant worker diets were comprised mainly of barley bread and oatmeal pottage, along with small amounts of herring, salted cod, cheese and bacon. The ration was calculated – for every 2 pounds of barley bread, workers received 2 ounces of cheese, 1 ounce of meat and 4.5 ounces of fish. It was estimated that 76% of their diet came from bread and porridge. (Dyer, 1994¹⁰)

In Northern Europe, Rieska, unleavened barley bread, was the earliest bread in Finland. Bread made from barley, and ‘small beer’, a low-alcohol product from fermented malt, formed the staple diet of the common people in medieval England, and as late as the beginning of the 20th century it was the main food in rural Denmark (Munck 1977¹¹). However, since the 18th century higher yielding barley has been progressively replaced by wheat as a food staple in Western cultures. This is due to wheat’s elastic proteins, superior baking and milling characteristics, taste and tradition, as well as its decreasing cost due to progressively increasing yields through improved breeding (MacGregor, 1993¹²).

Today barley is used for food in greatest quantities in geographic areas where other cereals grow poorly due to altitude, latitude, low rainfall or soil salinity (Nilan & Ullrich 1993¹³). It is a common food source in semi-arid regions of North Africa, the near and Middle East, Russia, and former Soviet Republics in Central Asia, Mongolia, China, Korea, India and Afghanistan. Not surprisingly, the greatest per capita consumption today is found in those regions. According to FAO reports for 2002, the world’s highest contemporary per capita levels of annual barley consumption for food were recorded in Morocco (35.6 kgs), Moldova

(19.5 kgs), Latvia (19.5 kgs), Lithuania (17.8 kgs), Algeria (15.4 kgs), Estonia (13.4 kgs) and Ethiopia (12.9 kgs). (FAO Stat data 2005¹⁴)

In addition to these countries, barley is widely grown as a food and animal feed crop in the highlands of Tibet, Nepal, Mongolia, China, Ethiopia and the Andes, as well as in regions with short seasons and very long summer daylight periods, including the State of Alaska, Canada, Norway, Sweden, Russia, Denmark, Poland, Finland and the Baltic states. (Newman, 2008¹⁵)

Barley can be manufactured into numerous products or blended with other foods for human consumption. It is prepared for food by removing the hull (if present), grinding into grits or flour for flat bread or barley cakes, cooking as gruel or porridge, or boiling or par boiling the whole grain or pearled grain as it appears in various barley soups. Naked or hull-less types (hulls fall off in harvest, similar to wheat) are extensively grown for food in the former Soviet Union, the Himalayas, Pakistan, Manchuria, China and Korea.

Barley is widely used in traditional dishes in many countries e.g.: kasha in Russia and Poland; giotta in Switzerland, tsampa and others in Tibet; miso in Japan and Korea; and chapattis, and sattu or popped barley in India. Barley miso is a Japanese staple food and more than one million tons of barley is consumed in this form annually. In Korea, barley is the second most important food crop (after rice) and is used in several ways: pearled barley as a rice extender, pearled barley inoculated with *Aspergillus* sp. for the production of soy paste and soy sauce, roasted barley used as a tea or coffee substitute, and barley-wheat composite flour used for making cookies, cakes and noodles. (Bhatty 1993¹⁶).

In the West Asia-North Africa region, barley is consumed as pearled grain in soups, flour in flat bread and ground grain in cooked porridge. In Western countries, small quantities of barley (pearled) are used in breakfast cereals, soups, stews, porridge, bakery blends and for baby foods. Barley is valued for these applications because its starch and protein are more readily digested than that from wheat or maize, has far less gluten than wheat, and because its starch gelatinizes at a temperature approximately 10°C lower than starch from other cereals.

Porridge was a staple food in Denmark around 1900, with rural families consuming in excess of 100 Kg annually. (Munck, 1977¹⁷) Roasted barley can be used as a coffee substitute, as it was widely used in the US during WW II, and this use in the German Federal Republic increased substantially in the late 1950s and early 1960s. It also gained wider consumer rediscovery in Poland during the 1970s.

Barley consumption for food has declined in nearly all modern developed countries as urbanization; rising incomes and an expanding array of food choices have altered demand in the era since WWII through 1990. Since then increasing knowledge of barley as a healthy food has begun to boost demand back onto a slowly increasing trend per capita in Europe.

Per capita consumption there has risen from less than 1 kg in 1991 to 1.6 kg in 2002. (FAO Stat Data 2005¹⁸).

U.S. data indicates per capita consumption is holding stable at about 1.1 pounds through the most recent data up to 2009. This is down from a recorded high of 10.7 pounds per capita in 1947 according to the available data. (USDA ERS Food Availability Data Sets, 2011)

B. Consumption of Cereal Beta-Glucan in Enriched Flours, Concentrates and Isolates

The current era of renewed interest in cereal beta-glucans (particularly from oats and barley) stems from the 1970's and 1980's when researchers around the world took note of epidemiological data that suggested a strong correlation between increased consumption of these grains and reduced risks of chronic diseases, such as heart disease and diabetes. Over the past 30 years hundreds of papers appeared documenting clinical and scientific studies confirming beneficial health effects from oats, barley and fractions, such as beta-glucan. A substantial portion of the clinical studies have shown significant efficacy in reducing blood lipids associated with cardiovascular disease (CVD), in modulating blood glucose, and beneficially affecting satiety and body weight. Health Claims for reduction of risk factors for developing CVD have been approved by FDA in the U.S. and by EFSA in Europe for both oat and barley beta-glucans. (FDA, 2005)(EFSA 2011)

Beginning in the early 1990's and especially after the initial FDA Health Claim for oat beta-glucan in 1997 and subsequent amendment to include barley beta-glucan (2005), an array of cereal beta-glucan ingredients have been introduced in the market. They range from whole grain flours or brans with background levels of beta-glucans (3-12% BG), to enriched concentrates (15-35% BG), and to higher purity isolates (50% - 80% BG).

Notable among these are Oat Trim (BetaTrim™), a 5–25% BG concentration (GRAS) by Quaker –Rhodia, OatWell® a 15-28% BG concentration (GRAS), by Crea Nutrition - SOF, Barliv® Barley Betafiber, a 70% BG isolate by Cargill (GRAS), OatVantage®, a 54% BG isolate by GTC (GRAS), Sustagrain®, a 12-15% BG Flour by ConAgra (GRAS), Barley Balance®, a 25% + BG concentrate (GRAS) by PolyCell Technologies, and a number of others. All of these ingredients have been incorporated into breakfast cereals, bars, beverages, breads, pasta, pizzas, juices, dairy products, soups, sauces, biscuits, desserts, and many other foods. In nearly all instances, incorporation rates in foods and beverages are at or slightly above the level needed to provide the 0.75 grams of beta-glucan needed to conform to Health Claim requirements (1.0 grams in Europe).

No reliable data concerning current aggregate or per capita consumption of beta-glucans from these cereal sources is available. We are not aware of any report in which oat or barley β -glucan consumption been shown to be harmful.

7. Safe Exposure Assessment of Proposed General Food Use of GlucagelTM Barley β-glucan Fiber

The addition of GlucagelTM barley β-glucan fiber to foods is expected to occur at levels consistent within the normal range of 100% to 200% of the recognized health claim minimum of at least 0.75 grams of beta-glucan (1 gram of GlucagelTM) per serving. Precise levels of use within this range depend on the specific character of the food or beverage item and one or more of the technical self-limiting factors previously outlined in this document.

The food and beverage items shown in Table 4 are primary categories where the use of GlucagelTM is expected to occur. Due to the diversity of these items, consumers will be able to exercise selectivity in choosing specific items to be consumed at a single eating event and we expect them to do that. For example, it would not be expected that an individual would consume cooked cereal and salad dressing at a single meal, although he/she might consume both items at separate meals over the course of a day.

Estimates of consumption of individual food and beverage items for children 2-19 years and adults ages 20-59 years are presented in Table 5. These estimates use USDA ARS estimated 2 day average intake data at the 50th percentile and the 95th percentile to determine consumption estimates for GlucagelTM barley β-glucan fiber.

Table 4 – Common Foods and Beverage Products Where Glucagel™ May be Used

Food Type	Serving Size	Amount	Glucagel™ per serve	Beta-glucan per serve
Whole Grain Bread	25 grams	1 slice	1 gram	0.75 grams
Muffins	75 grams	1 muffin	2 grams	1.50 grams
Crackers	50 grams	5 crackers	1 gram	0.75 grams
Biscuits	40 grams	1 biscuit	1 gram	0.75 grams
Cooked Cereal	250 grams	1 bowl	2 grams	1.50 grams
Ready to Eat Cereal	30 grams	1 cup	1 gram	0.75 grams
Soups	250 grams	1 cup	1 gram	0.75 grams
Fruit Drinks	240 ml	1 beverage	1 gram	0.75 grams
Salad Dressing	30 grams	2 tablespoons	1 gram	0.75 grams
Beverage Mix (dry)	240 ml (as mixed)	240 ml	1 gram	0.75 grams
Pasta	100 grams	1 serve	1 gram	0.75 grams
Ice Cream	150 grams	1 cup	1 gram	0.75 grams

Table 5 – Estimates of Potential Consumption of Glucagel™ in Children and Adults

F o o d C a t e g o r i e s *

For Children

Ages 2-19 in grams/day

	Mean Food consumed (grams)	50 th percentile Grams consumed	95 th Percentile Grams consumed	Consumption of Glucagel™ at 50 th percentile (g)	Consumption of Glucagel™ at 95 th percentile (g)
Whole grain bread	52	39-55	73-115	1.6-2.2	2.9-4.6
Quickbreads and muffins	76.7	54-111	112-291	1.4-3.0	3.0-7.2
Crackers	27	13-32	35-89	0.3-0.6	0.7-1.8
Biscuits	49.3	30-59	77-118	0.8-1.5	1.9-3.0
Cooked cereal	244.5	215-246	353-493	1.7-2.0	2.8-3.9
Ready-to-eat cereal	48.5	30-62	61-125	1.0-2.1	2.1-4.2
Pasta	121.6	70-155	189-398	0.7-1.6	1.9-4.0
Soups	339.8	240-479	480-722	1.0-1.9	1.9-2.9
Fruit drinks	411.3	249-480	613-1124	1.0-2.0	2.0-4.7
Pourable salad dressing	27.4	14-39	32-79	0.5-1.3	1.1-2.6
Ice cream	143.1	66-198	175-385	0.4-1.3	1.2-2.6

Food Categories*

For Adults

Aged 20-59 in g/day

	Mean Food consumed (grams)	50 th percentile Grams consumed	95 th percentile Grams consumed	Consumption of Glucagel™ at 50 th percentile (g)	Consumption of Glucagel™ at 95 th percentile (g)
Whole grain bread	64.3	50-56	91-122	2.0-2.2	3.6-4.9
Quickbreads and muffins	89.7	112-129	114-171	2.9-3.4	3.1-4.7
Crackers	31.1	18-28	53-83	0.4-0.6	1.1-1.7
Biscuits	47.7	50-67	97-153	1.2-1.7	2.4-3.8
Cooked cereal	272.5	204-245	361-498	1.6-2.0	2.9-4.0
Ready-to-eat cereal	65.2	49-65	93-140	1.6-2.2	3.0-4.7
Pasta	134.4	105-147	277-418	1.1-1.5	2.8-4.2
Soups	419.9	360-467	569-962	1.4-1.9	2.3-3.8
Fruit Drinks	487.7	251-494	751-1081	1.1-2.1	3.1-4.5
Pourable salad dressing	41.2	31	87-113	1.0-1.0	2.9-3.8
Ice cream	167.5	132-172	251-398	0.9-1.1	1.7-2.7

* Selected food categories where beta-glucan may be added – serving sizes are from Table 4. These are the daily consumptions of these foods listed in a 2-day consumer dietary study. The amounts consumed are the actual amounts of ready to eat foods in grams. The 50th percentile and 95th percentile of consumption are taken from the USDA ARS Survey.

These foods were selected because they are made of ingredients that may commonly contain significant amounts of beta-glucans, or they are potential product application areas for products that may have Glucagel™ added (e.g. salad dressings, cereals, baked goods, dairy, soups, beverages).

Smiciklas-Wright, H., Mitchell D.C., Mickle S.J., Cook A.J., and Goldman, J.D. 2002. Foods Commonly Eaten in the United States: Quantities Consumed Per Eating Occasion and in a Day. 1994-96, **USDA ARS**

Safe intake levels of barley beta-glucan in food and beverage preparations have been established by animal studies of safety-toxicology, by other animal studies and by numerous human studies. A study by Jonker et. al²⁴, of Glucagel™ with Wistar rats found the No-Observed-Adverse-Effects-Level (NOAEL) at intake levels of 7.7 g Glucagel™, 5.8 g and β-glucan per kg of body weight in male rats (extrapolated to 580g for 100kg men) and 7.8 g Glucagel™, 5.9 g/kg body weight in female rats (extrapolated to 468g for 60kg women) rats per day respectively (Jonker, 2010²¹). Other studies by Delaney, et.al.²² established NOAEL at an average of 5.1 grams of beta-glucan per kg of body weight in rats, and 19.0 grams and 23.6 grams per kg of body weight in male and female mice. (Delaney 2003a²²) (Delaney, 2003b²³) These levels are many times greater than the expected maximum consumption of Glucagel™ barley beta-glucan fiber by humans in prepared foods and beverages.

In numerous human trials, intake of beta-glucans at levels ranging from 3 grams to 13 grams per day have shown beneficial effects on subjects,(studies of men, women, and mixed gender groups of differing ages) (AbuMweiss, 2010²⁴). Short term digestive tract discomfort associated with substantial increases in fiber consumption (for virtually any type of fiber) have been noted in some studies, with symptoms of discomfort rapidly diminishing after 48 hours of exposure. This is an expected response to increased fiber in the diet. Interestingly, subjects on both the test diet and the control diets experienced similar effects, and there was no significant difference between them.

In trials using Glucagel™, Smith et.al, reported that it was “well tolerated” and that there was no significant difference in GI symptoms between active (with 6 grams of Glucagel™) and control groups (Smith 2008²⁵). A study of satiety with 14 subjects, using a 3 gram inclusion of Glucagel™ in a beverage by Lumaga²⁶ et.al. reported “The possibility that gastro-intestinal discomforts might have reduced food intakes at both ad libitum lunch and over all the experiment day following meal 2 and meal 3 was negligible as subjects reported no gastro-intestinal symptoms related to their consumption”. (Lumaga, 2012²⁶) Vitaglione reported that Glucagel™ was well tolerated when included in bread at 3% (Vitaglione, 2009²⁷). A cholesterol study in Japan of 44 men, with test subjects consuming 7 grams of beta-glucan/day found that level was well tolerated. (Shimizu, 2008²⁸) In a glycemic study of 44 subjects ages 30-70, a total of 69 adverse events were noted, 17 (7 subjects) in the placebo group, 18 (7 subjects) in the 3 gram/day beta-glucan test group and 34 (10 subjects). The most common complaints were of diarrhea, abdominal distension and flatulence, with all events being mild and self limiting. There were no significant differences among the study groups. (Bays, 2011²⁹)

Consumption of Glucagel™ barley beta-glucan fiber at the 50th percentile and 95th percentile of individual food items consistent with cGMP is well within safe limits for intake of beta-glucan. Further, consumption of this material in any likely combinations of several prepared individual food items at these levels fall well within demonstrated safe limits for intake.

8. Biological Studies

β -glucan is not digested by mammalian enzymes so the traditional ADME (Absorption, Disposition, Metabolism, and Excretion) studies are not relevant to establishment of product safety. The result is that animal feeding tests are conducted with comparison of the organ pathologies of the test group to the control group which has been fed a standard animal diet of established safety

A. Animal studies with extracted barley β -glucan

Besides the extensive history of safe use, there have been many studies feeding barley and barley β -glucan to animals, mostly looking at cholesterol lowering. This history was extensively reviewed in GRAS Notifications # 207 and 344 both by Cargill Inc*

There are only a few studies that focus solely on toxicological markers. Besides the general studies of barley and barley β -glucan, the two GRAS notifications referenced above reviewed in detail the toxicological studies on BarlivTM (barley beta-fiber), the product that was the subject of the notifications. This product is purified from processed barley with enzymatic hydrolysis, followed by extraction with food grade ethanol. The process details are not in the published GRAS notifications.

1) Rat studies

Delaney et al, 2003b Food Chem. toxicology conducted a 28 day feeding trial in Wistar rats. The animals were acclimatized to the laboratory for one week. They were approximately 6 weeks old at the start of the study. The rats were given unique ear tattoos and sorted to 4 groups of 5 each proportionate to weight, the sexes were separately housed in macrolon cages with sterilized wood shavings for bedding. They were housed in a well ventilated room (10 air exchanges/ hour) at 22-23°C. The artificial lighting was on a 12 hour light/dark cycle. Feed was provided in stainless steel containers and with water in polypropylene bottles, available ad lib (except the night before sacrificing and necropsy).

The diets were Rat and Mouse #3 from Special diets Services Witham, UK with 20% of the standard diet replaced with 0, 1, 5, or 10% β -glucan plus 20, 19, 15, 10 % pregelatinized potato starch respectively making up the rest of the diets.

The rats were observed twice a day for general clinical observations and neurobehavioral evaluated weekly.

*FDA GRAS Notifications #207 (2006) AND #344 (2010) both by Cargill Inc
After dissection, the adrenals, brain, cecum (full and empty), epididymides, heart, kidneys, liver, ovaries, spleen, testes and thymus were each weighed after dissection.

Samples of the weighed organs and of the colon, gut associated lymphoid tissue including Peyer's patches, lymph nodes, lungs, mammary gland, peripheral sciatic nerve, esophagus, parathyroids, pituitary, prostate, rectum, small intestines, spinal cord, sternum with bone marrow, urinary bladder, stomach, thyroid, trachea with bronchi, urinary bladder, uterus, vagina, and all gross lesions were preserved at neutral pH in buffered 4% formaldehyde. Histopathology was also done on all preserved organs.

From all of these measurements, differences were observed in only two places: dose dependant increase in caecal weights – which is characteristic of studies where the test animals are fed fermentable carbohydrates – and an increase in the number of circulating lymphocytes.

2) Mouse studies

In a separate publication, Delaney et al 2003b³⁰, fed β-glucan to CD-1 mice, animals were sacrificed at 28 or 42 days and histopathology was performed on the lymphoid organs and lymph nodes proximal and distal to the exposure area. No treatment related inflammation or other effects were observed on mice of either sex at any time point at any dose level .

GRN notification # 207 also provides an excellent review of the literature on the immune modulating effects of β-glucan. There have been other publications in this area since GRN 207, including the extensive review of D. El Khoury et al 2012. B-glucans can be useful immunomodulators and may be useful in a diet for controlling inflammation.

8.2 Animal studies using Glucagel™.

A. Toxicology

A 28-day toxicity study was performed in specific-pathogen-free bred Wistar rats (Charles River Deutschland, Sulzfeld, Germany) – (Jonker et al 2010³¹). The six week old rats were acclimatized for two weeks before the beginning of the study. The rats were divided into four groups of five animals each, sex segregated, and housed in macrolon cages with wood shavings for bedding.

Housing was in a controlled environment: 20-24°C, humidity was kept in the 40-70% range, on a 12 hr light/dark cycle; the air was changed about 10 times / hour. Feed was provided as a pelletized powder in stainless steel cans, both were available ad lib except during times like motor systems assessment, collection of blood and urine, etc.

The diet was a cereal-based, closed formula rodent diet. The basal diet (RM3 Special Diets Services, Witham, England) was supplemented with Glucagel™ and/or pregelatinized potato starch. Glucagel™ levels were 0%, 1%, 5% and 10% of the diet. The control diet was the basal diet plus 10% potato starch. In the test diets Glucagel™

replaced the same % potato starch. The β -glucan in the final diet was measured by AOAC Official Method 995.16.

During the study, motor activity was monitored and ophthalmic evaluations were made before the start of the experiment and at week 4. The animals were weighed weekly with feed consumption measured every 3-4 days. On days 23 and 24, the rats were deprived of water for 24 hours and for food for the last 16 hours of this period. Urine was collected from rats caged individually for the last 16 hours. Urine samples were analyzed for pH, protein, glucose, ketones, bilirubin, urobilinogen, and occult blood.

After sacrifice the blood was collected and the plasma was analyzed for alkaline phosphatases, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, total protein, albumin, glucose, total cholesterol, triglycerides, phospholipids, creatinine, urea, inorganic phosphatase, calcium, chloride, potassium, and sodium.

The adrenals, brain, full and empty caecum, epididymides, heart, kidneys, liver ovaries, spleen, testes, thymus, and uterus were weighed as soon as possible after sacrifice and standardized to g/kg body weight. Samples of the weighed organs and aorta, colon, eyes, gut associated lymphoid tissue including Peyer's patches, lymph nodes (both axillary and mesenteric), lungs, mammary gland, thigh muscle, peripheral sciatic nerve, esophagus, oviducts, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands, small intestine, spinal cord, sternum with bone marrow, stomach, thyroid, trachea with bronchi, urinary bladder, and vagina. All gross lesions were preserved in phosphate buffered 4% formaldehyde at neutral pH. Samples were embedded with paraffin, sectioned, stained and examined by light microscopy.

A few statistically significant differences between control and test groups were found, but they were not dose response related, and occurred in one sex only. It was concluded that they were not treatment related.

No obvious signs of toxicity were observed in the study.

The 10% level was equivalent to an overall intake of 7.7 and 7.8 g GlucagelTM /kg body weight/day in male and female rats respectively. This provided 5.8-5.9 g β -glucan/kg body weight/day. This level is 100 times higher than the level recommended for lowering of cholesterol. The absence of adverse effects supports the conclusion that GlucagelTM is a GRAS substance.

2) Additional Animal Studies.

In addition to Jonker (2010³¹), six studies assessing the safety and efficacy of GlucagelTM β -glucan have been done in pigs, chickens and rats, and the results have been published in six papers (Bang et al, 2004³²; Maqueda de Guevara et al 2000³³; Maqueda de Guevara et al 1999³⁴; Morel et al 2001³⁵; Padilla et al, 2002a³⁶, b³⁷).

Bang et al (2004)³² conducted a rat feeding trial with 24 Sprague-Dawley male rats. The rats were fed a casein based diet for 7 days, then randomized into three groups, a control group with no added β-glucan (replaced by cellulose) and two β-glucan diets with Glucagel™, one prepared with the addition of cellulase (Roxdale sample) and one without added cellulase (Werribee sample). All animals were healthy and no abnormalities in gross pathology or signs of toxicity were observed. Every parameter measured was the same with the exception that the animals eating the Roxdale Glucagel™ had lower kidney weights and higher intestine weights. The authors suggest this result could be due to relative tastes of the β-glucans, food consumption was measured during the last 14 days only.

Two separate studies using broiler chickens (Padilla 2002a³⁶, b; Maqueda de Guevara, et al 1999³⁴) examined the effect of high levels of β-glucan on digestive process and mucin secretion. In his study (Padilla 2002(a)³⁶, fed 20 chickens diets including up to 60 g/kg of barley with high β-glucan, which did not produce significant changes in mucin levels in the digestive tract. In the Maqueda de Guevara³⁶ study, 10 chickens were fed a diet including 150 g/kg of barley containing high β-glucan, a level which produced significantly ($P \leq 0.05$) increased mucin secretion. In both studies, animals on the high β-glucan diets were able to digest these diets readily, and no significant adverse effects were found.

In other studies, Morel et al. (2005)³⁵ studied the impact of soluble and insoluble fiber on rates of growth, organ weights and blood lipids with 25 five week old weaner pigs, over a 21 day period. Barley β-glucan was given at dosages of 4.0 g/kg and 7.5 g/kg in the diet, and other fibers compared were resistant starch, and insoluble cellulose, with wheat starch as a control. The addition of β-glucan in the diet caused increased mucin secretion, and increased endogenous nitrogen losses, but did not significantly affect weight compared to control.

Padilla (2002a)³⁶ studied the impact of barley β-glucan on mucin production in chickens over 4 weeks, which included diets with insoluble and soluble fibers, including β-glucan. The inclusion of barley β-glucan (Glucagel™) showed a significant effect in increased mucin secretions in chickens, in comparison to other fibers – arabinoseylan and cellulose.

Padilla (2002b)³⁷ also studied the effect of β-glucan in comparison to other fibers, cellulose and arabinoseylan, in pigs. 25 young weanling pigs were randomly assigned to diets rich in cellulose, arabinoseylan or beta-glucan (4g, 7.5g) for a 21 day period. β-glucan did not significantly change mucin secretion from the cellulose control, and did not increase endogenous nitrogen flow at 7.5g of β-glucan. In both studies no significant adverse effects or pathology were noted during the trial.

A cholesterol study by Maqueda de Guevara et al. (2000)³³ used 36 - 4 week old Sprague-Dawley rats in a 2 x 2 factorial design randomly assigned to a diet rich in lipids from coconut or flax oil, with test animals also given 100g/kg barley β-glucan (Glucagel™) and control

animals receiving a cornstarch–casein mixture in each case. The 26 day study indicated that cholesterol levels were 10% lower for rats on the β -glucan diet, regardless of whether rats were getting coconut or flax oil. β -glucan decreased triglycerides significantly for both lipid regimes, by 13% ($P<0.05$) for flax oil and by 40% ($P<.001$) for coconut oil. Analysis of fecal matter indicated that a decrease in fat digestibility in the presence of β -glucans, particularly for the rats consuming coconut oil. No significant adverse effects were found during this study.

The safety of GlucagelTM has been evaluated in a published rat study by Bang et al, (2004)³². This study was conducted at the Massey University using twenty-four, 3 week old male Sprague Dawley rats in a 21 day feeding study. The study used barley β -glucan (GlucagelTM) of a molecular weight range that is already in the food stream from steeped, cooked, baked, and other barley products. (Bang, et al, 2004 see Appendix III for data).

Rats were housed in family weaning group cages and fed a commercial rat pellets ad libitum for seven days, transferred to individual cages with mesh floors, and then randomly assigned to three groups with 8 21-23 day old rats per treatment, fed a semi-synthetic preliminary casein base diet for seven days and then fed either 1) a specially formulated control diet with 130 g/kg of cellulose, 2) a diet with 30g/kg of cellulose and 100 g/kg of higher molecular weight commercial beta-glucan made with the GlucagelTM process, or 3) a diet with 30 g/kg cellulose and 100 g/kg of lower molecular weight barley beta-glucan prototype made with a modified process. Water was available at all times for all groups. Live weights were recorded, food intakes and fecal outputs were calculated every seven days. At the conclusion of the trial rats were evaluated at autopsy in the Massey University – Institute of Veterinary, Animal and Biomedical Sciences to determine any significant effects. Major organs were removed, weighed and measured for comparison by groups. The samples including blood were then subject to histological examination.

The conclusions of the autopsy evaluation were that all groups of rats were deemed to be healthy, with normal organ development and characteristics, and no evidence of safety issues or toxic effects was found in either placebo or active test groups. Rats in the control and GlucagelTM groups had similar results in terms of organ weights and weight gain, with the only significant difference being a lesser rate of food intake in the commercial GlucagelTM test group. This is consistent with observations of a satiety effect with barley β -glucan (Hecker et al, 1998³⁷, Maqueda de Gueverra et al, 2000) in animal trials. This did not significantly affect weight gain. Rats in the low molecular weight β -glucan group did exhibit lower rates of food consumption and has lesser rates of weight gain, indicating an increased satiety response.

Total cholesterol, HDL and LDL cholesterol and triglycerides were determined at the trial's conclusion. The two β -glucan fractions had no significant effect on serum cholesterol, HDL

cholesterol, or triglycerides. Food intake, weight gain and organ size was greater in rats consuming the higher molecular weight β -glucan diet and the cellulose diet, than in those rats consuming the lower molecular weight β -glucan diet. This was attributed to measured differences in levels of food intake, relating to: 1) to differences in palatability between diets, and; 2) an increased satiety effect for rats consuming the lower molecular weight diet. The rats on each of the diets remained healthy, had normal organ formation and color, and no adverse effects were found in autopsies.

8.3. Human trials with GlucagelTM and other concentrated barley β -glucans as the source of soluble barley fiber.

Given that barley beta-glucans have a long history in the human diet, testing with a primary objective of toxicology has not been done with GlucagelTM, or with any other concentrated barley β -glucan material, to the best of our knowledge. However, many studies have been done with GlucagelTM and similar materials where the primary objectives have been to assess the effects of β -glucans on blood lipids, blood glucose, satiety, weight control, digestive function and immune function.

These trials have been conducted with clinical protocols that include ongoing monitoring of adverse events among the subjects. Commonly reported adverse events were digestion symptoms associated with increasing levels of dietary fiber intake. These included fullness, abdominal distension (bloating), flatulence and increased stool frequency, and symptoms were observed in both experimental test and control groups. These symptoms were self-correcting within several days as adjustment to increased fiber intake occurred. This response has shown up in studies with a wide range of dietary fibers.

When compared to control, adverse events between the experimental groups and controls did not reach statistical significance, and a finding of no significant adverse effects was reported in each instance.

Table 7 – Human trials of Glucagel™ and comparable barley beta-glucan materials

Journal Year	Lead Author Location	Subjects Test material	Delivery Medium Protocol	Duration	BG per day	Parameters Measured	Significant Adverse Events
JACN, 2008	Smith,* U MN US	90 MF 64 F/26M 45 mean age BMI =26	beverage double blind crossover	6 wks	6 g	metabolic, TC, LDL, HDL, C reactive P, Appetite, satiety	No significant adverse effects reported
Food Function 2012	Lumaga* U of Naples Italy	14 MF 8 M, 6 F 24-29 years BMI 22.4	beverage single blind randomized crossover	4 wks	3 g	glucose, insulin, ghrelin, PYY, GLP-1, PP, appetite, satiety	No significant adverse effects reported
Appetite 2009	Vitaglione* U of Naples Italy	14 MF 7 M, 7 F Aged 20-29 BMI 22.9	Bread double blind randommixed crossover	4 wks	3 g	blood glucose, appetite, satiety, Insulin, ghrelin, peptide YY	No significant adverse effects reported
JACN 2010	Vitaglione U of Naples Italy	20 MF 10 M, 10 F mean age 18 BMI 23.2	biscuits randomized, single blind	2 wks	2g 3g	fullness, satiety, appetite, energy	No significant adverse effects reported
Nutrition 2011	Chillo* Oxford Bks University, U.K.	9 MF 3 M, 6 F ages 18-60 BMI>30 Glucose>6.1 mm	spaghetti single blind crossover	2 wks	1g 2g 3g 4g 5g	blood glucose, Glycemic index, glycemic reponse	No significant adverse effects reported
Nutrition Research 2009	Thondre** Oxford Bks University, U.K.	8 MF 3 M 5 F 26-50 years BMI >30	chapattis Randomized single blind crossover	2 wks	2g 4g 6g 8g	blood glucose, Glycemic index, glycemic resp	No significant adverse effects reported
AJCN 2003	Keogh, *** Auckland U NZ	18 M 18-65 years BMI 22-39 Mildly hyperC	various foods live in unit in M school - double blind random crossover	12wks	8.1 to 11.9 g/d	TC, LDL, HDL, TG, Blood glucose	No significant adverse effects reported
EJCN 2011	Rondanelli** U Pavia Med U Milan Med	24 M 50.3 mean age BMI 24.9 Mild HyperC	pasta rice cakes soup double blind, rdm crossover	14 wks	5.99 g	TC, LDL, HDL, apo A-1, apo A-1/ apo B, glucose	No significant adverse effects reported
Nutrition & Metabolism 2011	Bays Louisville US	44 MF - 68% F 56 yrs mean age BMI 32	beverage randomized double blind crossover	12 wks	0, 3 & 6g	Glucose Insulin	No significant adverse effects reported
BJN 2007	Keenan UMN US	155 MF	cereal beverage	10 wks	3g, 5g	TC, LDL, HDL, TG	No significant adverse effects reported
JACN 2004	Behall USDA MD US	18 M 45.6 years BMI 28.5 mild hyperC	various foods double blind, randon crossover	17 wks	3g, 6 g	TC, LDL, TG, VLDL, HDL,	No significant adverse effects reported
AJCN 2004	Behall USDA MD US	25 M/F 9 pre-m F, 9 post-m F, 7 M 40-53 years BMI >26 mild hyperC	Various foods double blind, randon crossover	17 wks	3g, 6 g	TC, LDL, TG, VLDL, HDL	No significant adverse effects reported
FASEB 2000	Pins U MN US	60 M/F 45 years BMI >25 mild hyperC	Muffins double blind, random crossov	10 wks	7 g	TC, LDL, TG, HDL, satiety, weight	No significant adverse effects reported

* representative studies using barley beta-glucan - Glucagel™

** representative studies using barley beta-glucan concentrate – starting material for Glucagel™

*** studies done with early pre-commercial prototype of Glucagel™

8.4. Findings of Updated Literature Search

1 For the current notification, DKSH did an update, comprehensive search of the scientific literature for safety and toxicology information regarding Glucagel™ and constituent beta-glucans from January 2011 through June of 2012. Three relevant studies were found in this search. One study was published by Bays, et. al. *Reduced viscosity Barley β-Glucan versus placebo: a randomized controlled trial of the effects on insulin sensitivity for individuals at risk for diabetes mellitus*, in Nutrition and Metabolism, 2011. This study examined glycemic and metabolic effects of a similar barley beta-glucan extract (Barliv®) which has previously been designated as GRAS. Beneficial effects on glucose metabolism were shown, while no evidence of safety issues were found. A Meta-analysis by Tiwari et.al., *Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels*, in Nutrition, 2011³⁹ analyzed 30 clinical studies done with oat and barley beta-glucan with emphasis on cholesterol and glucose effects. The third study is by El Khoury, et. al. *Beta-glucan: health benefits in obesity and metabolic syndrome*, J Nutrition and Metabolism, 2012⁴⁰. This is a review article summarizing research showing effects of beta-glucan on markers of obesity and metabolic syndrome. The latter two reports emphasized the beneficial effects of β-glucan containing foods.

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Appendices

OPINION OF AN EXPERT PANEL ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) USE OF GLUCAGEL™ BARLEY β -GLUCAN IN ALL FOODS EXCEPT INFANT FORMULA AND MEAT PRODUCTS

An independent panel of experts (the Expert Panel), qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was requested by PolyCell® (on behalf of DKSH Italia) to determine the Generally Recognized as Safe (GRAS) status of Glucagel™, a barley β -glucan product made from dry milled barley β -glucan and extracted with water. This process is facilitated by the use of a bran fraction of the milled barley and its concomitant enzyme activity.

A comprehensive report of the literature prepared by PolyCell™ was made available to the panel along with their draft of the GRAS dossier.

Anticipated interest from a food manufacturer in using Glucagel™ would be for products in keeping with the beta-glucan health claim for the purpose of aiding the consumer in maintaining a healthy cholesterol level through the functionality the ingredient brings to the food product, e.g. gelling, to a beverage.

The expert panel conferred by phone as well as through e-mail over the dossier and rewrote parts of the dossier for clarity and asked PolyCell for clarification of details of the processing and uses of Glucagel™ if they were not clearly spelled out in the dossier. The panel looked especially at the toxicology publications on Glucagel™ to compare the properties of Glucagel™ to the published research on the FDA accepted product Barliv™. Due to a lack of information related to Barliv™ a direct comparison was not possible on processing, so the processing focus was on Glucagel™ as well as the process for the Glucagel™ precursor, Barley Balance® - which is made in an FDA-inspected facility. Glucagel™ is made in an ISO 22,000 plant that also makes pharmaceuticals, by a HACCP-certified process.

During its review of the Barliv™ notification, FDA was especially concerned about the potential exposure of consumers to the product and asked Cargill for more exposure information about Barliv™. The same exposure tables were used by PolyCell™ for preparation of this GRAS Dossier to ensure that the breadth of potential exposure was adequately addressed.

Based upon its independent and collective evaluation of the information summarized in the attached report, the GRAS notifications on Barliv™, FDA's comments on those notifications, and the scientific literature and history of safe use, the expert panel concluded that the DKSH product meets appropriate food grade specifications, is produced using current Good Manufacturing Practice, and therefore is judged to be GRAS by scientific procedures for use in foods with the exception of infant formula and

meat products, and will likely be useful in promoting healthy cholesterol levels through consumption in amounts currently recommended for other GRAS materials.



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APPENDIX B – Animal Safety Studies

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28-Day oral toxicity study in rats with high purity barley beta-glucan (Glucagel™)

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ABSTRACT

Beta-glucans are glucose polymers present in cereal grains, particularly barley and oat. Consumption of these grains or concentrated beta-glucan preparations has been shown to lower blood cholesterol. The present study was conducted to assess the safety of a high purity (>75%) barley beta-glucan (Glucagel™). The product was fed to Wistar rats (5/sex/group) at dietary levels of 0% (control), 1%, 5% and 10% for 28 days. Clinical and neurobehavioural observations, growth, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, necropsy and histopathological examination revealed no adverse effects of Glucagel™. High-dose males exhibited lower plasma cholesterol and phospholipids levels and a higher plasma urea level. These slight changes were considered of no toxicological significance. Full and empty caecum weights were increased in mid- and high-dose males. This caecal enlargement was a physiological response to the consumption of a high amount of indigestible carbohydrate and considered of no toxicological concern. In conclusion, feeding Glucagel™ at dietary levels up to 10% for 28 days was tolerated without any signs of toxicity. This dietary level was equivalent to 7.7 g Glucagel™ (5.8 g beta-glucan)/kg body weight/day in male rats and 7.8 g Glucagel™ (5.9 g beta-glucan)/kg body weight/day in female rats.

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1. Introduction

Cardiovascular disease (CVD) is the number one cause of death globally and is expected to remain the leading cause of death in the foreseeable future. According to the World Health Organization (2007) an estimated 17.5 million people died from CVD in 2005 which represents 30% of all global deaths. Elevated blood cholesterol levels are an important risk factor for CVD, and hence one way to reduce the risk of developing the disease is to lower blood cholesterol levels by making dietary changes such as reducing intake of total fat, saturated fat, and dietary cholesterol (FDA, 1993; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). In addition, blood cholesterol can be reduced by the consumption of dietary fibres, especially from cereal sources rich in beta-glucan (Brown et al., 1999). Epidemiological studies have shown that consumption of dietary fibre from cereals is inversely associated with risk of coronary heart disease (Theuwissen and Mensink, 2008; Pereira et al., 2004).

Beta-glucans are glucose polymers found in the cell wall of cereal grains, particularly oat and barley and are characterized by groups of contiguous ($1 \rightarrow 4$)- β -linkages and isolated ($1 \rightarrow 3$)- β -linkages. Beta-glucan forms between 2% and 7% by weight of the cereal grain and content varies depending mainly on the cultivar type (Morgan, 2000).

The first report in free-living volunteers describing the effect of oats on serum cholesterol levels was published in 1963 (de Groot et al., 1963). Since then, a large number of human intervention studies have demonstrated that consumption of oats and concentrated beta-glucan preparations from oats lowers serum cholesterol concentrations in healthy normocholesterolemic and hypercholesterolemic subjects (Bell et al., 1999; Theuwissen and Mensink, 2008). Most studies on beta-glucan in oats produced positive results but some studies reported no effect. Fewer studies have investigated barley fibre since barley is less palatable than oats and a less common dietary component. Nevertheless, there is a significant body of research literature linking barley consumption to the lowering of blood cholesterol. In 2006, the US FDA authorized a health claim for beta-glucan soluble fibre from whole grain barley and certain dry milled barley products for lowering serum total and LDL-cholesterol levels, and hence reducing risk of CVD (FDA, 2005, 2006). In 2008, this health claim was extended to cover also a more highly purified barley fibre extract (FDA,

Abbreviations: CVD, cardiovascular disease; Anova, one-way analysis of variance; FOB, functional observational battery.

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2008). Very recently, the NDA panel of the European Food Safety Authority (EFSA) supported a claim on beta-glucans contributing to maintenance of normal blood cholesterol concentrations (EFSA, 2009). With few exceptions, published human intervention studies demonstrate that the consumption of barley products including purified barley derived beta-glucan is an effective dietary approach for lowering total and LDL cholesterol (Ames and Rhymer, 2008).

Barley and oat as well as beta-glucan derived from these sources or soluble fibres as such are generally considered safe, however, there is limited safety data from toxicological studies on effects of repeated dose intake of concentrated beta-glucan extracts. A 28-day feeding study evaluating toxicity of a concentrated barley beta-glucan in rats suggested no obvious signs of toxicity following consumption of high doses (Delaney et al., 2003).

The present study evaluated the safety of a high purity beta-glucan (Glucagel™, beta-glucan content >75%) that was isolated by a solely water-based process from barley. The results of the study will be used to widen regulatory approval for Glucagel™ internationally, e.g., to obtain GRAS status in the USA. The study was conducted in accordance with OECD Guideline for Testing of Chemicals 407 Repeated dose 28-day oral toxicity study in rodents (adopted 27 July 1995) and EC guideline B.7 Repeated dose (28 days) toxicity (oral), EEC Directive 96/54/EC, Official Journal of the European Communities, No. L248 (30.9.96). Ophthalmoscopy, urinalysis and histopathology of some additional organs, were conducted in accordance with the OECD Guideline for Testing of Chemicals 408 Repeated dose 90-day oral toxicity study in rodents (adopted 21 September 1998).

2. Materials and methods

2.1. High purity barley beta-glucan

High purity beta-glucan (Glucagel™) is typically extracted from waxy hull-less barley varieties in an organic solvent-free process described in US patent 6,426,201 B1. The high purity beta-glucan (GraceLinc Ltd., Auckland, New Zealand) is very stable (24 months when stored in original packaging at room temperature) and the composition is presented in Table 1.

2.2. Analysis of beta-glucan in rat feed

The diets used in this study were analysed for beta-glucan to demonstrate homogeneity, achieved concentration and stability of this substance in the carrier. Immediately after diet mixing, five homogeneity samples, taken at different locations from the test diets, and one sample of the control diet were stored at -18 °C. Additionally, one sample of each diet was stored for 4 days in the animal room (see below for conditions) and subsequently frozen (-18 °C). All samples were shipped (on dry ice) from TNO Quality of Life to the analytical laboratory in Kantvik, Finland.

The concentration of beta-glucan in rat feed was determined by using the AOAC Official Method 995.16. The feed samples were homogenized and milled, after which 80–100 mg of each blend was weighed into high glass test tubes. Ethanol (0.2 ml) (95%, v/v) was added to wet the samples and after mixing well 4 ml of phosphate buffer (20 mM, pH 6.5) was added and again mixed well with Vortex mixer. The tubes were placed in a boiling water bath for 1 min, mixed, boiled for an additional 2 min and then incubated 5 min at 50 °C (water bath) and mixed well. After that 0.2 ml of Lichenase enzyme solution (endo-(1 → 3)(1 → 4)-beta-D-glucan

4 glucanohydrolase) and magnetic balls were added to the solutions and the incubation at 50 °C was continued for 60 min and stirred for the whole time. Acetate buffer (5 ml) (20 mM, pH 4.0) was added and the samples were left to cool at room temperature for 5–10 min. After careful mixing, 1 ml of the samples was centrifuged 10 min at ca. 1000g. Next, 0.1 ml of each supernatant was transferred to the bottom of three separate test tubes. Only two of the tubes were treated with beta-glucosidase and the tube that was not treated yielded the blank value. 0.1 ml of 50 mM acetate buffer was added to the blank supernatant and 0.1 ml beta-glucosidase solution was added to the remaining two tubes. The incubation was continued for 10 min at 50 °C. Three milliliters of glucose oxidase-peroxidase-buffer mixture was added to each tube and incubated for 20 min at 50 °C.

The absorbance of the mixtures was measured at 510 nm against reagent blank and the content of beta-glucan in the rat feeds was determined using the equation:

$$\text{Beta} - d - \text{Glucan} (\%, \text{w/w}) = dA \times F \times 94 \times 1/1000 \times 100/W \times 162/180 \\ = dA \times F/W \times 8.46$$

where dA = absorbance sample minus absorbance blank; F = factor to convert absorbance value to μg glucose; 94 = volume correction factor (0.1 ml from 9.4 ml was analysed); 1/100 = conversion from μg to mg; 100/W = conversion to express beta-glucan content as percentage (w/w); W = sample weight in mg; 162/180 = factor to convert from free glucose (as determined) to anhydroglucose (as occurs in beta-glucan).

2.3. Experimental diets

A cereal-based, closed formula rodent diet (Rat & Mouse No. 3 Breeding Diet, obtained from SDS Special Diets Services, Witham, England) was used as the basal diet. The experimental diets were prepared by supplementing the basal diet with Glucagel™ and/or pregelatinized potato starch (Paselli WA4 from AVEBE, the Netherlands). Glucagel™ was incorporated at dietary levels of 1% (low-dose), 5% (mid-dose) and 10% (high-dose). The supplement in the low- and mid-dose diet was made up to 10% with potato starch. The control diet consisted of basal diet supplemented with 10% potato starch. Glucagel™ was incorporated at the expense of added potato starch to minimize differences in the levels of nutrients and other substances between the different diets. Nutrient levels in the basal diet are in excess of the requirement for rats and allow 10% dilution. One batch of the experimental diets was prepared about 2 weeks before the start of the study and stored frozen (-18 °C) in portions sufficient for 4 days. The feed in the animal feeders was refreshed twice per week.

2.4. Animals and maintenance

Specific-pathogen-free bred Wistar rats (Cr:(WI)WU) were obtained from Charles River Deutschland, Sulzfeld, Germany, and acclimated to the laboratory conditions for nearly 2 weeks. At the start of the treatment period, they were about 6 weeks old (body weight: males mean 162 g, range 148–174 g; females mean 122 g, range 110–131 g). One day before initiation of treatment, the rats were allocated to four groups of five rats per sex, proportionately to body weight, using a computer randomization program. They were housed under conventional conditions in macron cages (five/cage separated by sex) with stainless steel grid covers and wood shavings as bedding material, in a controlled environment (temperature 20–24 °C, humidity 40–70%, 12-h light (fluorescent tubes)/dark cycle, about 10 air changes per hour). Feed (provided as a powder in stainless steel cans) and drinking water (tap water complying with Dutch and European drinking water regulations, provided in polypropylene bottles) were available *ad libitum*, except during FOB testing and motor activity assessment and collection of blood and urine from fasted rats. The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 86/609/EEC) and Dutch legislation (The Experiments on Animals Act, 1997). This included approval of the study by TNO's ethical review committee.

2.5. Experimental design and observations

The study included four groups of five rats per sex which were kept on the appropriate experimental diet (0%, 1%, 5% or 10% Glucagel™) from the start of the study (day 0) until scheduled sacrifice on day 28.

The animals were observed daily for abnormal clinical signs. Neurobehavioural functioning was evaluated by weekly, detailed clinical observations made outside the home cage in a standard arena, and a functional observational battery (FOB) and motor activity assessment conducted on days 22–23. The FOB was based on that used in the WHO/IPCS Collaborative Study on Neurotoxicity Assessment (Moser and MacPhail, 1992; Moser et al., 1997a,b). The FOB consisted of non-invasive observational and interactive measures designed to assess the neurobehavioural and functional integrity of the rat, using measures taken from different functional domains including autonomic and neuromuscular function, sensorimotor reactivity, arousal and excitability. Spontaneous motor activity was assessed during a 30-min test period using an automated quantitative microprocessor-based video image analysis system (Ethovision, Noldus Information Technology b.v., the Netherlands). During the test period, the rats were placed individually in open roofed cages (48.8

Table 1
Composition of high purity barley beta-glucan (Glucagel™).

	Test method	Concentration (% as is, w/w)
Beta-glucan	Mod. AOAC 995.16	75.6
Total carbohydrate	By difference	89.4
Protein (including other nitrogenous material)	AOAC 981.10	4.2
Fat	AOAC 960.39	1.5
Ash	AOAC 942.05	1.0
Moisture	AOAC 950.46	3.9
Total		100

$l \times 44.7 w \times 50 h$ cm) equipped with a video camera suspended above the test cage. The position of the rat was monitored continuously. The total distance moved during the 30-min test period and habituation of motor activity (distance moved during six 5-min time blocks) were evaluated as measures of motor activity. Ophthalmoscopic observations were made before initiation of treatment in all rats and in week 4 in the control and high-dose group using an ophthalmoscope after induction of mydriasis by a solution of atropine sulphate.

Body weights were recorded on days – 1 (weight used for allocation), 0, 7, 14, 21, 27 and 28. Feed consumption was measured per cage over successive periods of 3 or 4 days by weighing the feeders. The intake of Glucagel™ per kg body weight was calculated from the nominal dietary levels of Glucagel™, the feed consumption and the body weight. Water consumption was measured per cage by weighing the drinking bottles daily during 5-day periods in weeks 1 and 3.

On days 23–24, all rats were deprived of water for 24 h and of food during the last 16 h of this period. Urine was collected from individual rats whilst kept in stainless steel metabolism cages, during the last 16 h of deprivation. The volume (graduated tubes) and density (refractometer; Bellingham and Stanley Ltd., UK) of the urine were measured to assess the renal concentrating ability. The appearance of the urine was recorded and the samples were analysed semi-quantitatively (Combur-9-Test strips; Roche) for pH, protein, glucose, ketones, bilirubin, urobilinogen and occult blood. Centrifuged sediment was examined microscopically. Routine haematology and clinical chemistry were conducted on all rats at the end of the treatment period. Following overnight fasting (water freely available), the rats were anaesthetised with CO_2/O_2 and blood was collected from the abdominal aorta into tubes with K₃-EDTA for haematology and heparin for clinical chemistry. Plasma was prepared by centrifugation. Haemoglobin, packed cell volume, red blood cells, reticulocytes, total and differential white blood cells and thrombocytes were measured with an Advia 120 Haematology Analyser. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated from haemoglobin, packed cell volume and red blood cells. Prothrombin time was measured using the Normotest for EDTA blood (Nyegaard and Co. A/S, Norway). Plasma levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, total protein, albumin, glucose, total cholesterol, triglycerides, phospholipids, creatinine, urea, inorganic phosphate, calcium, chloride, potassium and sodium were measured using an Olympus AU-400 analyser. The albumin/globulin ratio was calculated from total protein and albumin.

At the end of the treatment period (day 28), the rats were sacrificed by exsanguination from the abdominal aorta under CO_2/O_2 anaesthesia, and a thorough necropsy was performed. The animals were sacrificed in the morning, in such a sequence (stratified randomly) that the average time of sacrifice was approximately the same for each group. The adrenals, brain, full and empty caecum, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus were weighed (paired organs together) as soon as possible after dissection. The relative organ weights (g/kg body weight) were calculated on the basis of the terminal body weight. Samples of the weighed organs and of the aorta, colon, eyes, gut associated lymphoid tissue including Peyer's patches, lymph nodes (axillary and mesenteric), lungs, mammary gland (female), muscle (thigh), peripheral nerve (sciatic), oesoph-

agus, oviducts, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands (parotid, sublingual, and submaxillary), seminal vesicles with coagulating glands, small intestines (duodenum, ileum, and jejunum), spinal cord (three levels), sternum with bone marrow, stomach, thyroid, trachea with bronchi, urinary bladder, vagina and all gross lesions were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde. Samples of the preserved organs from all rats of the control group and the high-dose group were processed, embedded in paraffin, sectioned at 5 μm , stained with haematoxylin and eosin, and examined by light microscopy. Additionally, all gross lesions observed in rats of the intermediate dose groups were examined microscopically.

2.6. Statistical analysis

Body weights, haematology, clinical chemistry and urinalysis results and organ weights were evaluated by one-way analysis of variance (ANOVA) after checking for homogeneity of variances (Bartlett test) and normality of data distribution (Shapiro-Wilk's test). If variances were not homogeneous or data were not normally distributed, the data were stepwise log or rank transformed prior to the ANOVA. If the ANOVA showed a significant inter-group difference ($P < 0.05$), the test groups were compared with the control group by Dunnett's multiple comparison test. Neurobehavioural data were analysed by ANOVA followed by Dunnett's multiple comparison test (continuous FOB data, total distance moved), repeated measures ANOVA on time blocks (habituation of activity), Kruskal-Wallis non-parametric ANOVA (rank order FOB data) or Pearson chi-square analysis (categorical FOB data). Histopathological changes were analysed by Fisher's exact probability test. All analyses were two-sided. Probability values of <0.05 were considered significant.

3. Results

3.1. Analysis of beta-glucan in rat feed

Analysis of beta-glucan in rat feed confirmed that Glucagel™ was homogeneously mixed into the feed at all dose levels as shown by relative standard deviations of 3–5% (Table 2). The concentrations of beta-glucan measured were close to the expected levels, both after storage in the animal room for 4 days and after storage at -18°C for up to about 4 weeks, confirming stability under the conditions of the animal study. The control diet (containing no Glucagel™) was found to contain about 0.8% beta-glucan. This observation can be explained by the presence of beta-glucan in the basal diet which is a natural ingredient diet containing 64% cereals (barley and wheat). Additionally, some glucose may have been released from other dietary components during the analytical procedure.

Table 2
Concentration of beta-glucan in the diets used in the 28-day rat study with Glucagel™.

Level of Glucagel™ in the diet (% w/w)	Sample for determination	Measured beta-glucan concentration (% as is, w/w)	Mean \pm SD of five homogeneity samples (% as is, w/w)	Relative SD of five homogeneity samples (%)
0	4 days animal room stability	0.80 \pm 0.09		
	$t = 0$	0.81 \pm 0.06		
1	4 days animal room stability	1.47 \pm 0.08		
	Homogeneity 1/ $t = 0$	1.52 \pm 0.12	1.46 \pm 0.05	3.2
	Homogeneity 2	1.40 \pm 0.13		
	Homogeneity 3	1.43 \pm 0.07		
	Homogeneity 4	1.49 \pm 0.07		
	Homogeneity 5	1.45 \pm 0.09		
5	4 days animal room stability	4.13 \pm 0.17		
	Homogeneity 1/ $t = 0$	4.34 \pm 0.21	4.38 \pm 0.13	3.0
	Homogeneity 2	4.33 \pm 0.09		
	Homogeneity 3	4.56 \pm 0.59		
	Homogeneity 4	4.22 \pm 0.29		
	Homogeneity 5	4.46 \pm 0.19		
10	4 days animal room stability	7.68 \pm 0.84		
	Homogeneity 1/ $t = 0$	7.26 \pm 0.19	7.61 \pm 0.39	5.1
	Homogeneity 2	7.43 \pm 0.50		
	Homogeneity 3	7.54 \pm 0.45		
	Homogeneity 4	8.27 \pm 1.02		
	Homogeneity 5	7.57 \pm 0.32		

All diets contained 90% (w/w) basal diet (RM3 diet) and 10% supplement (Glucagel™ and/or potato starch). The level of potato starch was 10%, 9%, 5% and 0% in the control, low-, mid- and high-dose diet, respectively.

Dietary concentrations of 1%, 5% and 10% Glucagel™ provided 0.76%, 3.78% and 7.56% beta-glucan, respectively (based on 75.6% beta-glucan in Glucagel™).

Measured concentrations are mean \pm SD of three separate measurements of the same samples (conducted about 2, 3 and 4 weeks after diet preparation; samples were kept at -18°C immediately after diet preparation or after storage for 4 days in the animal room).

Table 3
Mean body weight, feed and water consumption and Glucagel™ intake of rats fed Glucagel™ for 28 days.

	Level of Glucagel™ in the diet			
	0%	1%	5%	10%
<i>Males</i>				
Body weight (g):				
Day 0	162 ± 9	162 ± 8	161 ± 9	163 ± 7
Day 27	294 ± 16	295 ± 11	294 ± 12	281 ± 11
Feed consumption, mean weeks 1–4 (g/rat/day)	18.4	19.3	18.4	17.5
Water consumption, mean weeks 1 and 3 (g/rat/day)	29.4	31.5	32.4	30.6
Intake of Glucagel™, mean weeks 1–4 (g/kg body weight/day)	—	0.82	4.0	7.7
<i>Females</i>				
Body weight (g):				
Day 0	121 ± 6	123 ± 6	122 ± 6	122 ± 4
Day 27	194 ± 7	197 ± 15	197 ± 12	186 ± 7
Feed consumption, mean weeks 1–4 (g/rat/day)	13.0	13.3	13.7	12.4
Water consumption, mean weeks 1 and 3 (g/rat/day)	21.8	26.2	23.4	23.5
Intake of Glucagel™, mean weeks 1–4 (g/kg body weight/day)	—	0.80	4.1	7.8

Body weight values are mean ± SD for groups of five rats. Statistical analysis (Anova), conducted on the results in the individual weeks, showed no significant inter-group differences. Feed and water consumption values are cage means (1 cage/sex/group). Glucagel™ intake was calculated from the mean body weight and feed consumption results in each week and the nominal dietary concentrations of Glucagel™.

Table 4
Haematology values of rats fed Glucagel™ for 28 days.

Level of Glucagel™ in the diet	RBC (10E12/l)	Hb (mmol/l)	PCV (l/l)	MCV (fl)	MCH (fmol)	MCHC (mmol/l)	Thrombocytes (10E9/l)	Prothrombin time (s)	WBC (10E9/l)	Lymphocytes (10E9/l)	Neutrophils (10E9/l)
<i>Males</i>											
0%	8.48 ± 0.31	9.6 ± 0.3	0.515 ± 0.014	60.7 ± 1.1	1.13 ± 0.03	18.6 ± 0.2	1042 ± 56	40.7 ± 1.7	12.5 ± 1.8	11.6 ± 1.6	0.56 ± 0.21
1%	8.38 ± 0.22	9.5 ± 0.2	0.501 ± 0.012	59.8 ± 1.3	1.13 ± 0.02	19.0 ± 0.2	1063 ± 102	38.2 ± 0.9*	12.8 ± 1.5	11.6 ± 1.6	0.84 ± 0.18
5%	8.61 ± 0.39	9.7 ± 0.1	0.513 ± 0.017	59.6 ± 1.2	1.13 ± 0.05	18.9 ± 0.6	1051 ± 75	38.4 ± 0.8*	14.0 ± 1.7	12.8 ± 1.8	0.90 ± 0.17*
10%	8.55 ± 0.42	9.7 ± 0.4	0.515 ± 0.024	60.2 ± 0.5	1.13 ± 0.03	18.8 ± 0.3	1018 ± 93	39.6 ± 1.6	12.1 ± 1.9	11.0 ± 1.9	0.78 ± 0.15
<i>Females</i>											
0%	8.41 ± 0.38	9.4 ± 0.3	0.482 ± 0.020	57.3 ± 1.4	1.12 ± 0.04	19.5 ± 0.2	1078 ± 66	34.7 ± 2.0	12.5 ± 1.8	11.5 ± 1.7	0.70 ± 0.17
1%	8.61 ± 0.35	9.7 ± 0.5	0.502 ± 0.028	58.3 ± 1.0	1.12 ± 0.02	19.3 ± 0.2	1027 ± 134	35.9 ± 1.7	12.9 ± 3.3	12.1 ± 3.1	0.46 ± 0.15
5%	8.57 ± 0.23	9.7 ± 0.4	0.500 ± 0.017	58.3 ± 1.0	1.13 ± 0.02	19.4 ± 0.2	957 ± 82	37.3 ± 2.0	11.0 ± 1.9	10.3 ± 1.7	0.40 ± 0.10
10%	8.77 ± 0.22	9.7 ± 0.4	0.498 ± 0.020	56.8 ± 1.3	1.11 ± 0.03	19.6 ± 0.1	908 ± 101	36.4 ± 2.2	11.1 ± 2.5	10.3 ± 2.4	0.50 ± 0.23

RBC, red blood cells; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin.

MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells.

Reticulocytes, eosinophils, basophils and monocytes were comparable in all groups (data not shown).

Values are mean ± SD for groups of five rats.

* P < 0.05 (Anova + Dunnett's test).

3.2. Clinical, neurobehavioural and ophthalmoscopic observations

All rats survived until scheduled termination. Daily general clinical observations, weekly detailed clinical observations, FOB testing and motor activity assessment revealed no treatment-related changes in the appearance, general condition or behaviour of the animals. Ophthalmoscopy showed no treatment-related changes either (data not shown).

3.3. Body weight, feed and water consumption and intake of beta-glucan

There were no statistically significant inter-group differences in body weight (Table 3). Feed and water consumption were not affected by the administration of Glucagel™ (Table 3). The overall mean daily intake of Glucagel™ in the high-dose group was 7.7 g/kg body weight in male rats and 7.8 g/kg body weight in female rats (for other groups, see Table 3). These intake levels of provided 5.8–5.9 g beta-glucan/kg body weight/day (based on 75.6% beta-glucan in Glucagel™).

3.4. Haematology, clinical chemistry and urinalysis

Haematology showed no treatment-related changes in red blood cell values, coagulation values, and total and differential

white blood cell counts (Table 4). A few statistically significant differences between the control and test groups (lower prothrombin time in low- and mid-dose males; higher absolute number of neutrophils in mid-dose males) were not ascribed to treatment because they showed no dose-related response and occurred in one sex only.

Clinical chemistry values showed statistically significant changes in the plasma levels of total cholesterol, phospholipids and urea in high-dose males (Table 5). Compared to the starch control group, high-dose males had lower cholesterol and phospholipid levels and higher urea levels.

Renal concentrating ability was not affected as indicated by the absence of treatment-related changes in the volume and density of the urine (data not shown). Semi-quantitative urinary observations and microscopic findings in the urinary sediment showed no treatment-related changes either. Compared with controls, urinary pH was statistically significantly higher (mean ± SD: 6.0 ± 0.0 versus 6.4 ± 0.4, P < 0.05). This was considered a chance finding because the difference from controls was small and not confirmed in the other sex.

3.5. Organ weights and pathology

The weight of the empty caecum was increased by about 35% in mid-dose males and by about 60% in high-dose males (Table 6).

Table 5
Clinical chemistry values of rats fed GlucageTM for 28 days.

Level of Glucage TM in the diet	ALP (U/l)	ASAT (U/l)	ALAT (U/l)	GGT (μmol/l)	Bilirubin (μmol/l)	Total protein (g/l)	Albumin (mmol/l)	Glucose (mmol/l)	Cholesterol (mmol/l)	Phospholipids (mmol/l)	Triglycerides (mmol/l)	Creatinine (μmol/l)	Urea (mmol/l)	P (mmol/l)	Ca (mmol/l)	Cl (mmol/l)	K (mmol/l)	Na (mmol/l)
Males																		
0%	187 ± 32	67 ± 7	47 ± 4	0.4 ± 0.4	1.9 ± 0.2	67 ± 2	34 ± 2	7.80 ± 1.57	1.97 ± 0.14	1.64 ± 0.09	0.87 ± 0.19	28 ± 2	5.9 ± 1.0	3.15 ± 0.10	2.99 ± 0.09	99 ± 1	5.6 ± 0.5	149 ± 1
1%	179 ± 25	58 ± 8	46 ± 11	0.8 ± 0.5	1.9 ± 0.1	66 ± 2	33 ± 1	7.52 ± 1.45	1.86 ± 0.12	1.53 ± 0.08	0.87 ± 0.16	29 ± 1	6.8 ± 0.4	3.09 ± 0.20	2.98 ± 0.03	98 ± 2	5.3 ± 0.2	149 ± 1
5%	210 ± 22	60 ± 6	49 ± 5	0.7 ± 0.5	1.8 ± 0.1	66 ± 2	33 ± 1	8.65 ± 1.66	2.04 ± 0.10	1.63 ± 0.08	0.81 ± 0.21	26 ± 1	6.9 ± 0.7	3.20 ± 0.23	3.00 ± 0.05	99 ± 1	5.0 ± 0.6	148 ± 1
10%	208 ± 29	56 ± 5	47 ± 2	0.7 ± 0.6	1.7 ± 0.1	63 ± 2	32 ± 1	8.34 ± 1.13	1.74 ± 0.12*	1.44 ± 0.09**	0.81 ± 0.15	28 ± 4	7.4 ± 0.5**	3.25 ± 0.10	2.95 ± 0.01	98 ± 1	5.2 ± 0.3	149 ± 1
Females																		
0%	120 ± 22	54 ± 7	33 ± 6	0.4 ± 0.6	2.0 ± 0.2	64 ± 3	34 ± 1	6.28 ± 0.77	2.11 ± 0.22	1.94 ± 0.24	0.96 ± 0.36	33 ± 4	9.9 ± 2.5	2.94 ± 0.22	2.94 ± 0.10	100 ± 1	5.4 ± 0.3	147 ± 1
1%	133 ± 15	47 ± 10	36 ± 8	0.4 ± 0.4	1.9 ± 0.2	65 ± 2	34 ± 1	7.29 ± 1.10	2.12 ± 0.19	1.87 ± 0.14	0.97 ± 0.11	31 ± 2	8.2 ± 0.9	2.98 ± 0.14	2.96 ± 0.08	99 ± 1	5.5 ± 0.4	148 ± 1
5%	136 ± 22	48 ± 15	38 ± 6	0.5 ± 0.5	1.8 ± 0.2	65 ± 2	34 ± 1	6.68 ± 0.69	2.16 ± 0.16	1.82 ± 0.12	0.94 ± 0.29	28 ± 3	7.2 ± 1.9	2.77 ± 0.16	2.90 ± 0.05	99 ± 1	5.4 ± 0.4	148 ± 1
10%	138 ± 16	56 ± 7	38 ± 9	0.4 ± 0.6	1.7 ± 0.2	65 ± 4	34 ± 2	7.79 ± 1.40	2.05 ± 0.26	1.71 ± 0.19	0.67 ± 0.07	30 ± 2	7.9 ± 1.6	2.71 ± 0.25	2.91 ± 0.07	100 ± 1	5.4 ± 0.2	148 ± 1

ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; GGT, gamma-glutamyl transferase; P, inorganic phosphate.

Values are mean ± SD for groups of five rats.

* P < 0.05.

** P < 0.01 (Anova + Dunnett's test).

Table 6
Terminal body weights and relative organ weights of rats fed GlucageTM for 28 days.

Level of Glucage TM in the diet	Terminal body weight (g)	Brain (g/kg bw)	Heart (g/kg bw)	Adrenals (g/kg bw)	Kidneys (g/kg bw)	Liver (g/kg bw)	Spleen (g/kg bw)	Thymus (g/kg bw)	Testes/ovaries (g/kg bw)	Epididymides/ uterus (g/kg bw)	Caecum full (g/kg bw)	Caecum empty (g/kg bw)
0%	274 ± 18	6.6 ± 0.4	3.5 ± 0.1	0.16 ± 0.02	65 ± 0.4	29.4 ± 1.5	1.94 ± 0.13	2.11 ± 0.25	11.2 ± 1.1	3.1 ± 0.3	16.2 ± 1.2	3.2 ± 0.2
1%	274 ± 8	6.5 ± 0.1	3.5 ± 0.2	0.16 ± 0.01	65 ± 0.2	30.3 ± 1.7	1.96 ± 0.12	2.08 ± 0.39	10.7 ± 0.6	3.1 ± 0.3	15.3 ± 2.6	3.2 ± 0.4
5%	274 ± 13	6.8 ± 0.2	3.7 ± 0.1	0.18 ± 0.02	66 ± 0.3	29.9 ± 1.1	1.95 ± 0.08	2.07 ± 0.33	11.0 ± 0.5	3.2 ± 0.2	22.5 ± 4.0**	4.3 ± 0.5**
10%	259 ± 14	6.9 ± 0.4	3.7 ± 0.2	0.17 ± 0.01	64 ± 0.5	29.6 ± 1.0	2.05 ± 0.17	2.22 ± 0.25	11.8 ± 1.0	3.3 ± 0.1	20.9 ± 1.9*	5.1 ± 0.5**
0%	180 ± 6	9.7 ± 0.3	3.9 ± 0.1	0.32 ± 0.04	7.2 ± 0.3	28.6 ± 1.5	2.36 ± 0.04	2.09 ± 0.24	0.42 ± 0.05	3.2 ± 2.2	18.0 ± 3.2	4.2 ± 0.8
1%	185 ± 15	9.3 ± 0.5	3.9 ± 0.3	0.33 ± 0.05	7.6 ± 0.9	28.8 ± 1.8	2.31 ± 0.20	2.17 ± 0.31	0.39 ± 0.07	2.4 ± 0.7	20.2 ± 3.0	4.5 ± 0.7
5%	182 ± 10	9.3 ± 0.4	3.9 ± 0.4	0.27 ± 0.04	6.7 ± 0.3	27.5 ± 1.3	2.20 ± 0.18	2.21 ± 0.31	0.35 ± 0.02	2.9 ± 1.5	17.4 ± 2.5	4.9 ± 0.9
10%	172 ± 7	9.6 ± 0.5	3.8 ± 0.2	0.29 ± 0.02	7.1 ± 0.4	27.2 ± 0.6	2.22 ± 0.22	2.17 ± 0.15	0.34 ± 0.06	3.7 ± 1.8	25.2 ± 10.4	5.2 ± 0.8

Values are mean ± SD for groups of five rats.

* P < 0.05.

** P < 0.01 (Anova + Dunnett's test).

The weight of the full caecum was also increased in these groups of males but not dose-dependently. The increase in full caecum weight was due to increases in the weights of both the caecal contents and the caecal wall (empty caecum weight). In females, mean full caecum weight in the high-dose group was higher (about 40%) compared with the control group but this difference was not statistically significant due to high inter-animal variation in the high-dose group. The weights of the other organs were unremarkable.

Gross and microscopic examination revealed no changes attributable to the administration of Glucagel™. The examination revealed only common background pathology findings which occurred incidentally or at similar or random incidences between the control group and the high-dose group (data not shown).

4. Discussion and conclusion

The feeding of Glucagel™ at dietary levels up to 10% to male and female Wistar rats for 28 days was well tolerated, as evidenced by the absence of adverse changes in the general condition and appearance of the animals, neurobehavioural end-points, growth, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights and pathology findings.

Rats fed Glucagel™ showed a few changes. The most obvious change was caecal enlargement as shown by the increase in the weight of the full and empty caecum in mid- and high-dose males. Caecal enlargement is a common response of rats to the feeding of large amounts of poorly digestible or slowly absorbable carbohydrates (Newberne et al., 1988). Unabsorbed, fermentable carbohydrate is degraded by microbial fermentation in the large intestine. The fermentation results in the production of short chain fatty acids which represent an increased osmotic load attracting water. This is considered a main cause of the distension and increased weight of the large intestine with its contents (De Groot, 1987; Leegwater et al., 1974; Walker, 1978). Glucagel™ contains about 75% of beta-glucan, an indigestible carbohydrate which has been shown to be fermented to short chain fatty acids by bacterial microflora (Casterline et al., 1997). Therefore, the caecal enlargement seen in this study was considered a non-specific, physiological response to the ingestion of non-digestible carbohydrate. Such caecal enlargement is generally considered of no toxicological concern (JECFA, 1974; World Health Organization, 1987). In the present study, this opinion was supported by the absence of histopathological findings in the caecal wall.

Lower plasma levels of total cholesterol and phospholipids were observed in high-dose males. Lowering of cholesterol is a well-known effect of beta-glucan in animals (Kalra and Jood, 2000; Maqueda de Guevara et al., 2000; Wilson et al., 2004) and is an intended effect of this substance in humans (Keenan et al., 2007; FDA, 2005). The lower plasma lipid levels of high-dose males were within the normal range for rats of this strain and age. Because, moreover, the changes in plasma lipids were not accompanied by any relevant changes in other end-points including histopathology, the lower plasma lipid levels in high-dose males were considered not to be toxicologically relevant.

Another observation in high-dose males was a higher plasma level of urea. An increase in plasma urea might reflect an effect on the kidneys. However, the values in high-dose males were in the normal range, there were no corroborative changes in other end-points for renal toxicity (plasma creatinine, weight and morphology of the kidneys, urinalysis) and a similar change was not observed in females. Therefore, no toxicological significance was attached to the elevated urea level in high-dose males.

The results of our study were in agreement with those of a similar 28-day feeding study in rats with the concentrated (64%) barley beta-glucan preparation Barley Betafiber (Delaney et al., 2003).

Similar to our results, the feeding of Barley Betafiber was associated with caecal enlargement and higher plasma urea levels which were in the physiological range and not accompanied by corroborating signs of renal toxicity. Unlike Delaney et al. (2003), we did not observe an increase in the number of circulating lymphocytes. To assess the relevance of the latter finding, Delaney and co-workers conducted a 28-day feeding study with Barley Betafiber in CD-1 mice. In the mouse study, Barley Betafiber did not cause treatment-related changes in any of the immune or other parameters examined. In a micronucleus study in CD-1 mice administered Barley Betafiber by oral gavage at levels up to 2 g/kg body weight (single dose) the product was neither clastogenic nor cytotoxic to bone marrow cells (Delaney et al., 2004).

In conclusion, this study showed that consumption of diets containing up to 10% Glucagel™ for 28 days was not associated with any obvious signs of toxicity in Wistar rats. The 10% dietary concentration was equivalent to an overall intake of 7.7 and 7.8 g Glucagel™/kg body weight/day in male and female rats, respectively, and provided 5.8–5.9 g beta-glucan/kg body weight/day. This intake level of beta-glucan is at least 100-fold higher than that recommended for lowering blood cholesterol (namely, at least 3 g per person per day, corresponding to 0.05 g/kg body weight/day for a person weighing 60 kg (FDA, 2005)). The absence of adverse effects of Glucagel™ in rats at an intake level 100-fold above the anticipated human intake level supports the conclusion that Glucagel™ is safe under the conditions of intended use.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Investigation into the Toxicology of Barley β -Glucan in Rats

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ABSTRACT

The effect of two forms of barley β -glucan isolates of differing molecular weights Weribee (128 000 Da) and Roxdale (50 000 Da) on weight gain, food intake and organ pathology were assessed in a rat feeding trial. Twenty four weaned Sprague Dawley male rats (21-23 days, 45-50 g) were fed a casein diet (containing 50 g kg⁻¹) for 7 days before being randomized to receive experimental diets containing 130 g kg⁻¹ cellulose or 30 g kg⁻¹ cellulose plus 100 g kg⁻¹ of either Weribee or Roxdale barley β -glucan for 14 days. All animals were then sacrificed and autopsied. During the trial, overall weight gain, final live weight and food intake were reduced in rats fed the β -glucan diets compared with rats fed the cellulose-based diet. At autopsy, organ color and texture appeared normal. Rats fed the Roxdale β -glucan diet, but not the Weribee β -glucan diet, had lower kidney weights and higher intestine weights (when expressed as a proportion of metabolic bodyweight) than rats fed the cellulose diets. All animals appeared healthy and no abnormalities in gross pathology, or signs of toxicity as a result of β -glucan ingestion were observed.

Keywords: Barley, β -glucan, toxicity, rat

INTRODUCTION

Barley grain (*Hordeum vulgare* L.) has been a traditional staple in many human societies. Indeed, barley is, and has been, widely cultivated as a food source, and its use as a food in early civilisations is well documented (summarized in MacGregor et al. 1993;¹ Rasmusson 1985²). However, whereas barley is still used for food in large quantities in regions where other cereals grow poorly due to altitude, latitude, low rainfall or soil salinity,³ it has been progressively replaced during the last 2 centuries by

wheat as a food staple in Western cultures.¹ This is due primarily to wheat's elastic proteins, superior baking and milling characteristics, taste and its reputation as a "high class" food.¹

More recently, research has suggested that dietary fibre, and more importantly soluble fibre, can have significant therapeutic effects.^{4,5} In particular, a number of clinical and animal studies have demonstrated that the non-starch polysaccharide mixed-linked (1→3)(1→4) β-D-glucans can have beneficial effects on human health including lowering of both low-density lipoprotein (LDL) and total cholesterol,⁶⁻¹² a satiety effect,^{10,13-16} an immune effect¹⁷ and effects on digestion/colitis.^{18,19}

However, whereas β-glucans occur in all cereals, their concentrations are generally highest in oats and barley (up to 16%)²⁰. As such, the use of barley as a food ingredient has once again increased in popularity, although the incorporation of large percentages of barley grain to produce high fibre foods is not always practical or desirable.¹³

On a per capita basis, in the US 2.9 kg of barley was consumed annually in food, food ingredients and beverages in 2000 (as compared to 14.5 kg (32 pounds) per capita in 1947)²¹. Furthermore, upwards of 70 kg of barley per person are consumed in countries such as Morocco and Estonia yearly^{21,22} and, with the exceptions of rare barley intolerance disorders (barley allergy, and celiac sprue), there is no published suggestion of its consumption being unsafe.

However, whereas a number of clinical studies have investigated the effect of barley β-glucan enriched diets (up to 13 grams per day) on physiological effects and no diet-related adverse events have been reported,^{10,11,14,16,23-26} the safety issues surrounding the use of extracted/purified barley β-glucans in foodstuffs have not yet

been established and barley β -glucans are not an established food ingredient. Thus this study was designed to assess the effects of barley β -glucan on aspects of organ physiology when fed as the main source of dietary fibre to rats.

MATERIALS AND METHODS

Experimental Design

The experiment involved a rat feeding trial to assess the effect of two forms of β -glucan, compared with cellulose (Avicel PH102, Commerical Minerals Ltd, Auckland, New Zealand), on animal health when used as the source of dietary fibre. Two β -glucan samples (both supplied by Graceline, Lincoln, New Zealand) were extracted from barley using the Glucagel® process,²⁷ and a modified Glucagel® process in which cellulase was added as a processing aid. The first extraction was carried out at the Food Science Australia pilot plant in Werribee, Victoria, and yielded a product with a weight average molecular weight of 128,000 Daltons, while the second sample was produced by Roxdale Foods, and had a molecular weight of 50,000 Daltons. Both products consisted of 75% β -glucan, 20% starch and maltodextrins, 4% protein and 1% ash. Eight rats were used for each dietary treatment, with all treatments run in parallel.

Experimental Animals

Weaned Sprague Dawley male rats (21-23 days, 45-50 g) were used in this feeding trial and were bred at the Food Evaluation Unit, Crop & Food Research, Palmerston North, New Zealand. The trial was carried out at the Food Evaluation Unit in a room maintained at a temperature of $22\pm1^\circ\text{C}$, humidity of $60\pm5\%$, air exchange of 12 times/hour, and with a 12 hour light/dark cycle. This study was carried out with

ethics approval from the Palmerston North Crown Research Institutes' Animal Ethics Committee.

Experimental Diets

The experimental diets were formulated to contain all nutrients in excess of the dietary requirements for the growing laboratory rat.²⁸ All the diets contained 130 g/kg diet of dietary fibre supplied by cellulose only or a combination of cellulose and β-glucan. Lactic casein (NZMP, Wellington, New Zealand; 160 g/kg diet) was the sole source of dietary protein. Flax oil (Waihi Bush Organic Farm, Geraldine, New Zealand; 150 g/kg) was the source of dietary lipid in the experimental diets while corn oil (Davis Trading Company, Petone, New Zealand; 65 g/kg) supplied lipid in the preliminary diet.

All samples were stored in the dark in the Food Evaluation Unit food store room, Crop & Food Research, Palmerston North, at 23±2°C until required for the preparation of experimental diets. The compositions of the experimental diets are given in Table 1. The compositions of the vitamin and salt mixes are as described in James et al. (1998).²⁹

Experimental Procedure

Twenty-four rats were housed in family weaning groups in shoebox cages and fed commercial rat pellets *ad libitum* for seven days. The rats were transferred to individual raised stainless steel cages with mesh floors, and then randomly allocated to the experimental treatments. All rats were fed a semi-synthetic preliminary casein (120 g/kg casein, 50 g/kg cellulose) base diet (Table 1) *ad libitum* for seven days. The rats were then fed the experimental diets (Table 1) *ad libitum* for 14 days. Water was available at all times. Liveweights were recorded for each rat every seven days. Food

Table 1: Ingredient compositions (g kg⁻¹ diet) of preliminary and experimental diets.

Ingredient	Preliminary casein diet	Experimental diets		
		Cellulose Diet # 838	β-glucan Werribee Diet # 839	β-glucan Roxdale Diet # 840
Lactic casein ¹	120	160	160	160
Flax oil ²	-	150	150	150
Corn oil ³	65	-	-	-
Vitamin mix ⁴	50	50	50	50
Salt mix ⁴	50	50	50	50
Sucrose ⁵	80	70	70	70
Starch ⁶	585	390	390	390
Cellulose ⁷	50	130	30	30
β-glucan, Werribee ⁸	-	-	100	
β -glucan, Roxdale ⁸	-	-	-	100

¹ Lactic casein 60 mesh, Alacid, New Zealand Milk Products, Wellington, NZ

² Flax seed oil, Waihi Bush Organic Farm, Geraldine, New Zealand

³ 'Davco' brand, Davis Trading Company, Petone, NZ

⁴ As specified in James et al (1998)

⁵ Chelsea Sugar Refinery, Auckland, NZ

⁶ Wheaten cornflour, Goodman Fielder Industries Limited, Summerhill, NSW, Australia

⁷ Avicel PH102, Commercial Minerals Ltd, Auckland, New Zealand

⁸ Gracelinc, Lincoln, Auckland

intakes were calculated every seven day period for the final 14 days only. This is essentially the procedure for the measurement of Protein Efficiency Ratio (PER) as described in Method 960.48³⁰ but with specific details described in James & MacColl (1991).³¹

At the end of the 14 day experimental period, the rats were fasted overnight (from 16:00h), anaesthetised the following morning with diethyl ether, and killed immediately by decapitation. The partially dissected bodies were labelled and chilled (5°C) before being taken for autopsy to the Post-mortem Room, Institute of Veterinary,

Animal and Biomedical Sciences, Massey University. A routine autopsy technique enabling visual assessment of all major organs was performed on each rat. Three batches of eight rats at a time were examined to allow for the comparative assessment of organ colour and texture. The weights of liver, spleen, kidneys and small intestine were measured. These, with the exception of the pancreas (which in the rat is a diffuse organ), were first dissected free of mesenteric attachments. The pancreatic sample contained all structures of the mesoduodenal fan (i.e. pancreatic, mesothelial, connective, lymphoid, and fatty tissues). Brain (cerebral cortex/thalamus and cerebellum/medulla oblongata), lung, liver, kidney, spleen, thymus, stomach (fundus), small intestine, (duodenum, jejunum, ileum), large intestine, pancreas, caeco-colic lymph nodes, cardiac muscle, skeletal muscle and adipose tissue were stored in buffered 10% formalin solution for elective histological examination.

Statistical Analysis

A one-way analysis of variance (ANOVA) was carried out to test for the effect of dietary treatment on final liveweight, weight gain, food intake, organ weights and on the ratios of weight gain:food intake. Where the ANOVA indicated a significant effect of diet, comparisons were made among the treatments using the least significant difference calculated at the 5% level of significance. For each ANOVA, residual plots were studied to check the assumptions of homogeneity of variance and approximate normality. The statistical analyses were carried out using Minitab software.³²

RESULTS

The rats readily ate the experimental diets, grew well and were healthy during this study, except for two rats (#5 838₁, #9 839₅), that did not thrive during the course of the experiment. These rats did not gain weight or eat as well as the other rats and there were some differences in organ weights determined during autopsy. These differences did not appear to be caused by the experimental diets and the data for these two rats were deleted from subsequent analyses.

Weight Gain/Food Intake

Mean final live weights, weight gain, food intake and weight gain: food intake values for the three experimental fibre treatments are presented in Table 2. The rats fed the Roxdale β -glucan diet showed a lower overall weight gain (g/14 days; $p < 0.001$) and final live weight ($p = 0.004$) compared with rats fed the cellulose-based diet; however, no differences were observed between rats fed the Werribee β -glucan and cellulose diets.

Table 2: Final liveweight, weight gain and food intake data for rats fed the experimental diets.

Fibre source	Diet #	Final liveweight ¹ (g)	Weight gain ¹ (g/14 d)	Food intake ¹ (g/14 d)	Weight gain:food intake ¹
Cellulose	838	213 ^a ± 8.9	114 ^a ± 5.7	257 ^a ± 16.3	0.44 ± 0.022
β -glucan, Werribee	839	207 ^a ± 14.9	105 ^a ± 10.1	234 ^b ± 19.9	0.45 ± 0.014
β -glucan, Roxdale	840	189 ^b ± 13.7	92 ^b ± 8.8	207 ^c ± 18.1	0.45 ± 0.009
Significance ²		P=0.004	P<0.001	P<0.001	P=0.806
Least significant difference (df=19) ³		14.3	9.4	20.3	0.017

¹ Arithmetic mean ± standard deviation, n=7, except for β -glucan, Roxdale where n=8.

² Analysis of variance was carried out on the weight gain and food intake data to test for the effect of dietary treatment.

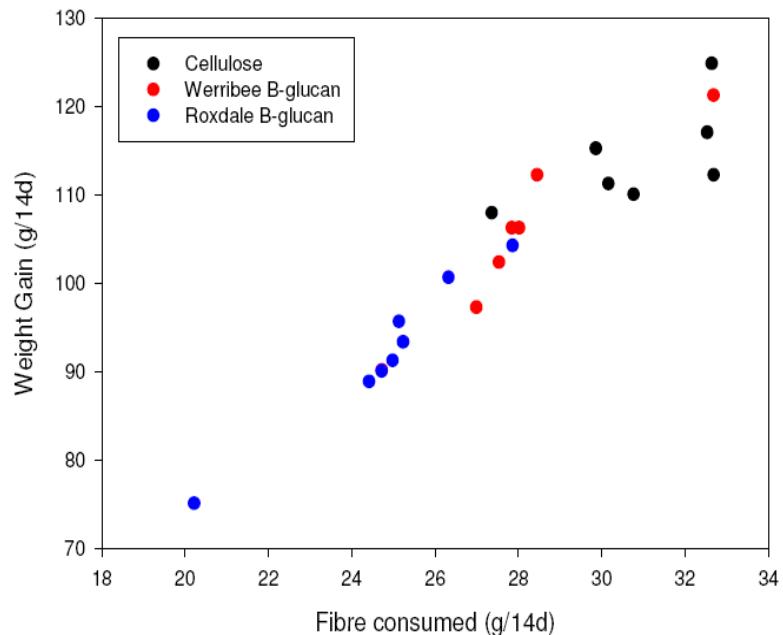
³ Comparisons were made among the dietary treatments using the least significant difference calculated at the 5% level of significance. Values with the same superscript are not significantly different at the 5% level.

Similarly, food intake was significantly reduced in rats fed the Roxdale β -glucan diet compared with the cellulose treatment ($p < 0.001$). Furthermore, rats fed on the

Werribee β -glucan diet also showed a significant reduction in food intake compared with the rats fed the cellulose diet ($p < 0.001$). Despite this, there was no significant effect of treatment on weight gain:food intake.

The dosage of β -glucan consumed by rats fed the Werribee diet and the Roxdale diet was similar during both week 1 (9.7 vs 9.3 g/7 days) and week 2 (11.9 vs 11.4 g/7 days). Furthermore, an analysis of overall weight gain (g/14 days) versus fibre dose consumed per 14 days (g) suggested that there were no adverse interactions at the higher β -glucan intakes when compared with cellulose intake (Figure 1).

Figure 1: Weight gain (g/14 days) versus fibre intake (g/14 days) for rats fed the cellulose (130g cellulose/130g fibre), Werribee β -glucan (100g β -glucan/130g fibre) and Roxdale β -glucan (100g β -glucan/130g fibre) diets.



Autopsy Results

There were no appreciable differences in organ colour or texture observed at autopsy. Mean gastrointestinal organ weights recorded during autopsy for the three experimental fibre treatments are presented in Table 3. Rats fed the Roxdale β -glucan diet, but not the Werribee β -glucan diet, had significantly lower kidney, liver and spleen weights than rats fed the cellulose diets. The reduction in organ weights (12–18%) is comparable to the reduction in final liveweight (12%) and overall weight gain (20%) observed (Table 2), suggesting that the observed reductions in organ weight may be directly related to the reduction in rat bodyweight.

Table 3: Organ weight data for rats fed the experimental diets.

Dietary fibre source	Organ weight ¹ (g)				
	Intestine	Kidney	Spleen	Liver	Pancreas
Cellulose	5.62 ± 0.580	1.70 ^a ± 0.145	0.38 ^a ± 0.046	6.64 ^a ± 0.845	0.85 ± 0.278
β -glucan, Werribee	5.74 ± 0.597	1.62 ^a ± 0.065	0.37 ^a ± 0.060	6.79 ^a ± 0.956	0.66 ± 0.196
β -glucan, Roxdale	5.76 ± 0.510	1.40 ^b ± 0.091	0.31 ^b ± 0.044	5.79 ^b ± 0.415	0.73 ± 0.116
Significance ²	P=0.876	P<0.001	P=0.024	P=0.039	P=0.229
Least significant difference (df=19) ³	0.628	0.117	0.056	0.850	0.228

¹ Arithmetic mean ± standard deviation, n=7, except for β -glucan, Roxdale where n=8.

² Analysis of variance was carried out on the organ weight data to test for the effect of dietary treatment.

³ Comparisons were made among the dietary treatments using the least significant difference calculated at the 5% level of significance. Values with the same superscript are not significantly different at the 5% level.

Indeed, when mean gastrointestinal organ weights were expressed as a proportion of metabolic bodyweight, the spleen and liver weights were no longer significantly different between dietary treatments (Table 4). However, kidney weight

remained significantly lower in rats fed the Roxdale β -glucan compared with the cellulose diet ($p = 0.011$). Furthermore, rats fed Roxdale β -glucan also demonstrated significantly higher intestine weights expressed as a proportion of metabolic bodyweight than the rats fed the cellulose diet ($p = 0.024$). There was no significant effect of diet on spleen, liver and pancreas weights expressed as a proportion of metabolic bodyweight.

Table 4: Organ weight data expressed as a proportion of metabolic bodyweight for rats fed the experimental diets.

Dietary fibre source	Organ weight ¹ (g kg ^{-0.75})				
	Intestine	Kidney	Spleen	Liver	Pancreas
Cellulose	17.9 ^b ± 1.48	5.4 ^a ± 0.33	1.2 ± 0.14	21.1 ± 2.30	2.7 ± 0.90
β -glucan, Werribee	18.7 ^{ab} ± 1.63	5.3 ^a ± 0.30	1.2 ± 0.17	22.1 ± 2.38	2.2 ± 0.74
β -glucan, Roxdale	20.1 ^a ± 1.23	4.9 ^b ± 0.28	1.1 ± 0.12	20.3 ± 1.32	2.6 ± 0.46
Significance ²	P=0.024	P=0.011	P=0.130	P=0.233	P=0.352
Least significant difference (df=19) ³	1.614	0.341	0.162	2.266	0.795

¹ Arithmetic mean ± standard deviation, n=7, except for β -glucan, Roxdale where n=8.

² Analysis of variance was carried out on the organ weight data to test for the effect of dietary treatment.

³ Comparisons were made among the dietary treatments using the least significant difference calculated at the 5% level of significance. Values with the same superscript are not significantly different at the 5% level.

DISCUSSION

As the results from this study have shown, intake of reduced molecular weight β -glucan at a level of 100g/kg diet can lead to reduced food intake and weight gain without any significant toxicological effects. Furthermore (with the exception of two animals), the reductions in food intake, weight gain and organ weights were obtained in

animals that appeared healthy and ate well. There were no indications of the rats being harmed by the diets, and all rats demonstrated normal gross pathology at their time of death. Instead, it is likely that the barley β -glucan may have affected either the palatability of the diet or influenced the gut environment such that satiety was reached more quickly, thus reducing food intake and resultant weight gain. Similar effects upon appetite suppression and weight loss have been reported after consumption of barley β -glucan in a number of other animal^{13,33} and clinical^{10,14-16,34} studies.

It is important to note, however, that kidney and intestinal weights (when expressed as a proportion of metabolic bodyweight) were significantly lower and higher, respectively, in rats fed the Roxdale β -glucan treatment compared with rats fed the cellulose diets. An increase in organ weight could indicate increased size due to increased metabolic demand and decreased organ weight could indicate reduced size due to a lower metabolic demand. Unfortunately, urine and faeces were not collected during this study, so there is no way to assess the nitrogen balance (and therefore the differences in the metabolism) of these dietary treatments. These changes in organ weights did not appear to have any detrimental effects on the animals involved. A similar effect of gelling β -glucan on relative kidney weight has been noted in grower pigs (Morel, pers comm.) The significance of this reduction in relative kidney size is unknown.

Interestingly, this study also demonstrated that there were different effects on food intake, weight gain, liveweight and organ weight depending on whether the animals were fed the Roxdale or the Werribee β -glucan. Significant reductions in all parameters were observed in rats fed the Roxdale β -glucan (compared with the cellulose diet), whereas rats fed the Werribee β -glucan exhibited a significant decrease in food intake only. However, as the data in Tables 2 show, final liveweight and weight gain

values were still numerically reduced in rats fed the Werribee β -glucan compared with the cellulose diet. It is possible, therefore, that the difference observed with the two β -glucan diets and with the cellulose may simply be due to palatability. Rats are simple animals that do alter their eating patterns depending on the type and taste of the food. The cellulose diet, for example, may have tasted better and was therefore consumed to a greater extent leading to a higher weight gain and final live weight. In contrast, the Werribee β -glucan diet was less palatable, leading to reduced intake, but still better than the Roxdale β -glucan diet.

However, given the larger intestines of rats fed the Roxdale β -glucan diet, it would appear that this is not the only explanation. Once consumed, β -glucan generally absorbs water in the gut, thereby gelling and increasing in size.⁹ This gelling/increased viscosity could lead to a satiety effect as discussed earlier, or it could trap nutrients thereby making them unavailable for absorption.

And as the two β -glucan samples were of different molecular weight, and therefore might exhibit different degrees of gelling, this may also explain why the two diets had different effects on food intake, weight gain etc. Other influences may include extent, rate and locus of β -glucan fermentation, influence on the digestibility of protein, lipid or other polysaccharide, and acute or chronic influences on the immune systems of the animals.

The results from this study suggest that barley β -glucan is unlikely to have a detrimental effect on human health or any significant adverse effects. The concentration of β -glucan fed in this study (10%) far exceeds both that likely be consumed in normal food products, and the levels that have been historically consumed as a result of having barley as a diet constituent. Indeed, the anticipated uses of β -glucan added to commercial food products is likely to be at low levels (0.5g to 3 g per serving), and well

below the fibre intake recommendations (38 g/day men, 25 g/day women) from the US National Academy of Sciences.³⁵ These levels are based upon food product testing that shows the food products to be technically achievable and palatable³⁶ and on the levels required to cause physiological effects.

A number of other studies have also reported on the safety of β-glucan consumption in humans.^{10,11,24-26} Dosages in these studies ranged from 3 to 13 g/day and only minor discomforts resulting from the increased fibre intake were reported (fullness, bloating, gas, loose stools). In additional animal studies, Morel et al (2001)³⁷ demonstrated that diets containing up to 7.5% barley β-glucan had no effect on organ size (except relative kidney size; Morel et al, unpublished) or blood lipid parameters when fed to weaner pigs over a 3-week period and Delaney (2004)³⁸ has recently demonstrated that concentrated preparations of barley β-glucan (~ 70% β-glucan) have no cytotoxic effects in mouse bone marrow cells when fed at a level of 74–2000 mg/kg of body weight (single dose). Furthermore, no treatment-related adverse effects were observed in hematological or clinical chemistry measurements or in organ weights and immunopathology after repeated dosing of β-glucan (0.7–10%) in CD-1 mice³⁹ or during feeding (0.7–10% β-glucan) in Wistar rats⁴⁰ over 28 day periods.

In conclusion, this study demonstrates that there are no significant safety issues attributable to β-glucan intake, even with relatively high levels of consumption. These findings are in agreement with other animal and clinical studies which have demonstrated significant effects on physiological endpoints.

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Appendix C - Glucagel barley beta-glucan fiber – analytical testing

1. Mycotoxins

Form A - TRP1
01/2004

VIMTA LABS®
Determining Quality

Analytical Division, Lifesciences facility : 5, Alexandria Knowledge Park, Genome Valley,
Hyderabad - 500 078, RR District, India
Ph : 91-40-6740 4040, Fax : 91-40-2726 3657, Email : quality@vimta.com

TEST REPORT

Report Number : 10194/11/VLL/000/01

Issue Date : 2011-10-05
Your Ref : PO NO:4503013755
and Date : 2011-08-17

Sample Particulars: GLUCAGEL

Sample Received date : 2011-09-03 Sample Registration Date : 2011-09-05

Analysis Starting date : 2011-09-23 Analysis Completion date : 2011-09-30

Sample Name : Glucagel; Batch No : GCL3180711;
Quantity received : 100gms x 1No;
Mfg date : July 2011, Retest date : June 2014;
Tests required : Ochratoxin A,Aflatoxin B1,Sum of Aflatoxin B1,B2,G1,G2,Zearalenone;
SAMPLE TESTED AS RECEIVED LAB REFERENCE_TAL/4056048

TEST RESULTS

Sl. No	Test Parameters	Unit of Measurement	Result
1	Zearalenon	ppb	Not detected
2	Ochratoxin A	ppb	Not detected
3	Aflatoxin B1	ppb	Not detected
4	Sum of Aflatoxin B1,B2,G1,G2	ppb	Not detected

Remarks : Instrument used :LC-MS/MS(AB Sciex,API 3000).
Detection limit : For Total Aflatoxins : 1ppb,For Ochratoxin A : 10 ppb,For Zearalenon : 5ppb;

Verified on : 03/10/2011
Verified by (QA) : *[Signature]*
[Signature]
A RAJASEKHARA RAO

2. Water Testing

ALKEM LABORATORIES LIMITED
MANDVA

MASTER



POTABLE WATER ANALYSIS RECORD		
SOP Ref No.: QC-044	Page No.: 1 of 4	Form No.: QC-115/C-00
Sampling Point : SP-11 PW 259	A.R. No. : MC/PW/12/259	
Date of Testing : 21/03/2012	Date of Sampling : 21/03/2012	
Date of Reporting : 26/03/2012	Sampled By : Abhishek	
Quantity Sampled: 1100 ml	Microbiological Analysis: 100 ml	Chemical Analysis: 1000 ml
Tests	Standard	Results
Chemical Analysis :		
Description	Clear, colourless & odourless liquid.	clear, colourless and odourless liquid.
Total Dissolved solid	Not more than 500 ppm	209 ppm
Total Hardness	Not more than 300 ppm	85 ppm
pH	Between 6.0 and 8.0	7.0
Microbiological Analysis :		
(A) Total Aerobic Microbial count	Alert limit : 250 CFU/ml Action limit : 400 CFU/ml Pharmacopeial Limit : 500 CFU/ml	71 CFU/ml
(B) Test for Specified microorganisms		
<i>Escherichia coli</i>	Should be absent	Absent
<i>Salmonella species</i>	Should be absent	Absent
<i>Pseudomonas aeruginosa</i>	Should be absent	Absent
<i>Staphylococcus aureus</i>	Should be absent	Absent
(C) Total coliforms	Should be absent	Absent
Remarks: Sample conforms /does not conform to the specification RM/W-001/02		
Microbiologist: <i>Abhi</i>	Checked By: <i>AB</i>	
Date: 26/03/2012	Date: 26/03/2012	

3. Analytical

LL-MSF-5.10-01-03

Subject to Indore Jurisdiction

CERTIFICATE ISSUED TO

ALKEM LABORATORIES LIMITED , MANDVA
AT-NAUGAMA ON NH NO-8,
MANDVA, ANKLESHWAR, GUJARAT
393 010

CHOKSI
LABORATORIES LIMITED
(CORPORATE OFFICE & CENTRAL LABORATORY)
63, Manoramangaj, Indore - 452 001, INDIA.
Tel : (0731) 4243888 (30 Lines), Fax : (0731) 2490593
E-mail : indore@choksilab.com, Website : www.choksilab.com

CERTIFICATE OF ANALYSIS

Particulars of Sample Submitted

ture	: GLUCAGEL	Your reference No.	: NM
tch No.	: GCL6180711	Code No.	: A1719
te of Mfg.	: N/A	Date of Receipt	: 10/08/2011
ianity	: 500.0 GM	Our Reference No.	: 10968/11-12/R&D
cking	:	Page No.	: 1 of 1
aled/Unsealed	:	Date	: 25/08/2011
XP-DI	: NM	BATCH SIZE	: NM

Description : Off white powder.

Calorific Value	: 383.05 kcal/100 gm
Protein	: 3.48 gm/100 gm
Carbohydrates	: 90.91 gm/100 gm
Fat	: 0.61 gm/100 gm
Crude fibers	: Nil
Saturated fatty acid (By GC)	: -
Caprylic acid	: 4.13%
Capric acid	: 4.90%
Myristic acid	: 0.23%
Palmitic acid	: 21.85%
Stearic acid	: 68.89%
Lead, as Pb (By ICP-MS)	: 0.33 ppm
Cadmium (By ICP-MS)	: Not detected
Sugar	: Nil
Salt (as Sodium Chloride)	: 0.88%

Note : 1. Date of Completion of Analysis: 25/08/2011
 2. Method of Analysis as per In-House.
 3. The Customer has not requested/provided any specification.
 4. This certificate of Analysis is intended for Region Z only.

Verified on : 28.08.2011
 Verified by (QA) : [Signature]

for CHOKSI LABORATORIES LTD.,

Vishay
Authorised Signatory
Authorised Signatory

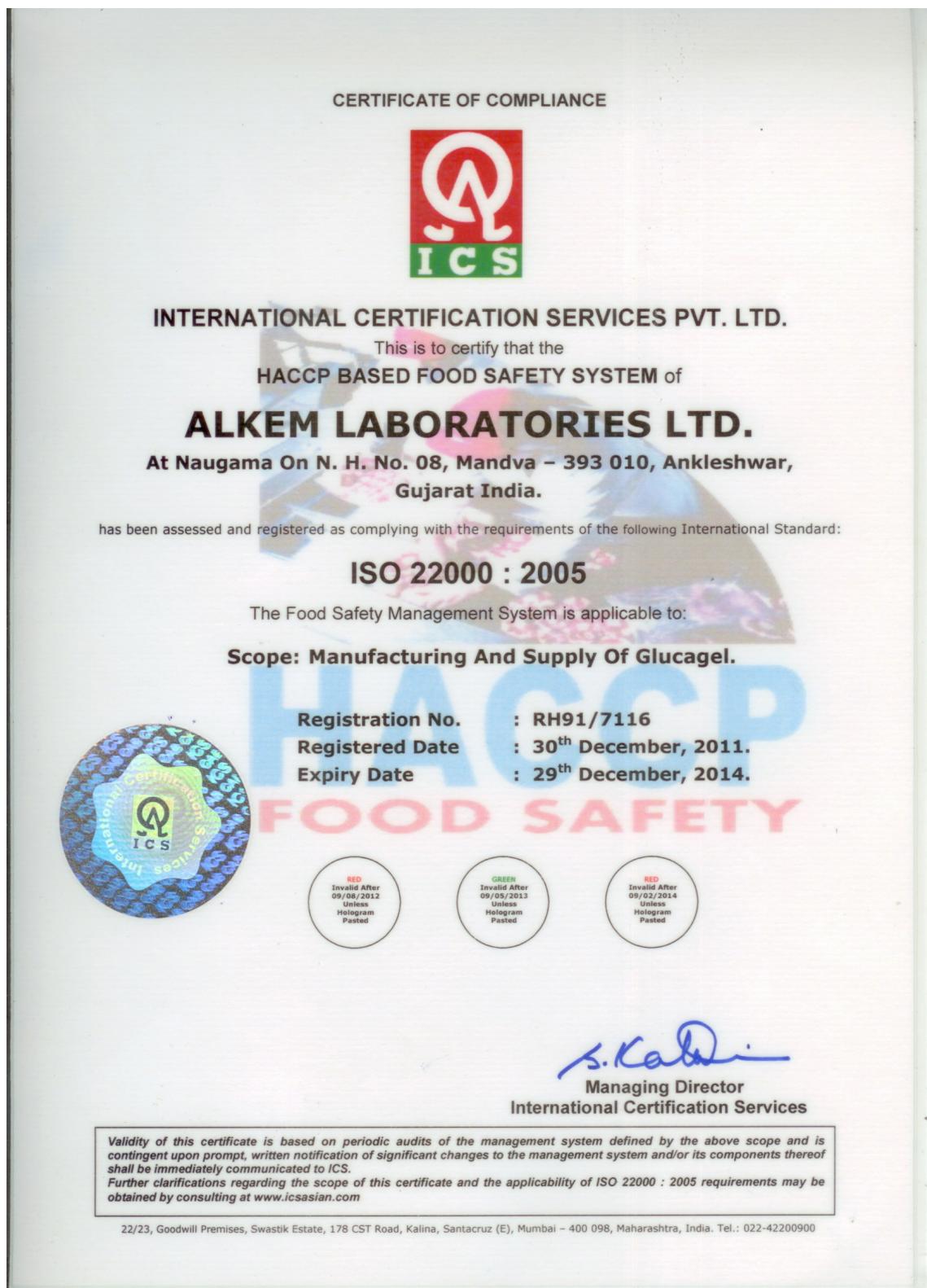
- 1. This report can neither be used as an evidence in the Court of Law, nor can it be used in part or full in any media without prior permission.
- 2. The result listed refer only to the tested samples and applicable parameters.
- 3. Perishable samples will be destroyed after testing; pesticide samples will be returned with the report.
- 4. Sample(s) not drawn by us, unless otherwise mentioned.

APPENDIX D - Certifications

ALKEM Laboratories – ISO 22000: 2005



ALKEM Laboratories – HACCP Certification



22/23, Goodwill Premises, Swastik Estate, 178 CST Road, Kalina, Santacruz (E), Mumbai – 400 098, Maharashtra, India. Tel.: 022-42200900

Alkem Laboratories – FDA Registration

DHHS/FDA Food Facility Registration

Page 1 of 3

DHHS/FDA - FOOD FACILITY REGISTRATION FORM

Please review the registration.

DATE: December 21, 2011 (MM/DD/YYYY)

Section 1 - TYPE OF REGISTRATION

Ia. Foreign Registration

Ib. FACILITY REGISTRATION NUMBER:
11371128512

PIN: B3Fe9Af7

Ic. PREVIOUS OWNER'S NAME:

PREVIOUS OWNER'S REGISTRATION NUMBER:

Section 2 - FACILITY NAME / ADDRESS INFORMATION

NAME: Alkem Laboratories limited, Ankleshwar, Gujarat, India

FACILITY STREET ADDRESS, Line 1: Alkem Laboratories Limited, Mandva,

FACILITY STREET ADDRESS, Line 2: Naugam, N.H. No. 8, Gujarat, India

CITY: Ankleshwar

STATE / PROVINCE: Gujarat

ZIP CODE (POSTAL CODE): 393010

COUNTRY/AREA: INDIA

PHONE NUMBER (Include Area/Country Code): 91 2646 284407 120

FAX NUMBER (OPTIONAL; Include Area/Country Code): 91 2646 284407

E-MAIL ADDRESS (OPTIONAL): pravin.patil@alkem.com

Section 3 - PREFERRED ADDRESS MAILING INFORMATION (Optional)

NAME: ALKEM LABORATORIES LIMITED.

ADDRESS, Line 1: Alkem Laboratories Limited, Mandva,

ADDRESS, Line 2: Naugam, N.H. No. 8, Gujarat, India

CITY: Ankleshwar

STATE / PROVINCE: Gujarat

ZIP CODE (POSTAL CODE): 393010

COUNTRY/AREA: INDIA

PHONE NUMBER (Include Area/Country Code): 91 2646 284407 120

FAX NUMBER (OPTIONAL; Include Area/Country Code): 91 2646 284407

E-MAIL ADDRESS (OPTIONAL): pravin.patil@alkem.com

Section 4 - PARENT COMPANY NAME / ADDRESS INFORMATION

NAME OF PARENT COMPANY: ALKEM LABORATORIES LIMITED.

STREET ADDRESS, Line 1: Alkem Laboratories Limited, Mandva,

STREET ADDRESS, Line 2: Naugam, N.H. No. 8, Gujarat, India

CITY: Ankleshwar

STATE / PROVINCE: Gujarat

ZIP CODE (POSTAL CODE): 399010

COUNTRY/AREA: INDIA

PHONE NUMBER (Include Area/Country Code): 91 2646 284407 120

FAX NUMBER (OPTIONAL; Include Area/Country Code): 91 2646 284407

E-MAIL ADDRESS (OPTIONAL): pravin.patil@alkem.com

Section 5 - FACILITY EMERGENCY CONTACT INFORMATION

INDIVIDUAL'S NAME (Optional): PRAVIN J PATIL

TITLE (Optional): DGM- QUALITY

EMERGENCY CONTACT PHONE (Include Area/Country Code): 91 2646 284407

E-MAIL ADDRESS (Optional): pravin.patil@alkem.com

Section 6 - TRADE NAMES

ALTERNATE TRADE NAME #1:
ALTERNATE TRADE NAME #2:
ALTERNATE TRADE NAME #3:
ALTERNATE TRADE NAME #4:

Section 7 - UNITED STATES AGENT

NAME OF U.S. AGENT: Tom Jorgens.	
TITLE (Optional): President	
STREET ADDRESS, Line 1: PolyCell Technologies, Valley Technology	
STREET ADDRESS, Line 2: park, 510 County Road 71, Crookston, MN	
CITY: Crookston	
STATE: Minnesota	ZIP CODE: 56716
U.S. AGENT PHONE NUMBER (Include Area Code): 612 2357207	
EMERGENCY CONTACT PHONE (Include Area Code): 218 2817071	
FAX NUMBER (OPTIONAL; Include Area Code): 218 4702005	
E-MAIL ADDRESS (Optional): tjorens@poly-cell.com	

Section 8 - SEASONAL FACILITY DATES OF OPERATION (Optional)

DATES OF OPERATION:

Section 9 - TYPE OF ACTIVITY CONDUCTED AT THE FACILITY (Optional)

Warehouse/Holding Facility (e.g. storage facilities, including storage tanks, grain elevators)
Manufacturer/Processor

Section 10 - TYPE OF STORAGE

Ambient Storage (Including heated Storage)
--

Section 11a - GENERAL PRODUCT CATEGORIES - FOOD FOR HUMAN CONSUMPTION

Food Additives, Generally Recognized as Safe (GRAS) Ingredients, or Other Ingredients Used for Processing

Section 11b - GENERAL PRODUCT CATEGORY - FOOD FOR ANIMAL CONSUMPTION**Section 12 - OWNER, OPERATOR, OR AGENT IN CHARGE INFORMATION**

NAME OF ENTITY OR INDIVIDUAL WHO IS THE OWNER, OPERATOR, OR AGENT IN CHARGE: Pravin Patil	
STREET ADDRESS, Line 1: Alkem Laboratories Limited, Mandva	
STREET ADDRESS, Line 2: Naugam, N.H. No. 8 Gujarat, India	
CITY: Ankleshwar	
STATE / PROVINCE: Gujarat	ZIP CODE (POSTAL CODE): 393010
COUNTRY/AREA: INDIA	
PHONE NUMBER (Include Area/Country Code): 91 2646 284407 120	
FAX NUMBER (OPTIONAL; Include Area/Country Code): 91 2646 284407	
E-MAIL ADDRESS (OPTIONAL): pravin.patil@alkem.com	

Section 13 - CERTIFICATION STATEMENT

The owner, operator, or agent in charge of the facility, or an individual authorized by the owner, operator, or agent in charge of the facility, must submit this form. By submitting this form to FDA, or by authorizing an individual to submit this form to FDA, the owner, operator, or agent in charge of the facility certifies that the above information is true and accurate. An individual (other than the owner, operator or agent in charge of the facility) who submits the form to the FDA also certifies that the above information submitted is true and accurate and that he/she is authorized to submit the registration on the facility's behalf. An individual authorized by the owner, operator, or agent in charge must below identify by name the individual who authorized submission of the registration. Under 18 U.S.C. 1001, anyone who makes a

materially false, fictitious, or fraudulent statement to the U.S. Government is subject to criminal penalties.	
NAME OF THE SUBMITTER: Pravin Patil	
CHECK ONE BOX:	
A. OWNER, OPERATOR OR AGENT IN CHARGE	
B. INDIVIDUAL AUTHORIZED TO SUBMIT THE REGISTRATION	
IF YOU CHECKED BOX B ABOVE, INDICATE WHO AUTHORIZED YOU TO SUBMIT THE REGISTRATION: OWNER, OPERATOR OR AGENT IN CHARGE (INDIVIDUAL AUTHORIZED TO SUBMIT THE REGISTRATION)	
FACILITY STREET ADDRESS, Line 1:	
FACILITY STREET ADDRESS, Line 2:	
CITY:	
STATE / PROVINCE:	ZIP CODE (POSTAL CODE):
COUNTRY/AREA:	
PHONE NUMBER (Include Area/Country Code):	
FAX NUMBER (OPTIONAL; Include Area/Country Code):	
E-MAIL ADDRESS (OPTIONAL):	

Barley Beta-glucan Concentrate – GMP/ HACCP Certification



Appendix B

Key Studies Cited

ORIGINAL ARTICLE

β -glucan from barley and its lipid-lowering capacity: a meta-analysis of randomized, controlled trials

SS AbuMweis¹, S Jew² and NP Ames²

¹Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, The Hashemite University, Zarqa, Jordan

²Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada

Background/Objectives: To more precisely quantify the effect of barley β -glucan on blood lipid concentrations in humans and to examine the factors that could affect its efficacy.

Subjects/Methods: Eleven eligible randomized clinical trials published from 1989 to 2008 were identified from nine databases. Weighted mean effect sizes were calculated for net differences in lipid profile using a random effect model (RevMan 4.2).

Results: Overall, barley and β -glucan isolated from barley lowered total and low-density lipoprotein (LDL) cholesterol concentrations by 0.30 mmol/l (95% confidence interval (CI): -0.39 to -0.21, $P < 0.00001$) and 0.27 mmol/l (95% CI: -0.34 to -0.20, $P < 0.00001$), respectively, compared with control. The pattern of cholesterol-lowering action of barley in this analysis could not be viewed as a dose-dependent response. There were no significant subgroup differences by type of intervention and food matrix.

Conclusions: Increased consumption of barley products should be considered as a dietary approach to reduce LDL cholesterol concentrations.

European Journal of Clinical Nutrition advance online publication, 6 October 2010; doi:10.1038/ejcn.2010.178

Keywords: meta-analysis; barley; β -glucan; cholesterol

Introduction

It is thought that barley cultivation began 8000–10 000 years ago in the 'Fertile Crescent' of the Middle East and thus barley has been considered to be one of the founding crops of Old World agriculture (Badr *et al.*, 2000). Barley consumption as food is still an important component for many regions, including several areas of North Africa and the Near East, the highlands of Central Asia, the Horn of Africa, the Andean countries and the Baltic States (2005). On the other hand, many Western countries now only use barley predominantly for animal feed and malting. However, there has

been renewed interest worldwide in barley as food due to its many health benefits, including its potential in reducing the risk of cardiovascular disease via cholesterol lowering (Behall *et al.*, 2004a, b; Keenan *et al.*, 2007; Shimizu *et al.*, 2008), and improvement of glucose tolerance (Pick *et al.*, 1998; Behall *et al.*, 2006; Hinata *et al.*, 2007). The active ingredient thought to provide barley its health benefits is β -glucan. β -Glucan is a type of soluble fiber, which is made up of unbranched polysaccharides with (1 → 4) and (1 → 3) linked β -D-glucopyranosyl units in varying proportions (Charalampopoulos *et al.*, 2002). Of cereal grains, oats and barley contain the highest level of β -glucan at 3–7 and 3–11% (dry weight basis), respectively (Charalampopoulos *et al.*, 2002). A meta-analysis by Ripsin *et al.* (1992) showed that the consumption of about 3 g/day of soluble fibers from oat products lowered serum total cholesterol concentrations by 0.13–0.16 mmol/l; likewise, barley is also now emerging to have similar health benefits. To date, a number of studies have been carried out to test the efficacy of barley or β -glucan derived from barley as cholesterol-lowering agents. However, some studies have demonstrated no benefits (Keogh *et al.*, 2003; Björklund *et al.*, 2005), whereas others have shown reductions in plasma lipids (McIntosh

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Contributors: SSA assessed the study quality, extracted data, performed statistical analysis, interpreted the results, and wrote and edited the paper. SJ was involved in literature search, electronic searches, study selection and quality assessment, data extraction and contributed to writing the paper. NA developed study concept, carried out initial study assessment and provided critical revision of the paper.

Received 28 October 2009; revised 8 June 2010; accepted 15 June 2010

et al., 1991; Li et al., 2003; Behall et al., 2004a,b; Keenan et al., 2007; Shimizu et al., 2008). The varying results in human studies may be due to factors such as dose size, food matrix, type of intervention, background diet and subjects' characteristics.

Meta-analysis is a statistical tool that generates pooled estimates of effects from the results of randomized, controlled trials (Pai et al., 2004). Therefore, a meta-analysis could be used to more precisely quantify the efficacy of barley and its products as lipid-lowering agents. Thus, the primary objective of this meta-analysis was to quantify the effect of β -glucan from barley on total and low-density lipoprotein (LDL) cholesterol concentrations, as well as on high-density lipoprotein (HDL) cholesterol and triacylglycerol concentrations. The secondary objectives of this meta-analysis were to test for the presence of a dose-response effect, and to identify and quantify the effects of food matrix, intervention type (barley vs β -glucan from barley) and background diet on the efficacy of barley as a cholesterol-lowering agent.

Methods

Search strategy

Nine electronic databases, including AGRICOLA, Agris, BIOSIS, CAB Abstracts, Foodline Science, Food Science and Technology Abstracts, PubMed, Scopus and Google Scholar, were searched using barley, cholesterol and heart/coronary disease terms to July 2008. Non-English-language articles were translated when possible.

Study selection

Studies conducted to examine barley and cardiovascular disease risk factors were first identified for this meta-analysis. Subsequently, all articles were reviewed to select studies with the following criteria: (1) randomized, controlled clinical trials with either a crossover or parallel design; (2) subjects from a healthy population, that is, not after myocardial infarction; (3) measured total and LDL cholesterol as outcomes; (4) subjects ingested β -glucan from barley; and (5) the intervention lasted for at least 3 weeks.

Validity assessment

Studies were then evaluated for study quality using a custom-built tool in collaboration with Health Canada. The custom-built tool was used to evaluate studies found via a systematic literature search that was conducted in order to review scientific evidence for a potential Health Canada health claim submission for barley β -glucan as a cholesterol-lowering food. The custom-built tool allocated a certain number of points for factors including: randomization (two points), subject-inclusion criteria (one point), measure of food exposure (one point), measure of health outcome (one point), justification of subject number/power to detect (one

point), description of background diet (two points), statistical analysis (two points), and accounted for confounding factors, including weight change, washout period and age (subtract up to three points). Studies were required to attain at least seven points to be deemed a pass to be included in the meta-analysis. These studies were then sent to expert reviewers for further re-evaluation. Expert reviewers were used to assess the data as per a step suggested in the Health Canada, 'Interim Guidance Document, Preparing a Submission for Foods with Health Claims.' (Health Canada, Bureau of Nutritional Sciences 2002). The reviewers chosen are all well-respected scientists in the field of nutrition and/or food science with emphasis on those who had an expertise in barley and soluble fiber. The reviewers were asked to comment on the following aspects: consistency of the observations and their effect, whether there is significant statistical evidence of lowering LDL cholesterol, dose-response relationship (for example— x causes y effect), feasibility of consuming the effective dose, target population that the evidence is aimed at (for example—general/hypercholesterolemic population), overall number or percentage of studies that show LDL cholesterol reduction and significance.

Data abstraction

For studies that met the inclusion criteria and that possessed a quality score of seven or more, data were extracted for parameters related to (i) trial design, (ii) type of intervention, that is, barley vs β -glucan from barley, (iii) dose (g/day), (iv) duration of treatment, (v) food matrix, that is, food carrier, (vi) characteristics of the study population, (vii) the mean values and the s.d. of lipid levels and (viii) sample size. Two reviewers independently extracted the data.

Quantitative data synthesis

For studies that reported multiple time points for the same subjects, only end points for the longest duration of the intervention were used (Li et al., 2003; Shimizu et al., 2008). In one instance, mean values and s.e. of the serum lipids were estimated from the figures because they were not reported in the text (Keogh et al., 2003).

The primary outcomes for this meta-analysis were the differences in total and LDL cholesterol levels due to barley treatment. For parallel arm designed trials, end points of lipid concentrations among the subjects ingesting barley were subtracted from those among the subjects consuming the control group (Deeks et al., 2005). For crossover trials, the lipid concentrations at the end of the treatment period were subtracted from those at the end of the control period (Deeks et al., 2005). Within-individual changes were used when presented; otherwise, group means were used. The s.d.'s were extracted from the studies or, if not reported, derived from s.e. of the mean, paired *t*- or *P*-value as provided (Deeks et al., 2005). For a number of studies (Keogh et al., 2003; Behall et al., 2004a,b) approximate paired analysis was performed

using imputed correlation coefficients describing the similarity of outcomes within each subject as was previously described (Deeks *et al.*, 2005). Within-individual correlations between the treatment and control periods were calculated from two studies (Clinical Study Report, 2005; Sundberg, 2008).

If different treatments were tested within the same trial, they were evaluated as separate strata, as is described by 'i, ii, iii and iv' suffixes in Tables and Figures. To obtain the pooled treatment effect size, effect size estimates and s.e. were entered into RevMan 4.2 under the 'generic inverse variance' outcome. Heterogeneity between trial results was tested for by using a standard χ^2 -test. A *P*-value <0.1 was used to indicate that significant heterogeneity was present (Deeks *et al.*, 2005). Calculations used in this meta-analysis were previously presented in more detail (AbuMweis *et al.*, 2008). Estimates of the pooled treatment effect sizes and 95% confidence intervals (CIs) were calculated by using both fixed effect and random effect models. If the test for heterogeneity was significant, we presented the results of the random effect models. Otherwise, estimated results based on a fixed effect model are presented. The presence of publication bias was examined using a funnel plot in which the s.e.'s of the studies were plotted against their corresponding effect.

Results

Characteristics of the studies

A total of 266 articles were identified from the first search strategy. Of these, only 11 studies with 17 strata met the *a priori* inclusion criteria and passed the quality assessment test (Figure 1). Table 1 describes the studies included in the calculation of the effect size. Seven studies utilized a crossover design, and four used a parallel design. The duration of the intervention lasted from 4 to 12 weeks and 10–62 subjects were enrolled in the studies. Mean age ranged from 20 to 63 years and mean baseline body mass index ranged from 19 to 35 kg/m². Five studies recruited only males and one study recruited only women, whereas the remaining studies comprised 28–52% male subjects. In five studies (McIntosh *et al.*, 1991; Keogh *et al.*, 2003; Clinical Study Report, 2005; Björklund *et al.*, 2005; Sundberg, 2008), the subjects had high blood cholesterol concentrations at baseline according to the definitions of the ATP III (Cleeman *et al.*, 2001). All the studies except one (Shimizu *et al.*, 2008) reported no significant weight changes.

The median intervention dose of β -glucan given to the subjects was 5 g/day. The interventions were incorporated into different foods including liquid and solid food. In five studies, the subjects consumed their habitual diet (Newman *et al.*, 1989; Clinical Study Report, 2005; Björklund *et al.*, 2005; Shimizu *et al.*, 2008; Sundberg, 2008), and in two studies, the subjects were provided with an American Heart Association Step I diet (Behall *et al.*, 2004a,b). Two other

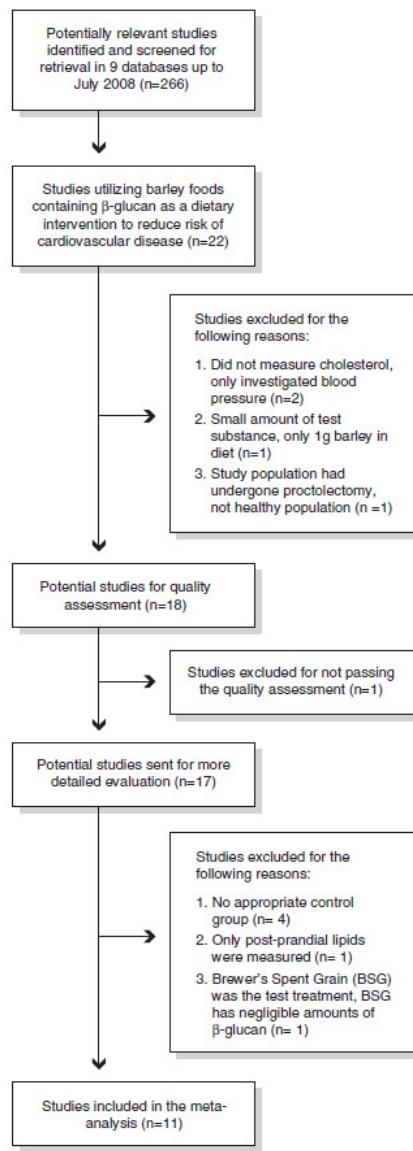


Figure 1 Systematic review flow diagram. Numbers in parentheses represent *n*.

Table 1 Design and subjects characteristics of randomized, controlled studies of barley and lipid concentrations

Study	Design duration sample size (n)	Baseline serum cholesterol (mmol/l)	Mean age (years)	Males (%)	Treatment type	Dose (g/day) ^a	Food carrier	Control	Background diet
Behall <i>et al.</i> (2004a) i (Behall <i>et al.</i> , 2004a)	Crossover 5 weeks n=16	6.12	46.9	100	Barley flakes, flour, pearled barley	3 barley SDF	Pancake, spice cake, no-bake cookies, hot cereal, toasted flakes, steamed pilaf, muffins	Test foods made with wheat and rice	Controlled feeding of AHA Step 1
Behall <i>et al.</i> (2004a) ii (Behall <i>et al.</i> , 2004a)	Crossover 5 weeks n=16	6.12	46.9	100	Barley flakes, flour, pearled barley	6 barley SDF	Pancake, spice cake, no-bake cookies, hot cereal, toasted flakes, steamed pilaf, muffins	Test foods made with wheat and rice	Controlled feeding of AHA Step 1
Behall <i>et al.</i> (2004b) i (Behall <i>et al.</i> , 2004b)	Crossover 5 weeks n=27	5.18–6.2	43–50	28	Barley flakes, flour, pearled barley	3	Pancake, spice cake, no-bake cookies, hot cereal, granola, steamed grain, tabbouleh, muffins	Test foods made with wheat and rice	Controlled feeding of AHA Step 1
Behall <i>et al.</i> (2004b) ii (Behall <i>et al.</i> , 2004b)	Crossover 5 weeks n=27	5.18–6.2	43–50	28	Barley flakes, flour, pearled barley	6	Pancake, spice cake, no-bake cookies, hot cereal, granola, steamed grain, tabbouleh, muffins	Test foods made with wheat and rice	Controlled feeding of AHA Step 1
Björklund <i>et al.</i> (2005) i (Björklund <i>et al.</i> , 2005)	Parallel 5 weeks n=39	6.49	56	49.4	β -Glucan	5	Beverage	Placebo beverage	Habitual diet
Björklund <i>et al.</i> (2005) ii (Björklund <i>et al.</i> , 2005)	Parallel 5 weeks n=36	6.49	56	49.4	β -Glucan	10	Beverage	Placebo beverage	Habitual diet
Clinical Study Report (2005)	Crossover 4 weeks n=39	4.6–8.6	58	40.8	Barley powder	3	Non-alcoholic beverage	Wheat flour	Habitual diet
Keenan <i>et al.</i> (2007) i (Keenan <i>et al.</i> , 2007)	Parallel 6 weeks n=62	6.11	58.6	46.9	β -Glucan HMW	5	Cereal + juice	Placebo cereal and juice	Dietary instructions to follow low saturated fat and low trans fat diet
Keenan <i>et al.</i> (2007) ii (Keenan <i>et al.</i> , 2007)	Parallel 6 weeks n=60	6.11	52.8	36.7	β -Glucan LMW	5	Cereal + juice	Placebo cereal and juice	Dietary instructions to follow low saturated fat and low trans fat diet
Keenan <i>et al.</i> (2007) iii (Keenan <i>et al.</i> , 2007)	Parallel 6 weeks n=62	6.11	53.9	50	β -Glucan HMW	3	Cereal + juice	Placebo cereal and juice	Dietary instructions to follow low saturated fat and low trans fat diet
Keenan <i>et al.</i> (2007) iv (Keenan <i>et al.</i> , 2007)	Parallel 6 weeks n=61	6.11	55	51.6	β -Glucan LMW	3	Cereal + juice	Placebo cereal and juice	Dietary instructions to follow low saturated fat and low trans fat diet
Keogh <i>et al.</i> (2003)	Crossover 4 weeks n=18	5.9	38.8	100	β -Glucan gelling form	9.9	Bread, waffles, muffins, bread, savoury dishes, cakes and cookies	Glucose control supplement	Controlled feeding of Western diet

Table 1 Continued

Study	Design duration sample size (n)	Baseline serum cholesterol (mmol/l)	Mean age (years)	Males (%)	Treatment type	Dose (g/day) ^a	Food carrier	Control	Background diet
Li <i>et al.</i> (2003)	Crossover 4 weeks n=10	Tx = 3.61 Co = 3.66	20.4	0	Barley	8.9 barley SDF	Different foods	Rice	Controlled feeding of typical Japanese diet
McIntosh <i>et al.</i> (1991)	Crossover 4 weeks n=21	6.23	44.2	100	Bran and flaked barley	8	Bread, muesli, spaghetti, biscuits	Test foods made with wheat	Not clear
Newman <i>et al.</i> (1989)	Parallel 4 weeks n=14	Tx = 4.64 Co = 4.58	NR	100	Barley flour	12	Muffin, bread, cookies, bars, cereal	Test foods made with wheat	Habitual diet
Shimizu <i>et al.</i> (2008)	Parallel 12 weeks n=39	Tx = 6.10 Co = 6.39	40.9–42.1	100	Pearl barley	7	Pearl barley	Rice	Habitual diet
Sundberg (2008)	Crossover 4 weeks n=44	6.64	63.4	29.2	Barley flake	3.66	Barley flake	Wheat flake	Habitual diet

Abbreviations: AHA, American Heart Association; Co, control; HMW, high molecular weight; LMW, low molecular weight; NR, not reported; SDF, soluble dietary fibers; Tx, treatment.

^aUnless otherwise indicated

studies were controlled feeding trials of typical Japanese (Li *et al.*, 2003) and Western (Keogh *et al.*, 2003) diets. In one study (Keenan *et al.*, 2007) subjects were given instructions to follow a low saturated fat and low trans fat diet. Most of the studies did not report all data on the actual nutrient intakes by the subjects. Most control groups received wheat or rice products and therefore barley products were not isocalorically substituted for fat.

Changes in serum lipid concentrations

Overall, β-glucan from barley lowered total and LDL cholesterol concentrations by 0.30 mmol/l (95% CI: -0.39 to -0.21, $P<0.00001$) and 0.27 mmol/l (95% CI: -0.34 to -0.20, $P<0.00001$), respectively, compared with the control (Figure 2). The reductions in total and LDL cholesterol concentrations were significant in 10 and 11 strata, respectively. The ingestion of β-glucan from barley did not affect HDL cholesterol and triacylglycerol concentrations (Figure 2). The test for heterogeneity was not significant ($P>0.1$) except for the subgroup analysis of the effect of β-glucan from barley and use of beverage as food carrier on total cholesterol concentrations (Table 2).

Subgroup analysis

Subgroup analysis based on dose size (Tables 2 and 3) showed that the reductions in total cholesterol were 0.25 mmol/l (95% CI: -0.36 to -0.14, $P<0.00001$), 0.45 mmol/l (95% CI: -0.69 to -0.20, $P=0.0003$) and 0.28 mmol/l (95% CI: -0.50 to -0.07, $P=0.01$) for intakes of 3–5, 5.1–7 and

>7.1 g/day, respectively. The reductions in LDL were 0.22 mmol/l (95% CI: -0.31 to -0.12, $P<0.00001$), 0.33 mmol/l (95% CI: -0.47 to -0.19, $P<0.00001$) and 0.24 mmol/l (95% CI: -0.37 to -0.10, $P=0.0005$) for intakes of 3–5, 5.1–7 and >7.1 g/day, respectively. However, there were no significant subgroup differences, which could be due to the limited number of studies used in the analysis. When studies were analyzed according to the type of the intervention, that is, barley vs β-glucan from barley, the reduction of total cholesterol was only significant in barley, but both barley and β-glucan from barley reduced LDL concentrations. Subgroup analysis by food matrix revealed that β-glucan from barley reduced total and LDL cholesterol whether they were incorporated into a beverage or solid foods. However, results of the subgroup analyses should be interpreted with caution.

The effect of the background diet on efficacy of β-glucan from barley as a cholesterol-lowering agent was not tested due to the limited information provided by the studies. For example, studies reported that subjects were given controlled feeding diets but did not report the actual nutrient intake of the subjects. Therefore, it is not known whether subjects adhered to the dietary instructions.

On the basis of subgroup analysis, baseline cholesterol levels did not affect the cholesterol-lowering action of β-glucan from barley ($P>0.05$). The decreases in LDL were 0.31 mmol/l (95% CI: -0.55 to -0.07, $P=0.01$), 0.33 mmol/l (95% CI: -0.46 to -0.20, $P<0.00001$) and 0.22 mmol/l (95% CI: -0.30 to -0.13, $P=0.01$) for studies in which subjects had optimal, borderline high and high baseline values of serum cholesterol, respectively.

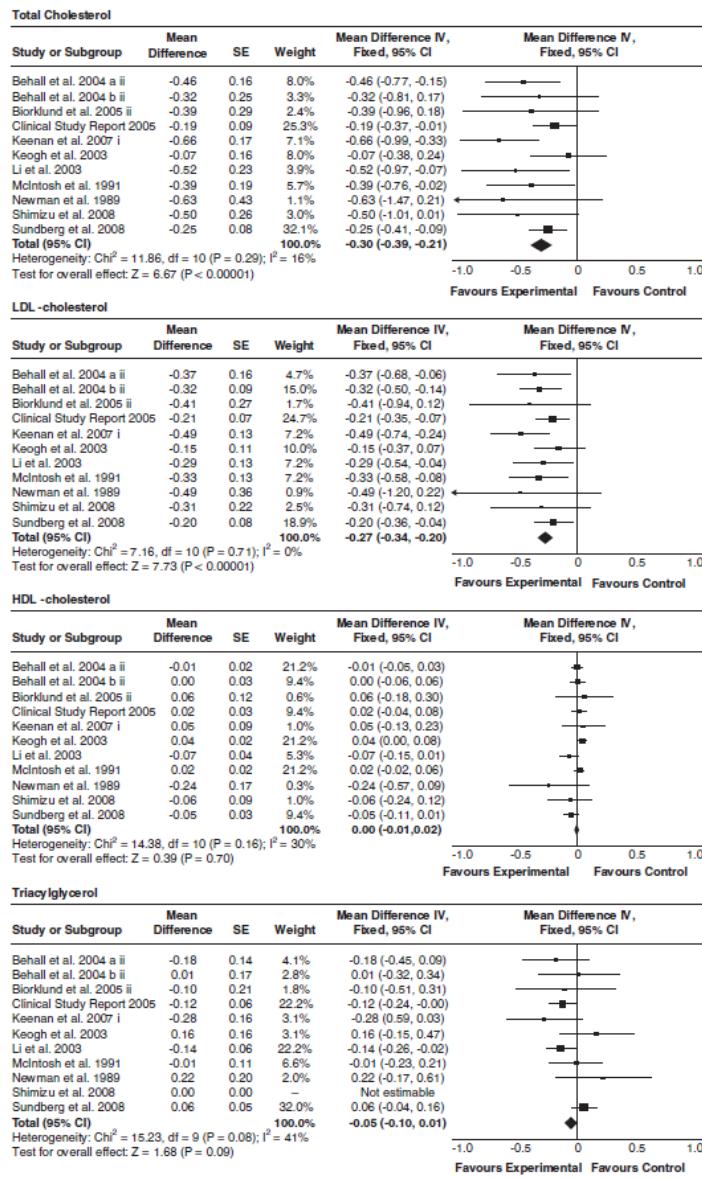


Figure 2 Mean difference (mmol/l) and 95% CI in total, LDL and HDL cholesterol and triacylglycerol concentrations associated with consumption of barley and β -glucan isolated from barley.

Table 2 Pooled estimates of treatment effect on total cholesterol in subgroups of trials defined by study design features

Variables	Number of strata	Effect size (95% CI), mmol/l	Test for overall effect, P-value	Test for heterogeneity, P-value
<i>Dose size (g/day)</i>				
3–5	4	–0.25 (–0.36, –0.14)	<0.00001	0.60
5.1–7	3	–0.45 (–0.69, –0.20)	0.0003	0.84
> 7	4	–0.28 (–0.50, –0.07)	0.005	0.28
<i>Intervention type</i>				
Barley	8	–0.29 (–0.39, –0.19)	<0.00001	0.64
β -Glucan from barley	3	–0.37 (–0.77, 0.03)	0.07	0.04
<i>Food matrix</i>				
Beverage	3	–0.39 (–0.73, –0.06)	0.02	0.05
Solid foods	8	–0.31 (–0.42, –0.20)	<0.00001	0.54

Abbreviation: CI, confidence interval.

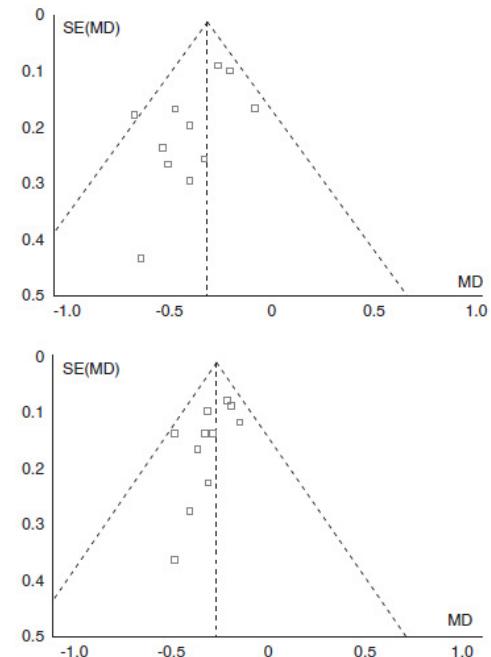
Table 3 Pooled estimates of treatment effect on LDL cholesterol in subgroups of trials defined by study design features

Variables	Number of strata	Effect size (95% CI), mmol/l	Test for overall effect, P-value	Test for heterogeneity, P-value
<i>Dose size (g/day)</i>				
3–5	4	–0.22 (–0.31, –0.12)	<0.00001	0.93
5.1–7	3	–0.33 (–0.47, –0.19)	<0.00001	0.96
> 7	4	–0.24 (–0.37, –0.10)	0.0005	0.63
<i>Intervention type</i>				
Barley	8	–0.26 (–0.34, –0.19)	<0.00001	0.90
β -Glucan from barley	3	–0.32 (–0.58, –0.07)	0.01	0.12
<i>Food matrix</i>				
Beverage	3	–0.33 (–0.54, –0.12)	0.002	0.15
Solid foods	8	–0.26 (–0.34, –0.18)	<0.00001	0.86

Abbreviations: CI, confidence interval; LDL, low-density lipoprotein.

Publication bias

Funnel plots for total and LDL cholesterol concentrations are shown in Figure 3. Visual examination of the funnel plots shows its asymmetrical appearance with a gap in a bottom corner of the graph. Thus, it is clear that small studies remain unpublished.

**Figure 3** Funnel plots of s.e. vs effect size for total and LDL cholesterol concentrations.

Discussion

This is the first meta-analysis of randomized clinical trials yielding information on the effect of β -glucan from barley on lipid profile. The meta-analysis showed that the consumption of β -glucan from barley decreased blood cholesterol concentrations in subjects with different dietary backgrounds. Owing to limited information available in the studies, we were not able to study the cholesterol-lowering effect of other dietary components present in barley, such as arabinoxylans, plant sterols and tocots.

Barley intake did not affect HDL cholesterol or triacylglycerol concentrations. Similarly, the consumption of other soluble fibers, including those from oats, psyllium and pectin, have been shown not to have an effect on HDL cholesterol and triacylglycerol concentrations (Brown *et al.*, 1999).

The pattern of cholesterol-lowering action of β -glucan from barley in this analysis cannot be viewed as a dose-dependent response. There are various reasons that may help to explain the lack of dose-dependent response. First, the dose-dependent response may be observable with a wider range of doses, other than the one used in our analysis.

In other words, the range of doses used here may have showed only a plateau effect. Second, differences in the molecular weight of β -glucan may influence the dose-response effect. It has been suggested that β -glucan characteristics including its solubility and molecular weight are important determinants of its cholesterol-lowering action (Theuwissen and Mensink, 2008). Highly water-soluble β -glucan, with moderate to high molecular weight, may reduce serum LDL cholesterol levels better than β -glucan with a low water-solubility and low molecular weight (Theuwissen and Mensink, 2008). On the other hand, sensory properties of foods enriched with β -glucan are more positively received at a lower β -glucan molecular weight (Keenan et al., 2007). In one study, the consumption of either high molecular weight or low molecular weight concentrated barley β -glucan at both 3 and 5 g doses for 6 weeks decreased LDL cholesterol compared with control (Keenan et al., 2007). The exact molecular weight of β -glucan was not reported in the majority of studies and therefore we could not assess its impact on the cholesterol-lowering action of barley products. Third, the power might have been insufficient to prove dose-response effect at the included dose range. High consumption of β -glucan (>7 g/day) did not appear to have substantially greater effects than modest consumption (3–5 g/day). Therefore, consumption of at least 3 g/day of barley β -glucan will reduce blood cholesterol concentrations.

Similarly, other studies have shown that food processing of β -glucan affect its cholesterol-lowering effect as a consequence of changes in β -glucan structure or solubility. A daily intake of a beverage providing 5 g of β -glucan from barley for 5 weeks did not reduce LDL concentrations (Biorklund et al., 2005). In another study, intake of 3 g/day of β -glucan supplied as barley powder and mixed in a beverage reduced LDL concentrations compared with control (Clinical Study Report, 2005). The consumption of about 8–12 g/day of barley β -glucan extract incorporated into baked products for 4 weeks did not improve the lipid profiles of hypercholesterolemic men (Keogh et al., 2003). While, consumption of barley based baked products reduced LDL cholesterol in other studies (Newman et al., 1989; McIntosh et al., 1991). In spite of some conflicting results from individual clinical trials, pooling data from 11 studies in this analysis showed that incorporating barley into different food products could be used as an efficacious way to increase the consumption of viscous soluble fibers in order to achieve the desired reduction in LDL cholesterol concentration.

The cholesterol-lowering effect of barley reported in this meta-analysis is greater than that reported previously for oats (Ripsin et al., 1992), 0.25 mmol/l (95% CI: –0.36 to –0.14) and 0.13 mmol/l (95% CI: –0.19 to –0.017), respectively, for barley and oats. One difference between the two meta-analyses is that Ripsin et al. included many studies that used lower doses (<3 g/day) but in this study all studies used doses ≥ 3 g. Moreover, there are differences with respect to other aspects such as the inclusion criteria and quality assessment of the trials. For example, this meta-analysis

assessed the quality of the trials, whereas that by Ripsin et al. did not. Upon quality assessment of trials in this current analysis, seven trials were excluded because they were not of sufficient quality.

Another meta-analysis by Brown et al. (1999) showed that various soluble fibers from oats, psyllium or pectin reduce total and LDL cholesterol by similar amounts. We cannot directly compare our results to the result from Brown et al. analysis as the latter analysis was expressed as reduction in LDL per gram of fiber consumed. In addition, heterogeneity was evident in the Brown et al. (1999) analysis, whereas heterogeneity was not observed in this study.

Both barley and oats contain β -glucan, which is thought to be the active ingredient responsible for their cholesterol-lowering effect (Truswell, 2002). One human study has concurrently examined the effect of β -glucan from oats or barley on total cholesterol concentration (Biorklund et al., 2005). The Biorklund et al. (2005) study showed that the consumption of a beverage containing 5 g of β -glucan from oats lowered total cholesterol concentrations by 7.4% compared with a control beverage. No cholesterol-lowering effect of a beverage with 5 g of β -glucan from barley was found. The lack of cholesterol-lowering effect of β -glucan from barley in the study by Biorklund et al. (2005) was attributed to its lower molecular weight compared with that of β -glucan from oats.

In conclusion, this meta-analysis of 11 studies indicates that the consumption of barley or β -glucan from barley incorporated into different food products is associated with a significant reduction in total and LDL cholesterol concentrations. Increased consumption of barley products should be considered as a dietary approach to reduce LDL cholesterol concentrations.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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The Effects of Barley-Derived Soluble Fiber on Serum Lipids

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ABSTRACT

PURPOSE We wanted to determine the association between consumption of barley and changes in plasma lipids in healthy and hypercholesterolemic men and women.

METHODS A systematic literature search was conducted from the earliest possible date through January 2008. Trials were included in the analysis if they were randomized controlled trials of barley that reported efficacy data on at least 1 lipid endpoint. A DerSimonian and Laird random-effects model was used in calculating the weighted mean difference (WMD) and its 95% confidence interval (CI). Statistical heterogeneity was addressed using the I^2 statistic. Visual inspection of funnel plots, Egger's weighted regression statistics, and the trim and fill method were used to assess for publication bias.

RESULTS We found 8 trials ($n = 391$ patients) of 4 to 12 weeks' duration evaluating the lipid-reducing effects of barley. The use of barley significantly lowered total cholesterol (weighted mean difference [WMD], -13.38 mg/dL ; 95% CI, $-18.46 \text{ to } -8.31 \text{ mg/dL}$), low-density lipoprotein (LDL) cholesterol (WMD, -10.02 mg/dL ; 95% CI, $-14.03 \text{ to } -6.00 \text{ mg/dL}$) and triglycerides (WMD, -11.83 mg/dL ; 95% CI, $-20.12 \text{ to } -3.55 \text{ mg/dL}$) but did not appear to significantly alter high-density lipoprotein (HDL) cholesterol ($P = .07$).

CONCLUSION Barley-derived β -glucan appears to beneficially affect total cholesterol, LDL-cholesterol, and triglycerides, but not HDL-cholesterol.

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INTRODUCTION

According to the guidelines of the National Cholesterol Education Program (NCEP), approximately 30% of Americans have undesirably high serum cholesterol concentrations.¹ High serum lipid levels, including total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, are a major cause of coronary atherosclerosis.¹ Any LDL cholesterol concentration above 100 mg/dL appears to be atherogenic and the higher the level, the greater the risk.¹ Although elevated LDL cholesterol plays a role in the development of the coronary plaque instability, lowering LDL cholesterol stabilizes plaques and reduces the likelihood of acute coronary syndromes.¹ Lowering serum cholesterol reduces the risk of coronary heart disease.

The effect of dietary fiber on cholesterol metabolism has been studied extensively.^{2,3} Barley and oats have a similar concentration of soluble fibers called β -glucan (3.5%-5.9% of the dry matter), whereas wheat and rice do not possess this constituent type of fiber.⁴ Unlike wheat and rice,⁵⁻¹⁰ a diet high in β -glucan has been shown to slow gastric emptying, digestion, and absorption.¹¹ These effects are associated with increased excretion of bile acids and neutral sterols, increased catabolism of cholesterol, and reduced absorption of cholesterol and fat.^{12,13}

Although the antihyperlipidemic effects of oats have been extensively studied, there are fewer barley studies, and findings have shown more

Conflicts of interest: none reported

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apparent inconsistency in cholesterol effects.^{14,15} Some reasons for inconsistencies in the barley studies may be explained by differences in the β -glucan dose, the molecular size of β -glucan, the composition of dietary food, the process of food preparation, and the initial variation in cholesterol level. Even though several clinical trials^{10,16-29} have investigated the impact of barley β -glucan on total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides, a meta-analysis assessing these effects has not been published. We therefore sought to perform a meta-analysis of randomized controlled trials of barley to better characterize its effect on various lipid parameters.

METHODS

Was conducted a systematic literature search of MEDLINE, EMBASE, CINAHL, Web of Science, the Cochrane Library, and the Natural Medicines Comprehensive Database from the earliest possible date through January 2008. Our search strategy used the Medical Subject Headings (MeSH) and text key words: " β -glucan," "barley" and "lipids," "serum cholesterol," "total cholesterol," "low-density lipoproteins," "high-density lipoproteins," "LDL," "HDL," "triglycerides," or "hypercholesterolemia." This search was then limited to clinical trials in humans. We also performed a manual search of references from retrieved articles. When applicable, we made an effort to contact investigators for clarification or additional data (although no additional data were acquired).

To be included in this meta-analysis, studies had to be randomized controlled trials of barley and report data on at least 1 of the following lipid parameters: total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides. Both parallel and crossover trials were eligible for inclusion; however, crossover trials had to have at least a 4-week washout period. If this criterion was not met, when possible, we included only the first phase of each crossover trial.

A more detailed description of the methods can be found in the Supplemental Appendix, available online at <http://www.annfammed.org/cgi/content/full/7/2/157/DC1>.

We treated the mean change in lipid parameters from baseline as a continuous variable, and the weighted mean difference (WMD) and its 95% confidence interval (CI) were calculated as the difference between the mean in the β -glucan and control groups using a DerSimonian and Laird

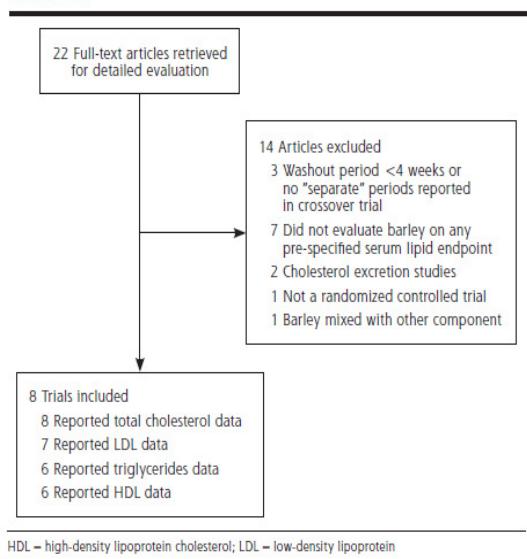
random-effects model.³⁰ For parallel trials, we calculated net changes in each of these study parameters as the difference (β -glucan minus control) of the changes (baseline minus follow-up) in the mean values (also referred to as the change score). For crossover trials, we calculated net changes as the mean difference in values at the end of the β -glucan and control periods. Standard statistical methods were used to impute change scores, as suggested by Follman and colleagues.³¹

Statistical heterogeneity was addressed using the I^2 statistic. Visual inspection of funnel plots, Egger's weighted regression statistics, and the trim and fill method was used to assess for the presence of publication bias.³² Sensitivity analysis was conducted to assess the impact of double-blinding, the use of crossover methodology, and the use of a fixed-effects model (Mantel-Haenszel methodology).³³ Additionally, subgroup analyses were conducted to assess the effect of using or not using concurrent dietary modifications and to assess the effect on only hypercholesterolemic patients. Statistics were performed using StatsDirect statistical software, version 2.4.6 (StatsDirect Ltd, Cheshire, England). A P value of $<.05$ was considered statistically significant for all analyses.

RESULTS

The initial search yielded a total of 22 studies for full-text review. For reasons depicted in Figure 1, 14 of

Figure 1. Flow diagram of trial identification, inclusion, and exclusion.



the 22 studies were excluded; therefore, a total of 8 randomized controlled trials^{10,16-22} (evaluating 391 participants) were included in this meta-analysis (Table 1). Five of the studies^{10,16-18,20} were conducted using a parallel study design, whereas 2 studies^{19,22} used a crossover design with a 4-week washout period, and 1 study²¹ used a crossover design with no washout period and was treated as a parallel trial by taking into account only the first phase of the study data.

Each study enrolled relatively few participants (median sample size, 30 participants; range, 10-155 participants) and had a short duration of treatment (median duration, 4 weeks; range, 4-12 weeks). The dosage of β -glucan reported in included studies ranged from 3 to 10 g/d (median dose, 7 g/d) and was administered in various forms, including pearl barley, barley bran flour, oil extracts in capsules, barley concentrates, barley-containing beverages, and gelling agents. Only 2 studies^{17,20} administered barley along with some type of dietary modification. Of the 8 studies, 6 were not double-blind.^{10,18-22} Three of the 8 studies were industry funded.^{16,17,22}

Upon meta-analysis, participants consuming barley had significantly greater reductions in total cholesterol, LDL cholesterol, and triglycerides, but not HDL cholesterol compared with control participants (Table 2, Figure 2). No statistical heterogeneity was observed in any of these analyses ($I^2 = 0\%$ for all). Visual inspec-

tion of funnel plots (not shown) suggested a low likelihood of publication bias. This finding was further supported by Egger's weighted regression statistic P values, which also suggested that publication bias was unlikely for all analyses except total cholesterol ($P = .02$). After recalculating effect size estimates using trim and fill methods, barley's effect was not significantly altered for triglycerides. For total cholesterol, LDL cholesterol, and HDL cholesterol, the trim and fill analysis suggests that as many as 4 studies for total cholesterol and 3 studies for LDL cholesterol and HDL cholesterol could potentially exist for each endpoint; however, barley still had a significant, although reduced, effect when these theoretically "missing" studies were imputed for total cholesterol and LDL cholesterol. For HDL cholesterol, the original analysis did not show significance, but after imputing the 3 "missing" studies from the trim and fill, it was statistically significant for this endpoint.

Upon subgroup and sensitivity analysis, similar results were seen for all of the study endpoints when crossover or non-double-blinded studies were excluded, except the effect of barley on triglycerides lost statistical significance (Table 2). When a fixed-effects model was used, the results did not change. When studies evaluating barley in only hypercholesterolemic patients were analyzed, the effect of barley on triglycerides lost statistical significance but still

Table 1. Characteristics of Included Randomized Controlled Trials of Barley

Reference	Design	Type of Patient	Double-Blinding	N ^a	Duration of Treatment (wk)	Preparation of Barley	β -Glucan Intake per Day	Concurrent Diet Modification
Shimizu et al, ¹⁶ 2007	Parallel	Hyper-cholesterolemic	Yes	39	12	Pearled barley	7 g	None
Keenan et al, ¹⁷ 2007	Parallel	Hyper-cholesterolemic	Yes	155	6	Barley concentrate in cereal and juice	3 or 5 g of either HMW or LMW	Low saturated (<10%) & low trans unsaturated fat diet
Björklund et al, ¹⁸ 2005	Parallel	Hyper-cholesterolemic	No	55	5	Barley concentrate as beverage	5 or 10 g	None
Keogh et al, ¹⁹ 2003	Crossover	Hyper-cholesterolemic	No	18	4	Naturally extracted barley β -glucan as a gel	9.9 g	None
Li et al, ²² 2003	Crossover	Healthy	No	10	4	Barley bran in whole grain	NR	None
Lupton et al, ²⁰ 1994	Parallel	Hyper-cholesterolemic	No	79	4	Barley bran flour or oil extract in capsules	NR	Step I diet
McIntosh et al, ²¹ 1991	Parallel ^b	Hyper-cholesterolemic	No	21	4	Barley grain (bran and flakes)	8 g	None
Newman et al, ¹⁰ 1989	Parallel	Healthy	No	14	4	Barley grain flour in cereal and baked goods	4.5 g	None

HMW or LMW = high or low molecular weight β -glucan; NR = not reported; step I diet = diet consisting total fat to $\leq 30\%$ of total calories, saturated fat to $\leq 10\%$ of total calories, and cholesterol to 300 mg/d.

^a Number of patients evaluated.

^b Crossover trial treated as parallel trial with only the first phase of the study data taken into account.

Table 2. Results of the Meta-Analysis of Randomized Controlled Trials Evaluating Effect of Barley Cholesterol Levels

Study Type	Total Cholesterol mg/dL (95% CI)	LDL Cholesterol mg/dL (95% CI)	HDL Cholesterol mg/dL (95% CI)	Triglycerides mg/dL (95% CI)
All studies	-13.38 (-18.46 to -8.31) [8 studies]	-10.02 (-14.03 to -6.00) [7 studies]	0.99 (-0.09 to 2.06) [6 studies]	-11.83 (-20.12 to -3.55) [6 studies]
Fixed-effects model	-13.38 (-18.46 to -8.31) [8 studies]	-10.02 (-14.03 to -6.00) [7 studies]	0.99 (-0.09 to 2.06) [6 studies]	-11.83 (-20.12 to -3.55) [6 studies]
Excluding crossover studies	-13.75 (-19.24 to -8.26) [6 studies]	-9.76 (-14.64 to -4.88) [5 studies]	-0.97 (-3.31 to 1.36) [4 studies]	-13.68 (-12.74, 0.39) [4 studies]
Excluding studies not double-blind	-17.39 (-26.05 to -8.74) [2 studies]	-13.43 (-20.58 to -6.29) [2 studies]	0.85 (-4.71 to 6.41) [1 studies]	-22.45 (-50.65 to 5.76) [1 studies]
Excluding studies in patients without hypercholesterolemia	-12.56 (-17.89 to -7.24) [6 studies]	-9.38 (-14.13 to -4.63) [5 studies]	1.08 (-0.01 to 2.17) [4 studies]	-11.06 (-24.97 to 2.85) [4 studies]
Trim and fill	-10.49 (-15.09 to -5.89) [+4 studies]	-8.45 (-12.21 to -4.69) [+3 studies]	1.34 (0.31 to 2.37) [+3 studies]	-11.83 (-20.12 to -3.55) [+0 studies]
Studies evaluating barley with diet modification	-17.14 (-25.02 to -9.23) [2 studies]	-14.57 (-21.69 to -17.45) [2 studies]	1.53 (-2.98 to 6.05) [2 studies]	-17.36 (-40.66 to 5.94) [2 studies]
Studies evaluating barley without diet modification	-10.75 (-17.38 to -4.12) [6 studies]	-7.89 (-12.75 to -3.04) [5 studies]	-0.16 (-2.33 to 2.02) [4 studies]	-11.03 (-19.90 to -2.17) [4 studies]

CI = confidence interval; LDL = low-density lipoprotein; HDL = high-density lipoprotein; + = analysis-imputed missing studies.

Note: All results reported as weighted mean differences.

trended toward a reduction. When studies using and not using dietary modification were assessed separately, the effect of barley on serum lipids qualitatively appeared more robust when combined with dietary modifications.

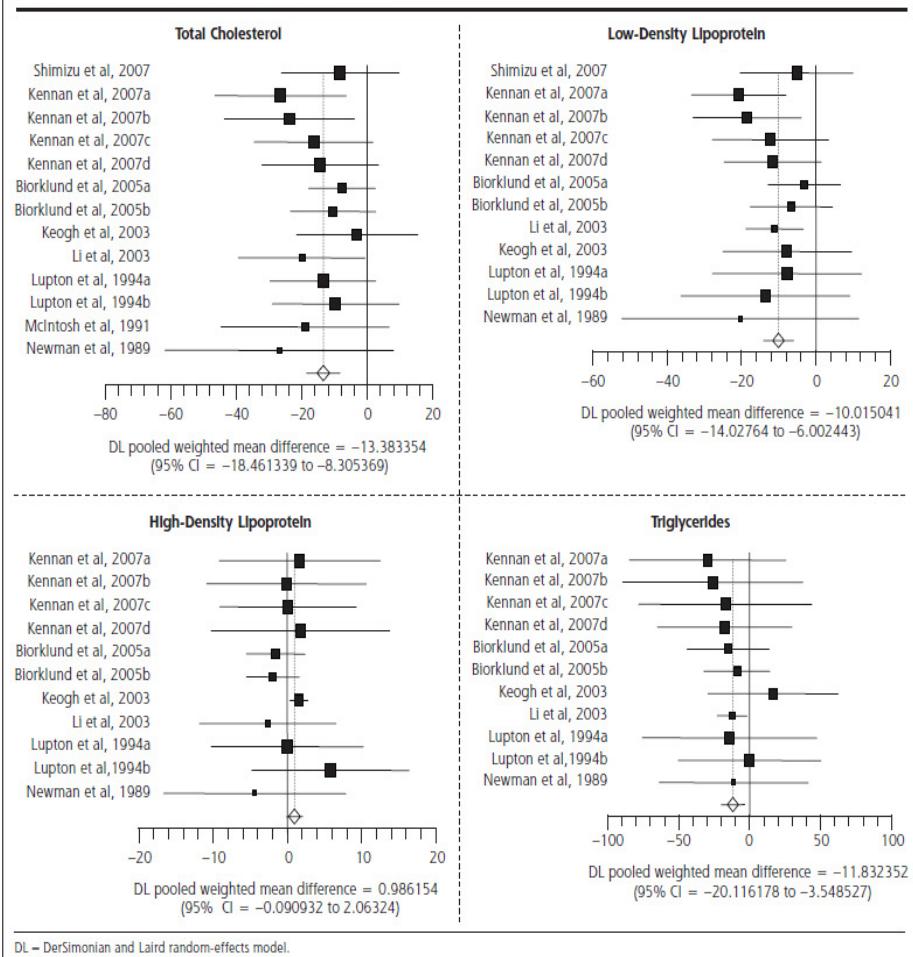
DISCUSSION

In our meta-analysis of 8 randomized controlled trials, participants receiving barley had statistically significant reductions in total cholesterol (-13 mg/dL), LDL cholesterol (-10 mg/dL), and triglycerides (-12 mg/dL) compared with control group participants. Because studies have shown that for each milligram per deciliter reduction in a patient's LDL cholesterol level, their relative risk of having a coronary heart disease event is decreased by 1%, this modest reduction in LDL cholesterol observed with barley is likely clinically significant as well.³⁴

This reduction in total cholesterol and LDL cholesterol is in line with that found for oat-derived β-glucan. In a meta-analysis of oats containing 2 to 10 g/d of β-glucan, there was a net change resulting from soluble fiber ingested of -3.1 mg/dL to -15.5 mg/dL for total cholesterol, and -2.9 mg/dL to -14.3 mg/dL for LDL

cholesterol.³⁵ Barley and oats have similar concentrations of β-glucans (3.5%–5.9% of the dry matter), the proposed active ingredient in both soluble fibers, so a similar magnitude of total cholesterol or LDL cholesterol reductions is plausible.⁴ In the meta-analysis of oats by Brown and colleagues, however, changes of -0.08 to -0.4 mg/dL were noted for HDL cholesterol, and changes of 1.06 to 5.3 mg/dL were noted for triglycerides, which is in contrast to our meta-analysis, in which with barley-derived β-glucan we saw a nonsignificant increase of 1 mg/dL for HDL cholesterol and a significant reduction of 12 mg/dL in triglycerides compared with a control group.³⁵ In addition, Brown and colleagues found a dose-response relationship when evaluating studies of soluble fibers in the practical dose range (<10 g/d).³⁵ That review, however, included 67 clinical trials evaluating a variety of soluble fibers (not including barley). Thus, their analysis was appropriately powered to evaluate dose response. In comparison, our meta-analysis included 8 studies, only 6 of which reported a β-glucan dose (75% of total patient population), making it difficult to conduct a dose-response analysis. At least 10 studies are recommended to provide adequate power.³⁶

The Food and Drug Administration (FDA) has stated that daily intakes of 3 g or more of soluble fiber

Figure 2. Impact of barley on serum lipids.

(β -glucan) in whole oats or barley may reduce the risk of heart disease by its ability to lower total cholesterol and LDL cholesterol.^{37,38} Our meta-analysis results support this FDA decision, because 3 to 10 g of β -glucan from various forms of barley lowered total cholesterol, LDL cholesterol, and triglycerides in the study participants. Furthermore, a significant reduction in total cholesterol and LDL cholesterol was found regardless of whether a low-fat or step I diet was mandated equally in both arms of the studies. This finding is important because of the potential for a dietary substitution effect. If study participants are replacing their normal foods (eg, eggs, bacon, sausage) with barley, it may be difficult to discern whether the improvements in cholesterol resulted from the healthier diet or from barley.

That significant reductions in total cholesterol and LDL cholesterol were seen regardless of whether diet modifications were mandated equally in both study groups helps guard against the issue of dietary substitution and strengthens the beneficial effects of barley use.

There are some limitations to this meta-analysis that should be noted. First, we included crossover and parallel studies. Crossover studies have methodological advantages compared with parallel studies, because patients act as their own controls; however, an adequate washout period is necessary. As such, we did not include trials that did not explicitly state the presence and duration of the washout period or trials that had a washout period of fewer than 4 weeks, in which case, we only included the first phase of the

study when possible. The only noteworthy change seen upon conducting a sensitivity analysis excluding crossover studies was loss of statistical significance in the triglycerides endpoint.

Second, as with any meta-analysis, the potential for publication bias is a concern. Although visual inspection of our meta-analysis' funnel plot could not rule out the possibility of publication bias, review of Egger's weighted regression statistics and trim and fill analyses showed that it was unlikely that publication bias significantly affected our study results. Finally, we did not evaluate the potential for harms with barley. Based upon available data, barley appears to be well tolerated, with flatulence and abdominal discomfort being reported as the most common adverse effects, but there is not adequate power to look for other less common adverse effects.¹⁷

The results of our study support the routine use of soluble fibers in the diets of adult patients with and without hypercholesterolemia. Barley adds another source of soluble fibers, in addition to oats, psyllium, pectin, and guar gum that patients can consume as part of a healthy diet.³⁵ Larger randomized clinical trials are warranted to better characterize the potential for a dose-response relationship with barley β -glucan. Health practitioners should feel comfortable recommending barley β -glucan to their patients to help reduce total cholesterol and LDL cholesterol concentrations as recommended by the NCEP guidelines.¹

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28-Day oral toxicity study in rats with high purity barley beta-glucan (Glucagel™)

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ABSTRACT

Beta-glucans are glucose polymers present in cereal grains, particularly barley and oat. Consumption of these grains or concentrated beta-glucan preparations has been shown to lower blood cholesterol. The present study was conducted to assess the safety of a high purity (>75%) barley beta-glucan (Glucagel™). The product was fed to Wistar rats (5/sex/group) at dietary levels of 0% (control), 1%, 5% and 10% for 28 days. Clinical and neurobehavioural observations, growth, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights, necropsy and histopathological examination revealed no adverse effects of Glucagel™. High-dose males exhibited lower plasma cholesterol and phospholipids levels and a higher plasma urea level. These slight changes were considered of no toxicological significance. Full and empty caecum weights were increased in mid- and high-dose males. This caecal enlargement was a physiological response to the consumption of a high amount of indigestible carbohydrate and considered of no toxicological concern. In conclusion, feeding Glucagel™ at dietary levels up to 10% for 28 days was tolerated without any signs of toxicity. This dietary level was equivalent to 7.7 g Glucagel™ (5.8 g beta-glucan)/kg body weight/day in male rats and 7.8 g Glucagel™ (5.9 g beta-glucan)/kg body weight/day in female rats.

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1. Introduction

Cardiovascular disease (CVD) is the number one cause of death globally and is expected to remain the leading cause of death in the foreseeable future. According to the World Health Organization (2007) an estimated 17.5 million people died from CVD in 2005 which represents 30% of all global deaths. Elevated blood cholesterol levels are an important risk factor for CVD, and hence one way to reduce the risk of developing the disease is to lower blood cholesterol levels by making dietary changes such as reducing intake of total fat, saturated fat, and dietary cholesterol (FDA, 1993; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001). In addition, blood cholesterol can be reduced by the consumption of dietary fibres, especially from cereal sources rich in beta-glucan (Brown et al., 1999). Epidemiological studies have shown that consumption of dietary fibre from cereals is inversely associated with risk of coronary heart disease (Theuwissen and Mensink, 2008; Pereira et al., 2004).

Abbreviations: CVD, cardiovascular disease; Anova, one-way analysis of variance; FOB, functional observational battery.

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Beta-glucans are glucose polymers found in the cell wall of cereal grains, particularly oat and barley and are characterized by groups of contiguous (1 → 4)- β -linkages and isolated (1 → 3)- β -linkages. Beta-glucan forms between 2% and 7% by weight of the cereal grain and content varies depending mainly on the cultivar type (Morgan, 2000).

The first report in free-living volunteers describing the effect of oats on serum cholesterol levels was published in 1963 (de Groot et al., 1963). Since then, a large number of human intervention studies have demonstrated that consumption of oats and concentrated beta-glucan preparations from oats lowers serum cholesterol concentrations in healthy normocholesterolemic and hypercholesterolemic subjects (Bell et al., 1999; Theuwissen and Mensink, 2008). Most studies on beta-glucan in oats produced positive results but some studies reported no effect. Fewer studies have investigated barley fibre since barley is less palatable than oats and a less common dietary component. Nevertheless, there is a significant body of research literature linking barley consumption to the lowering of blood cholesterol. In 2006, the US FDA authorized a health claim for beta-glucan soluble fibre from whole grain barley and certain dry milled barley products for lowering serum total and LDL-cholesterol levels, and hence reducing risk of CVD (FDA, 2005, 2006). In 2008, this health claim was extended to cover also a more highly purified barley fibre extract (FDA,

2008). Very recently, the NDA panel of the European Food Safety Authority (EFSA) supported a claim on beta-glucans contributing to maintenance of normal blood cholesterol concentrations (EFSA, 2009). With few exceptions, published human intervention studies demonstrate that the consumption of barley products including purified barley derived beta-glucan is an effective dietary approach for lowering total and LDL cholesterol (Ames and Rhymer, 2008).

Barley and oat as well as beta-glucan derived from these sources or soluble fibres as such are generally considered safe, however, there is limited safety data from toxicological studies on effects of repeated dose intake of concentrated beta-glucan extracts. A 28-day feeding study evaluating toxicity of a concentrated barley beta-glucan in rats suggested no obvious signs of toxicity following consumption of high doses (Delaney et al., 2003).

The present study evaluated the safety of a high purity beta-glucan (GlucagelTM, beta-glucan content >75%) that was isolated by a solely water-based process from barley. The results of the study will be used to widen regulatory approval for GlucagelTM internationally, e.g., to obtain GRAS status in the USA. The study was conducted in accordance with OECD Guideline for Testing of Chemicals 407 Repeated dose 28-day oral toxicity study in rodents (adopted 27 July 1995) and EC guideline B.7 Repeated dose (28 days) toxicity (oral), EEC Directive 96/54/EC, Official Journal of the European Communities, No. L248 (30.9.96). Ophthalmoscopy, urinalysis and histopathology of some additional organs, were conducted in accordance with the OECD Guideline for Testing of Chemicals 408 Repeated dose 90-day oral toxicity study in rodents (adopted 21 September 1998).

2. Materials and methods

2.1. High purity barley beta-glucan

High purity beta-glucan (GlucagelTM) is typically extracted from waxy hull-less barley varieties in an organic solvent-free process described in US patent 6,426,201 B1. The high purity beta-glucan (GraceLinc Ltd, Auckland, New Zealand) is very stable (24 months when stored in original packaging at room temperature) and the composition is presented in Table 1.

2.2. Analysis of beta-glucan in rat feed

The diets used in this study were analysed for beta-glucan to demonstrate homogeneity, achieved concentration and stability of this substance in the carrier. Immediately after diet mixing, five homogeneity samples, taken at different locations from the test diets, and one sample of the control diet were stored at -18 °C. Additionally, one sample of each diet was stored for 4 days in the animal room (see below for conditions) and subsequently frozen (-18 °C). All samples were shipped (on dry ice) from TNO Quality of Life to the analytical laboratory in Kantvik, Finland.

The concentration of beta-glucan in rat feed was determined by using the AOAC Official Method 995.16. The feed samples were homogenized and milled, after which 80–100 mg of each blend was weighed into high glass test tubes. Ethanol (0.2 ml) (95%, v/v) was added to wet the samples and after mixing well 4 ml of phosphate buffer (20 mM, pH 6.5) was added and again mixed well with Vortex mixer. The tubes were placed in a boiling water bath for 1 min, mixed, boiled for an additional 2 min and then incubated 5 min at 50 °C (water bath) and mixed well. After that 0.2 ml of lichenase enzyme solution (endo-(1 → 3)(1 → 4)-beta-D-glucan

4 glucanohydrolase) and magnetic balls were added to the solutions and the incubation at 50 °C was continued for 60 min and stirred for the whole time. Acetate buffer (5 ml) (20 mM, pH 4.0) was added and the samples were left to cool at room temperature for 5–10 min. After careful mixing, 1 ml of the samples was centrifuged 10 min at ca. 1000g. Next, 0.1 ml of each supernatant was transferred to the bottom of three separate test tubes. Only two of the tubes were treated with beta-glucosidase and the tube that was not treated yielded the blank value. 0.1 ml of 50 mM acetate buffer was added to the blank supernatant and 0.1 ml beta-glucosidase solution was added to the remaining two tubes. The incubation was continued for 10 min at 50 °C. Three milliliters of glucose oxidase-peroxidase-buffer mixture was added to each tube and incubated for 20 min at 50 °C.

The absorbance of the mixtures was measured at 510 nm against reagent blank and the content of beta-glucan in the rat feeds was determined using the equation:

$$\text{Beta} - d - \text{Glucan} (\%, \text{w/w}) = dA \times F \times 94 \times 1/1000 \times 100/W \times 162/180 \\ = dA \times F/W \times 8.46$$

where dA = absorbance sample minus absorbance blank; F = factor to convert absorbance value to µg glucose; 94 = volume correction factor (0.1 ml from 9.4 ml was analysed); 1/100 = conversion from µg to mg; 100/W = conversion to express beta-glucan content as percentage (w/w); W = sample weight in mg; 162/180 = factor to convert from free glucose (as determined) to anhydroglucose (as occurs in beta-glucan).

2.3. Experimental diets

A cereal-based, closed formula rodent diet (Rat & Mouse No. 3 Breeding Diet, obtained from SDS Special Diets Services, Witham, England) was used as the basal diet. The experimental diets were prepared by supplementing the basal diet with GlucagelTM and/or pregelatinized potato starch (Paselli WA4 from AVEBE, the Netherlands). GlucagelTM was incorporated at dietary levels of 1% (low-dose), 5% (mid-dose) and 10% (high-dose). The supplement in the low- and mid-dose diet was made up to 10% with potato starch. The control diet consisted of basal diet supplemented with 10% potato starch. GlucagelTM was incorporated at the expense of added potato starch to minimize differences in the levels of nutrients and other substances between the different diets. Nutrient levels in the basal diet are in excess of the requirement for rats and allow 10% dilution. One batch of the experimental diets was prepared about 2 weeks before the start of the study and stored frozen (-18 °C) in portions sufficient for 4 days. The feed in the animal feeders was refreshed twice per week.

2.4. Animals and maintenance

Specific-pathogen-free bred Wistar rats (CrI:(WI)WU) were obtained from Charles River Deutschland, Sulzfeld, Germany, and acclimatized to the laboratory conditions for nearly 2 weeks. At the start of the treatment period, they were about 6 weeks old (body weight: males mean 162 g, range 148–174 g; females mean 122 g, range 110–131 g). One day before initiation of treatment, the rats were allocated to four groups of five rats per sex, proportionately to body weight, using a computer randomization program. They were housed under conventional conditions, in macrolon cages (five/cage separated by sex) with stainless steel grid covers and wood shavings as bedding material, in a controlled environment (temperature 20–24 °C, humidity 40–70%, 12-h light (fluorescent tubes)/dark cycle, about 10 air changes per hour). Feed (provided as a powder in stainless steel cans) and drinking water (tap water complying with Dutch and European drinking water regulations, provided in polypropylene bottles) were available *ad libitum*, except during FOB testing and motor activity assessment and collection of blood and urine from fasted rats. The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 86/609/EEC) and Dutch legislation (The Experiments on Animals Act, 1997). This included approval of the study by TNO's ethical review committee.

2.5. Experimental design and observations

The study included four groups of five rats per sex which were kept on the appropriate experimental diet (0%, 1%, 5% or 10% GlucagelTM) from the start of the study (day 0) until scheduled sacrifice on day 28.

The animals were observed daily for abnormal clinical signs. Neurobehavioural functioning was evaluated by weekly, detailed clinical observations made outside the home cage in a standard arena, and a functional observational battery (FOB) and motor activity assessment conducted on days 22–23. The FOB was based on that used in the WHO/IPCS Collaborative Study on Neurotoxicity Assessment (Moser and MacPhail, 1992; Moser et al., 1997a,b). The FOB consisted of non-invasive observational and interactive measures designed to assess the neurobehavioural and functional integrity of the rat, using measures taken from different functional domains including autonomic and neuromuscular function, sensorimotor reactivity, arousal and excitability. Spontaneous motor activity was assessed during a 30-min test period using an automated quantitative microprocessor-based video image analysis system (Ethovision, Noldus Information Technology b.v., the Netherlands). During the test period, the rats were placed individually in open roofed cages (48.8

Table 1
Composition of high purity barley beta-glucan (GlucagelTM).

	Test method	Concentration (% as is, w/w)
Beta-glucan	Mod. AOAC 995.16	75.6
Total carbohydrate	By difference	89.4
Protein (including other nitrogenous material)	AOAC 981.10	4.2
Fat	AOAC 960.39	1.5
Ash	AOAC 942.05	1.0
Moisture	AOAC 950.46	3.9
Total		100

$1 \times 44.7 \text{ w} \times 50 \text{ h cm}$) equipped with a video camera suspended above the test cage. The position of the rat was monitored continuously. The total distance moved during the 30-min test period and habituation of motor activity (distance moved during six 5-min time blocks) were evaluated as measures of motor activity. Ophthalmoscopic observations were made before initiation of treatment in all rats and in week 4 in the control and high-dose group using an ophthalmoscope after induction of mydriasis by a solution of atropine sulphate.

Body weights were recorded on days -1 (weight used for allocation), 0, 7, 14, 21, 27 and 28. Feed consumption was measured per cage over successive periods of 3 or 4 days by weighing the feeders. The intake of Glucagel™ per kg body weight was calculated from the nominal dietary levels of Glucagel™, the feed consumption and the body weight. Water consumption was measured per cage by weighing the drinking bottles daily during 5-day periods in weeks 1 and 3.

On days 23–24, all rats were deprived of water for 24 h and of food during the last 16 h of this period. Urine was collected from individual rats whilst kept in stainless steel metabolism cages, during the last 16 h of deprivation. The volume (graduated tubes) and density (refractometer; Belltingham and Stanley Ltd., UK) of the urine were measured to assess the renal concentrating ability. The appearance of the urine was recorded and the samples were analysed semi-quantitatively (Combur-9-Test strips; Roche) for pH, protein, glucose, ketones, bilirubin, urobilinogen and occult blood. Centrifuged sediment was examined microscopically. Routine haematology and clinical chemistry were conducted on all rats at the end of the treatment period. Following overnight fasting (water freely available), the rats were anaesthetised with CO_2/O_2 and blood was collected from the abdominal aorta into tubes with $\text{K}_3\text{-EDTA}$ for haematology and heparin for clinical chemistry. Plasma was prepared by centrifugation. Haemoglobin, packed cell volume, red blood cells, reticulocytes, total and differential white blood cells and thrombocytes were measured with an Advia 120 Haematology Analyser. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were calculated from haemoglobin, packed cell volume and red blood cells. Prothrombin time was measured using the Normotest for EDTA blood (Nyegaard and Co. A/S, Norway). Plasma levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, total protein, albumin, glucose, total cholesterol, triglycerides, phospholipids, creatinine, urea, inorganic phosphate, calcium, chloride, potassium and sodium were measured using an Olympus AU-400 analyser. The albumin/globulin ratio was calculated from total protein and albumin.

At the end of the treatment period (day 28), the rats were sacrificed by exsanguination from the abdominal aorta under CO_2/O_2 anaesthesia, and a thorough necropsy was performed. The animals were sacrificed in the morning, in such a sequence (stratified randomly) that the average time of sacrifice was approximately the same for each group. The adrenals, brain, full and empty caecum, epididymides, heart, kidneys, liver, ovaries, spleen, testes, thymus and uterus were weighed (paired organs together) as soon as possible after dissection. The relative organ weights (g/kg body weight) were calculated on the basis of the terminal body weight. Samples of the weighed organs and of the aorta, colon, eyes, gut associated lymphoid tissue including Peyer's patches, lymph nodes (axillary and mesenteric), lungs, mammary gland (female), muscle (thigh), peripheral nerve (sciatic), oesoph-

agus, oviducts, pancreas, parathyroids, pituitary, prostate, rectum, salivary glands (parotid, sublingual, and submaxillary), seminal vesicles with coagulating glands, small intestines (duodenum, ileum, and jejunum), spinal cord (three levels), sternum with bone marrow, stomach, thyroid, trachea with bronchi, urinary bladder, vagina and all gross lesions were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde. Samples of the preserved organs from all rats of the control group and the high-dose group were processed, embedded in paraffin, sectioned at 5 μm , stained with haematoxylin and eosin, and examined by light microscopy. Additionally, all gross lesions observed in rats of the intermediate dose groups were examined microscopically.

2.6. Statistical analysis

Body weights, haematology, clinical chemistry and urinalysis results and organ weights were evaluated by one-way analysis of variance (ANOVA) after checking for homogeneity of variances (Bartlett test) and normality of data distribution (Shapiro-Wilk's test). If variances were not homogeneous or data were not normally distributed, the data were stepwise log or rank transformed prior the ANOVA. If the ANOVA showed a significant inter-group difference ($P < 0.05$), the test groups were compared with the control group by Dunnett's multiple comparison test. Neurobehavioural data were analysed by ANOVA followed by Dunnett's multiple comparison test (continuous FOB data, total distance moved), repeated measures ANOVA on time blocks (habituation of activity), Kruskal-Wallis non-parametric ANOVA (rank order FOB data) or Pearson chi-square analysis (categorical FOB data). Histopathological changes were analysed by Fisher's exact probability test. All analyses were two-sided. Probability values of <0.05 were considered significant.

3. Results

3.1. Analysis of beta-glucan in rat feed

Analysis of beta-glucan in rat feed confirmed that Glucagel™ was homogeneously mixed into the feed at all dose levels as shown by relative standard deviations of 3–5% (Table 2). The concentrations of beta-glucan measured were close to the expected levels, both after storage in the animal room for 4 days and after storage at -18°C for up to about 4 weeks, confirming stability under the conditions of the animal study. The control diet (containing no Glucagel™) was found to contain about 0.8% beta-glucan. This observation can be explained by the presence of beta-glucan in the basal diet which is a natural ingredient diet containing 64% cereals (barley and wheat). Additionally, some glucose may have been released from other dietary components during the analytical procedure.

Table 2
Concentration of beta-glucan in the diets used in the 28-day rat study with Glucagel™.

Level of Glucagel™ in the diet (% w/w)	Sample for determination	Measured beta-glucan concentration (% as is, w/w)	Mean \pm SD of five homogeneity samples (% as is, w/w)	Relative SD of five homogeneity samples (%)
0	4 days animal room stability	0.80 \pm 0.09		
	$t = 0$	0.81 \pm 0.06		
1	4 days animal room stability	1.47 \pm 0.08		
	Homogeneity 1/ $t = 0$	1.52 \pm 0.12	1.46 \pm 0.05	3.2
	Homogeneity 2	1.40 \pm 0.13		
	Homogeneity 3	1.43 \pm 0.07		
	Homogeneity 4	1.49 \pm 0.07		
5	4 days animal room stability	1.45 \pm 0.09		
	Homogeneity 1/ $t = 0$	4.13 \pm 0.17		
	Homogeneity 2	4.34 \pm 0.21	4.38 \pm 0.13	3.0
	Homogeneity 3	4.33 \pm 0.09		
	Homogeneity 4	4.56 \pm 0.59		
10	4 days animal room stability	4.22 \pm 0.29		
	$t = 0$	4.46 \pm 0.19		
	Homogeneity 1/ $t = 0$	7.68 \pm 0.84		
	Homogeneity 2	7.26 \pm 0.19	7.61 \pm 0.39	5.1
	Homogeneity 3	7.43 \pm 0.50		
	Homogeneity 4	7.54 \pm 0.45		
	Homogeneity 5	8.27 \pm 1.02		
		7.57 \pm 0.32		

All diets contained 90% (w/w) basal diet (RM3 diet) and 10% supplement (Glucagel™ and/or potato starch). The level of potato starch was 10%, 9%, 5% and 0% in the control, low-, mid- and high-dose diet, respectively.

Dietary concentrations of 1%, 5% and 10% Glucagel™ provided 0.76%, 3.78% and 7.56% beta-glucan, respectively (based on 75.6% beta-glucan in Glucagel™).

Measured concentrations are mean \pm SD of three separate measurements of the same samples (conducted about 2, 3 and 4 weeks after diet preparation; samples were kept at -18°C immediately after diet preparation or after storage for 4 days in the animal room).

Table 3
Mean body weight, feed and water consumption and Glucagel™ intake of rats fed Glucagel™ for 28 days.

	Level of Glucagel™ in the diet			
	0%	1%	5%	10%
<i>Males</i>				
Body weight (g):				
Day 0	162 ± 9	162 ± 8	161 ± 9	163 ± 7
Day 27	294 ± 16	295 ± 11	294 ± 12	281 ± 11
Feed consumption, mean weeks 1–4 (g/rat/day)	18.4	19.3	18.4	17.5
Water consumption, mean weeks 1 and 3 (g/rat/day)	29.4	31.5	32.4	30.6
Intake of Glucagel™, mean weeks 1–4 (g/kg body weight/day)	—	0.82	4.0	7.7
<i>Females</i>				
Body weight (g):				
Day 0	121 ± 6	123 ± 6	122 ± 6	122 ± 4
Day 27	194 ± 7	197 ± 15	197 ± 12	186 ± 7
Feed consumption, mean weeks 1–4 (g/rat/day)	13.0	13.3	13.7	12.4
Water consumption, mean weeks 1 and 3 (g/rat/day)	21.8	26.2	23.4	23.5
Intake of Glucagel™, mean weeks 1–4 (g/kg body weight/day)	—	0.80	4.1	7.8

Body weight values are mean ± SD for groups of five rats. Statistical analysis (ANOVA), conducted on the results in the individual weeks, showed no significant inter-group differences. Feed and water consumption values are cage means (1 cage/sex/group). Glucagel™ intake was calculated from the mean body weight and feed consumption results in each week and the nominal dietary concentrations of Glucagel™.

Table 4
Haematology values of rats fed Glucagel™ for 28 days.

Level of Glucagel™ in the diet	RBC (10E12/l)	Hb (mmol/l)	PCV (l/l)	MCV (fl)	MCH (fmol)	MCHC (mmol/l)	Thrombocytes (10E9/l)	Prothrombin time (s)	WBC (10E9/l)	Lymphocytes (10E9/l)	Neutrophils (10E9/l)
<i>Males</i>											
0%	8.48 ± 0.31	9.6 ± 0.3	0.515 ± 0.014	60.7 ± 1.1	1.13 ± 0.03	18.6 ± 0.2	1042 ± 56	40.7 ± 1.7	12.5 ± 1.8	11.6 ± 1.6	0.56 ± 0.21
1%	8.38 ± 0.22	9.5 ± 0.2	0.501 ± 0.012	59.8 ± 1.3	1.13 ± 0.02	19.0 ± 0.2	1063 ± 102	38.2 ± 0.9*	12.8 ± 1.5	11.6 ± 1.6	0.84 ± 0.18
5%	8.61 ± 0.39	9.7 ± 0.1	0.513 ± 0.017	59.6 ± 1.2	1.13 ± 0.05	18.9 ± 0.6	1051 ± 75	38.4 ± 0.8*	14.0 ± 1.7	12.8 ± 1.8	0.90 ± 0.17*
10%	8.55 ± 0.42	9.7 ± 0.4	0.515 ± 0.024	60.2 ± 0.5	1.13 ± 0.03	18.8 ± 0.3	1018 ± 93	39.6 ± 1.6	12.1 ± 1.9	11.0 ± 1.9	0.78 ± 0.15
<i>Females</i>											
0%	8.41 ± 0.38	9.4 ± 0.3	0.482 ± 0.020	57.3 ± 1.4	1.12 ± 0.04	19.5 ± 0.2	1078 ± 66	34.7 ± 2.0	12.5 ± 1.8	11.5 ± 1.7	0.70 ± 0.17
1%	8.61 ± 0.35	9.7 ± 0.5	0.502 ± 0.028	58.3 ± 1.0	1.12 ± 0.02	19.3 ± 0.2	1027 ± 134	35.9 ± 1.7	12.9 ± 3.3	12.1 ± 3.1	0.46 ± 0.15
5%	8.57 ± 0.23	9.7 ± 0.4	0.500 ± 0.017	58.3 ± 1.0	1.13 ± 0.02	19.4 ± 0.2	957 ± 82	37.3 ± 2.0	11.0 ± 1.9	10.3 ± 1.7	0.40 ± 0.10
10%	8.77 ± 0.22	9.7 ± 0.4	0.498 ± 0.020	56.8 ± 1.3	1.11 ± 0.03	19.6 ± 0.1	908 ± 101	36.4 ± 2.2	11.1 ± 2.5	10.3 ± 2.4	0.50 ± 0.23

RBC, red blood cells; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin.

MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells.

Reticulocytes, eosinophils, basophils and monocytes were comparable in all groups (data not shown).

Values are mean ± SD for groups of five rats.

* P < 0.05 (ANOVA + Dunnett's test).

3.2. Clinical, neurobehavioural and ophthalmoscopic observations

All rats survived until scheduled termination. Daily general clinical observations, weekly detailed clinical observations, FOB testing and motor activity assessment revealed no treatment-related changes in the appearance, general condition or behaviour of the animals. Ophthalmoscopy showed no treatment-related changes either (data not shown).

3.3. Body weight, feed and water consumption and intake of beta-glucan

There were no statistically significant inter-group differences in body weight (Table 3). Feed and water consumption were not affected by the administration of Glucagel™ (Table 3). The overall mean daily intake of Glucagel™ in the high-dose group was 7.7 g/kg body weight in male rats and 7.8 g/kg body weight in female rats (for other groups, see Table 3). These intake levels of provided 5.8–5.9 g beta-glucan/kg body weight/day (based on 75.6% beta-glucan in Glucagel™).

3.4. Haematology, clinical chemistry and urinalysis

Haematology showed no treatment-related changes in red blood cell values, coagulation values, and total and differential

white blood cell counts (Table 4). A few statistically significant differences between the control and test groups (lower prothrombin time in low- and mid-dose males; higher absolute number of neutrophils in mid-dose males) were not ascribed to treatment because they showed no dose-related response and occurred in one sex only.

Clinical chemistry values showed statistically significant changes in the plasma levels of total cholesterol, phospholipids and urea in high-dose males (Table 5). Compared to the starch control group, high-dose males had lower cholesterol and phospholipid levels and higher urea levels.

Renal concentrating ability was not affected as indicated by the absence of treatment-related changes in the volume and density of the urine (data not shown). Semi-quantitative urinary observations and microscopic findings in the urinary sediment showed no treatment-related changes either. Compared with controls, urinary pH was statistically significantly higher (mean ± SD: 6.0 ± 0.0 versus 6.4 ± 0.4, P < 0.05). This was considered a chance finding because the difference from controls was small and not confirmed in the other sex.

3.5. Organ weights and pathology

The weight of the empty caecum was increased by about 35% in mid-dose males and by about 60% in high-dose males (Table 6).

Table 5
Clinical chemistry values of rats fed Glucage™ for 28 days.

Level of Glucagel™ in the diet	ALP (U/l)	ASAT (U/l)	ALAT (U/l)	GGT (μmol/l)	Bilirubin (μmol/l)	Total protein (g/l)	Albumin (mmol/l)	Glucose (mmol/l)	Cholesterol (mmol/l)	Phospholipids (nmol/l)	Triglycerides (nmol/l)	Creatinine (μmol/l)	Urea (mmol/l)	P (mmol/l)	Ca (mmol/l)	Cl (mmol/l)	K (mmol/l)	Na (mmol/l)
Males																		
0%	187 ± 32	67 ± 7	47 ± 4	0.4 ± 0.4	1.9 ± 0.2	67 ± 2	34 ± 2	7.80 ± 1.57	1.97 ± 0.14	1.64 ± 0.09	0.87 ± 0.19	28 ± 2	5.9 ± 1.0	3.15 ± 0.10	2.99 ± 0.09	99 ± 1	5.6 ± 0.5	149 ± 1
1%	179 ± 25	58 ± 8	46 ± 11	0.8 ± 0.5	1.9 ± 0.1	66 ± 2	33 ± 2	7.52 ± 1.45	1.86 ± 0.12	1.53 ± 0.08	0.87 ± 0.16	29 ± 1	6.8 ± 0.4	3.09 ± 0.20	2.98 ± 0.03	98 ± 2	5.3 ± 0.2	149 ± 1
5%	210 ± 22	60 ± 6	49 ± 5	0.7 ± 0.5	1.8 ± 0.1	66 ± 2	33 ± 1	8.65 ± 1.65	2.04 ± 0.10	1.63 ± 0.08	0.81 ± 0.21	26 ± 1	6.9 ± 0.7	3.20 ± 0.23	3.00 ± 0.05	99 ± 1	5.0 ± 0.6	148 ± 1
10%	208 ± 29	56 ± 5	47 ± 2	0.7 ± 0.5	1.7 ± 0.1	63 ± 2	32 ± 1	8.34 ± 1.13	1.74 ± 0.12*	1.44 ± 0.09**	0.81 ± 0.15	28 ± 4	7.4 ± 0.5*	3.25 ± 0.10	2.95 ± 0.01	98 ± 1	5.2 ± 0.3	149 ± 1
Females																		
0%	120 ± 22	54 ± 7	33 ± 6	0.4 ± 0.6	2.0 ± 0.2	64 ± 3	34 ± 1	6.26 ± 0.77	2.11 ± 0.22	1.94 ± 0.24	0.96 ± 0.36	33 ± 4	9.9 ± 2.5	2.94 ± 0.22	2.94 ± 0.10	100 ± 1	5.4 ± 0.3	147 ± 1
1%	133 ± 15	47 ± 10	36 ± 8	0.4 ± 0.4	1.9 ± 0.2	65 ± 2	34 ± 1	7.20 ± 1.11	2.12 ± 0.19	1.87 ± 0.14	0.97 ± 0.11	31 ± 2	8.2 ± 0.3	2.98 ± 0.14	2.96 ± 0.08	99 ± 1	5.5 ± 0.4	148 ± 1
5%	136 ± 22	48 ± 15	38 ± 6	0.5 ± 0.5	1.8 ± 0.2	65 ± 2	34 ± 1	6.68 ± 0.85	2.16 ± 0.16	1.82 ± 0.12	0.94 ± 0.26	28 ± 3	7.2 ± 1.3	2.77 ± 0.16	2.90 ± 0.05	99 ± 1	5.4 ± 0.4	148 ± 1
10%	138 ± 16	56 ± 7	38 ± 9	0.4 ± 0.6	1.7 ± 0.2	65 ± 4	34 ± 2	7.79 ± 1.40	2.05 ± 0.26	1.71 ± 0.19	0.67 ± 0.07	30 ± 2	7.9 ± 1.6	2.71 ± 0.25	2.91 ± 0.07	100 ± 1	5.4 ± 0.2	148 ± 1

ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; GGT, gamma-glutamyl transferase; P, inorganic phosphate.

Values are mean ± SD for groups of five rats.

* P < 0.05.

** P < 0.01 (Anova + Dunnett's test).

Table 6
Terminal body weights and relative organ weights of rats fed Glucage™ for 28 days.

Level of Glucagel™ in the diet	Terminal body weight (g)	Brain (g/kg bw)	Heart (g/kg bw)	Adrenals (g/kg bw)	Kidneys (g/kg bw)	Liver (g/kg bw)	Spleen (g/kg bw)	Thymus (g/kg bw)	Testes/ovaries (g/kg bw)	Epididymides/ uterus (g/kg bw)	Caecum full (g/kg bw)	Caecum empty (g/kg bw)
0%	274 ± 18	6.6 ± 0.4	3.5 ± 0.1	0.16 ± 0.02	6.5 ± 0.4	29.4 ± 1.5	1.94 ± 0.13	2.11 ± 0.25	11.2 ± 1.1	3.1 ± 0.3	16.2 ± 1.2	3.2 ± 0.2
1%	274 ± 8	6.5 ± 0.1	3.5 ± 0.2	0.16 ± 0.02	6.5 ± 0.2	30.3 ± 1.7	1.96 ± 0.12	2.08 ± 0.39	10.7 ± 0.6	3.1 ± 0.3	15.3 ± 2.6	3.2 ± 0.4
5%	274 ± 13	6.8 ± 0.2	3.7 ± 0.1	0.18 ± 0.02	6.6 ± 0.3	29.9 ± 1.1	1.95 ± 0.08	2.07 ± 0.33	11.0 ± 0.5	3.2 ± 0.2	22.5 ± 4.0**	4.3 ± 0.5**
10%	259 ± 14	6.9 ± 0.4	3.7 ± 0.2	0.17 ± 0.01	6.4 ± 0.5	29.6 ± 1.0	2.05 ± 0.17	2.22 ± 0.25	11.8 ± 1.0	3.3 ± 0.1	20.9 ± 1.9*	5.1 ± 0.5*
0%	180 ± 6	9.7 ± 0.3	3.9 ± 0.1	0.32 ± 0.04	7.2 ± 0.3	28.6 ± 1.5	2.36 ± 0.04	2.09 ± 0.24	0.42 ± 0.05	3.2 ± 2.2	18.0 ± 3.2	4.2 ± 0.8
1%	185 ± 15	9.3 ± 0.5	3.9 ± 0.3	0.33 ± 0.05	7.6 ± 0.9	28.8 ± 1.8	2.31 ± 0.20	2.17 ± 0.31	0.39 ± 0.07	2.4 ± 0.7	20.2 ± 3.0	4.5 ± 0.7
5%	182 ± 10	9.3 ± 0.4	3.9 ± 0.4	0.27 ± 0.04	6.7 ± 0.3	27.5 ± 1.3	2.20 ± 0.18	2.21 ± 0.31	0.35 ± 0.02	2.9 ± 1.5	17.4 ± 2.5	4.9 ± 0.9
10%	172 ± 7	9.6 ± 0.5	3.8 ± 0.2	0.29 ± 0.02	7.1 ± 0.4	27.2 ± 0.6	2.22 ± 0.22	2.17 ± 0.15	0.34 ± 0.06	3.7 ± 1.8	25.2 ± 10.4	5.2 ± 0.8

Values are mean ± SD for groups of five rats.

* P < 0.05.

** P < 0.01 (Anova + Dunnett's test).

The weight of the full caecum was also increased in these groups of males but not dose-dependently. The increase in full caecum weight was due to increases in the weights of both the caecal contents and the caecal wall (empty caecum weight). In females, mean full caecum weight in the high-dose group was higher (about 40%) compared with the control group but this difference was not statistically significant due to high inter-animal variation in the high-dose group. The weights of the other organs were unremarkable.

Gross and microscopic examination revealed no changes attributable to the administration of GlucagelTM. The examination revealed only common background pathology findings which occurred incidentally or at similar or random incidences between the control group and the high-dose group (data not shown).

4. Discussion and conclusion

The feeding of GlucagelTM at dietary levels up to 10% to male and female Wistar rats for 28 days was well tolerated, as evidenced by the absence of adverse changes in the general condition and appearance of the animals, neurobehavioural end-points, growth, feed and water consumption, ophthalmoscopy, haematology, clinical chemistry, urinalysis, organ weights and pathology findings.

Rats fed GlucagelTM showed a few changes. The most obvious change was caecal enlargement as shown by the increase in the weight of the full and empty caecum in mid- and high-dose males. Caecal enlargement is a common response of rats to the feeding of large amounts of poorly digestible or slowly absorbable carbohydrates (Newberne et al., 1988). Unabsorbed, fermentable carbohydrate is degraded by microbial fermentation in the large intestine. The fermentation results in the production of short chain fatty acids which represent an increased osmotic load attracting water. This is considered a main cause of the distension and increased weight of the large intestine with its contents (De Groot, 1987; Leegwater et al., 1974; Walker, 1978). GlucagelTM contains about 75% of beta-glucan, an indigestible carbohydrate which has been shown to be fermented to short chain fatty acids by bacterial microflora (Casterline et al., 1997). Therefore, the caecal enlargement seen in this study was considered a non-specific, physiological response to the ingestion of non-digestible carbohydrate. Such caecal enlargement is generally considered of no toxicological concern (JECFA, 1974; World Health Organization, 1987). In the present study, this opinion was supported by the absence of histopathological findings in the caecal wall.

Lower plasma levels of total cholesterol and phospholipids were observed in high-dose males. Lowering of cholesterol is a well-known effect of beta-glucan in animals (Kalra and Jood, 2000; Maqueda de Guevara et al., 2000; Wilson et al., 2004) and is an intended effect of this substance in humans (Keenan et al., 2007; FDA, 2005). The lower plasma lipid levels of high-dose males were within the normal range for rats of this strain and age. Because, moreover, the changes in plasma lipids were not accompanied by any relevant changes in other end-points including histopathology, the lower plasma lipid levels in high-dose males were considered not to be toxicologically relevant.

Another observation in high-dose males was a higher plasma level of urea. An increase in plasma urea might reflect an effect on the kidneys. However, the values in high-dose males were in the normal range, there were no corroborative changes in other end-points for renal toxicity (plasma creatinine, weight and morphology of the kidneys, urinalysis) and a similar change was not observed in females. Therefore, no toxicological significance was attached to the elevated urea level in high-dose males.

The results of our study were in agreement with those of a similar 28-day feeding study in rats with the concentrated (64%) barley beta-glucan preparation Barley Betafiber (Delaney et al., 2003).

Similar to our results, the feeding of Barley Betafiber was associated with caecal enlargement and higher plasma urea levels which were in the physiological range and not accompanied by corroborating signs of renal toxicity. Unlike Delaney et al. (2003), we did not observe an increase in the number of circulating lymphocytes. To assess the relevance of the latter finding, Delaney and co-workers conducted a 28-day feeding study with Barley Betafiber in CD-1 mice. In the mouse study, Barley Betafiber did not cause treatment-related changes in any of the immune or other parameters examined. In a micronucleus study in CD-1 mice administered Barley Betafiber by oral gavage at levels up to 2 g/kg body weight (single dose) the product was neither clastogenic nor cytotoxic to bone marrow cells (Delaney et al., 2004).

In conclusion, this study showed that consumption of diets containing up to 10% GlucagelTM for 28 days was not associated with any obvious signs of toxicity in Wistar rats. The 10% dietary concentration was equivalent to an overall intake of 7.7 and 7.8 g GlucagelTM/kg body weight/day in male and female rats, respectively, and provided 5.8–5.9 g beta-glucan/kg body weight/day. This intake level of beta-glucan is at least 100-fold higher than that recommended for lowering blood cholesterol (namely, at least 3 g per person per day, corresponding to 0.05 g/kg body weight/day for a person weighing 60 kg (FDA, 2005)). The absence of adverse effects of GlucagelTM in rats at an intake level 100-fold above the anticipated human intake level supports the conclusion that GlucagelTM is safe under the conditions of intended use.

Conflict of interest

The authors declare that there are no conflicts of interest.

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A novel barley β -glucan extract (GlucagelTM) in combination with flax or coconut oil influences cholesterol and triglyceride levels in growing rats

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Summary

A 2 x 2 factorial arrangement of treatments was used to test the hypothesis that inclusion of a novel β -glucan extract (GlucagelTM) in cholesterol-free synthetic diets containing coconut oil or flax oil would lower circulating total cholesterol (TC) and triglyceride (TG) levels in growing rats. Inclusion of GlucagelTM (100 g/kg) tended to decrease TC levels ($P=0.07$), however TC level was not influenced by oil type ($P>0.05$). A significant interaction ($P<0.05$) occurred for TG levels, with the addition of β -glucan to diets containing coconut oil and flax oil decreasing TG levels by 40% ($P<0.01$) and 13% ($P>0.05$), respectively. Faecal digestibility of fat was reduced by 7% ($P=0.08$) in rats fed coconut oil plus β -glucan. These data suggest that reduced TG levels caused by addition of β -glucan may be mediated in part by reduced fat digestion in the small intestine, an effect most likely caused by the unique gel-forming properties of GlucagelTM.

Introduction

High levels of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol are recognised as significant risk factors for human cardiovascular disease. The ability of several dietary fibre sources, such as β -glucan from barley, to lower plasma cholesterol levels, especially those that are water soluble, has been demonstrated previously. Barley fed to rats has been reported to lower TC and LDL concentrations (1), while the inclusion of β -glucanase in diets for rats reversed the hypocholesterolemic effects of barley (2). This supports the notion that soluble β -glucan is the component responsible for these cholesterol-lowering properties. Some dietary lipids may also regulate TC levels. In general, studies have shown that saturated fatty acids (SFA) raise TC levels and high levels of polyunsaturated fatty acids (PUFA) reduce the (mainly) high-density lipoprotein (HDL) cholesterol (3).

The effects of a β -glucan extract (GlucagelTM) obtained from New Zealand barley were evaluated in this study by including it as the only source of soluble fibre in synthetic diets fed to growing rats. In addition, the effect of coconut oil, a rich source of SFA, and flax oil, a source rich in PUFA, on plasma TC and TG levels were also evaluated. The hypotheses tested in this study were twofold: (i) rats consuming diets containing β -glucan will have lower TC and TG levels, and (ii) TC levels of rats fed diets with coconut oil will be higher than those of rats fed diets containing flax oil.

Materials and Methods

Thirty-six, 4-week-old male Sprague Dawley rats were allocated in a 2 x 2 factorial arrangement of treatments with factors being flax oil or coconut oil in the diet, and the presence or absence of GlucagelTM. Treatments were assigned as follows: F100: Flax oil+β-glucan; F0: Flax oil minus β-glucan; C100: Coconut oil+β-glucan; and C0: Coconut oil minus β-glucan. The cholesterol-free synthetic diets were based on cornstarch and casein, with GlucagelTM added at 100 g/kg. GlucagelTM (4) is a white odourless powder containing 700 g/kg β-glucan. It has a low molecular weight, is partially depolymerised and, when dispersed in water, forms a soft gel (4). The oils used in the diets were New Zealand flax oil and imported coconut oil. The diets contained Cr₂O₃ (3 g/kg) as a dietary marker.

Rats were housed individually in stainless steel, wire mesh cages in a controlled-temperature room having an ambient temperature of 22 ± 2° C, with a 12 hour light/dark reverse cycle. At 1700 hours on the day before the experiment commenced, all feed was withdrawn, rats were fasted for the next 16 h, and then bled (1 ml) from the tail vein for baseline measurements. Rats were fed the experimental diets in stainless-steel feeders for 26 d. During the last five days, samples of faeces were carefully collected from the floor of each rat's cage and immediately frozen at -20° C. On d 25 of the trial, all rats were fasted from 1700 until 0900 h the following day (d 26), at which time all rats were bled again.

Blood samples were centrifuged and TC and TG were assayed. Crude fat content of the diet and pooled faeces samples was determined using Soxhlet extraction with toluene. All samples were analysed for chromium content. A linear model with the fixed effects of oil type (coconut vs. flax), β-glucan (minus vs. plus), and their interaction was fitted to the data using the GLM procedure of SAS.

Results

Total cholesterol levels in blood decreased about 10% with β-glucan (1.58 vs. 1.76 mmol/L, P=0.07). The type of oil fed had no significant effect on the plasma levels of TC in rats. A significant interaction (P<0.05) existed between β-glucan level and the type of oil added to diets for TG levels. In diets with coconut oil, addition of β-glucan decreased TG levels by 40% (P<0.001). In diets containing flax oil, β-glucan inclusion reduced TG levels by 13% (P>0.05). Rats fed diet F0 had lower TG levels than rats fed diet C0 (P<0.001). The same trend was observed for coconut and flax oil diets containing β-glucan (P=0.053) (Table 1).

An interaction (P=0.08) existed between β-glucan inclusion and the oil type added for faecal fat digestibility. Addition of β-glucan to diets with coconut oil reduced faecal fat digestibility by 7.6 %, whereas addition of β-glucan to diets containing flax oil had no influence on fat digestibility (Table 2).

Table 1: Least-squares interaction means for total cholesterol (TC) and triglyceride (TG) levels in plasma of rats fed different diets (see text for details)

	Diet			RSD ¹
	F100	F0	C100	C0
TC (mmol/l)	1.65	1.71	1.50	1.80
TG (mmol/L)	0.46 ^a	0.53 ^a	0.75 ^a	1.25 ^b

¹RSD - residual standard deviation.

^{a,b} Within rows, values not having the same superscript are significantly different (*P<0.05)

Table 2: Least-squares interaction means for faecal digestibility of fat of rats fed different

	Diet			RSD ¹
	F100	F0	C100	C0
Digestibility, %	93.6	94.5	89.6	97.2

¹RSD - residual standard deviation.

Discussion

The addition of Glucagel™ to a cholesterol-free synthetic diet decreased the levels of TC in the plasma of growing rats by around 10% after 26 d of feeding, although this effect was significant only at the 7% level. These data support our hypothesis, and concur with other work demonstrating the hypocholesterolemic effect of soluble β-glucan (5). We proposed also that TC levels of rats fed diets containing coconut oil would be higher than those fed flax oil, but this was not supported. The fact that flax oil, a rich source of n-3 PUFA, did not have a significant effect on blood TC levels concurs with reports by other workers who showed that the major response to n-3 PUFA intake was a reduction in plasma TG levels but not TC levels (6). In the current experiment, rats fed coconut oil had higher (P<0.05) levels of TG than those fed flax oil irrespective of Glucagel™ addition. Other workers (7) have found higher TG concentrations in the serum of rats fed coconut oil versus corn oil. Coconut oil is a rich source of lauric acid whereas flax oil is a rich source of α-linoleic acid. In numerous metabolic studies, n-3 PUFA's have shown significant hypolipidemic effects, with the major response being a reduction in plasma TG levels (8).

Numerous mechanisms may explain how soluble β-glucan lowers serum cholesterol. These include binding of bile acids in the lumen, inhibition of HMG-CoA reductase in the liver by short-chain fatty acids, delayed gastric emptying, physico-chemical properties of the fibre sources, and interference with fat absorption by increased intestinal viscosity (9). Data from the current experiment, albeit on a small sample size, shows a reduction in faecal fat digestibility, especially in rats fed coconut oil, in the presence of β-glucan. In broiler chickens, the digestibility of saturated fats is decreased in the presence of soluble arabinoxylans. This can be circumvented by the addition of exogenous xylanases (13), suggesting that soluble fibre sources interfere with the process of emulsification, lipid digestion or absorption, or a combination of these factors. Increased viscosity, therefore, may be an important factor responsible for decreased lipid digestion and absorption (14). Gel formation has also been proposed as a mechanism that may delay lipid digestion and absorption (15). Gelling fibres may modify the resistance of the surface-associated unstirred water layer of the small intestine, which in turn can

influence nutrient flux and absorption. They may also interfere with the diffusion of lipid-containing micelles within the small intestine. Glucagel™ forms a soft gel rather than a viscous solution when mixed with water (4), such that the formation of this gel may be the primary factor responsible for the reductions in TC and TG of rats fed the diets containing Glucagel™.

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Original Research

Physiological Effects of Concentrated Barley β -Glucan in Mildly Hypercholesterolemic Adults

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Objective: Barley fiber rich in beta-glucans lowers serum lipids, but is difficult to incorporate into products acceptable to consumers. We investigated the physiological effects of two concentrated barley β -glucans on cardiovascular disease (CVD) endpoints and body weight in human subjects.

Methods: Hypercholesterolemic men and women ($n = 90$) were randomly assigned to one of two treatments: low molecular weight (low-MW) or high molecular weight (high-MW) concentrated barley β -glucan consumed as a daily supplement containing 6 grams beta-glucan/day. Fasting blood samples were collected at baseline and week 6 and analyzed for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, homocysteine and C-reactive protein (CRP). Dietary intakes, body weights, blood pressure, hunger ratings, and gastrointestinal symptoms were measured at baseline and 6 weeks.

Results: The only difference between treatments in lipid outcomes at week 6 was a reduction of the cholesterol/HDL ratio in the low-MW group and a small increase in the high-MW group. No changes were found in blood pressure, glucose, insulin, and gastrointestinal symptoms. Body weight decreased from baseline to 6 weeks in the high-MW group while body weight increased in the low-MW group. Levels of hunger decreased slightly in the low-MW group and decreased significantly in the high-MW group ($P = 0.02$).

Conclusion: Overall, supplementation with isolated barley β -glucans of different molecular weights had small effects on cardiovascular disease markers. Molecular weight of the barley fiber did alter effects on body weight with the high-MW fiber significantly decreasing body weight.

INTRODUCTION

Diets recommended for improvement of cardiovascular risk factors include a diet high in dietary fiber. Cereal fibers that are high in viscous fiber, such as β -glucan, may improve cardiovascular disease risk through improvements in serum cholesterol and other intermediary risk factors. β -glucan is thought to be the active component for the cholesterol lowering effect of barley. Few studies have been conducted on the effectiveness of concentrated β -glucan from barley and changes in physiological endpoints.

The United States Food and Drug Administration (FDA) allows the claim that consumption of soluble fiber from oats or psyllium in a diet low in saturated fat and cholesterol may decrease risk of cardiovascular disease. The health claim was amended to allow inclusion of barley and barley products [1] and there is interest in Sweden to accept a generic health claim

for barley [2]. The incorporation of viscous (soluble) fibers into diet reduces serum cholesterol concentrations. Meta-analysis by Brown et al. [3] showed that daily intake of 2–10 grams of soluble fiber significantly lowered serum total cholesterol and LDL-cholesterol concentrations. The majority of these studies showed no change in HDL-cholesterol or triacylglycerol concentrations with soluble fiber.

β -glucan is thought to be a primary hypocholesterolemic component of barley. More clinical trials have used oats rather than barley even though barley contains more β -glucan than oats. More recent trials have used concentrated beta-glucans from oats or barley. Results of these trials have been inconsistent [4]. Potential reasons for these inconsistencies include low effectiveness because of processing techniques used to isolate beta-glucans, the molecular weight and/or viscosity of the beta-glucans, and the delivery method of the beta-glucans.

Higher molecular weight (MW) fibers are associated with

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increased viscosity. Higher viscosities may be linked to greater reductions in serum cholesterol concentrations and cardiovascular disease risk, but this relationship is not well established. Keenan et al [5] reported a 9–15% decrease in LDL-cholesterol with a 6-week intervention of low and high MW barley beta-glucan when given at doses of 3 and 5 g/day in a parallel study of 155 subjects. The high MW barley was most effective in cholesterol lowering, although the difference was not statistically significant. In contrast, Keogh et al [6] found no changes in cholesterol when 10g/d of isolated barley beta-glucan was fed in a metabolic study.

No differences in effects on blood lipids were found when both high and low molecular weight beta-glucans isolated from oats were given to human subjects [7]. Isolated beta-glucan from oats (5 g/day) lowered LDL-cholesterol when incorporated into a fruit drink [8], but when oats and barley were compared in a similar design only the lower dose of oat beta-glucan (5 g/day) lowered serum lipids while the 10 g/day dose did not [9]. In contrast to oats, barley beta-glucans did not lower serum lipids in this study. Concentrated oat beta-glucan (6 g/day) lowered serum cholesterol in hypercholesterolemic adults [10].

Soluble fiber increases the viscosity of the contents of the stomach and digestive tract. Higher molecular weight (MW) fibers are thought to increase viscosity most. This altered viscosity may be responsible for effects on body weight and attenuated glucose and insulin response because nutrients become trapped and emptying from the stomach is delayed. Few studies have been published on the effectiveness of isolated beta-glucans and glucose and insulin control [11]. Poppitt et al [12] found that high dose barley beta-glucan supplement improved glucose control when added to a high carbohydrate starch food, but not when added to a high carbohydrate beverage. Compared to control, 5 g of beta-glucans from oats significantly lowered postprandial concentrations of glucose and insulin, while barley beta-glucan did not [9]. Barley beta-glucan reduced plasma glucose and insulin responses in male subjects [13]. Incorporation of beta-glucan rich barley into bread lowered glycemic and insulinemic responses [14].

A barrier to use of soluble fiber in the past has been the poor acceptability of foods high in soluble fiber for an extended period. New technologies allow the beta-glucan to be isolated in barley so smaller amounts of the functional fiber can be fed. We measured the effect of 6 g/day of concentrated barley beta-glucan, either low molecular weight or high molecular weight, on cardiovascular biomarkers and body weight.

MATERIALS AND METHODS

Ninety hypercholesterolemic subjects were recruited for the study. To be included in the study, subjects needed to be healthy, non-smoking men and women between the ages of 22 and 65 years at moderate risk for CVD (as defined by a total

cholesterol greater than 200 mg/dl), with spoken and written English literacy. Subjects exclusion criteria included BMI > 30 upon admission to study; CVD, (fasting blood sugar > 126 mg/dl), chronic inflammatory diseases (e.g. Crohn's rheumatoid arthritis), cancer in prior 5 years, renal or hepatic disease, recent bacterial infection (<2 weeks), acute febrile illness in prior 2 months, history of drug or alcohol abuse in prior 6 months, lipid-lowering, anti-hypertensive or anti-inflammatory steroid medication use, and active weight loss >5 kg in prior 3 months.

Study Design

The University of Minnesota Institutional Review Board Human Subjects Committee approved all aspects of this research. The study was a randomized, double blind parallel group design. A total of 90 patients were enrolled with 45 patients per treatment arm, stratified by age and sex. Subjects in either the low-MW or the high-MW treatment group consumed their "usual" diet during the 6 weeks of the study. Participants in the low-MW group consumed six grams of isolated β -glucan, (9 grams of total barley β -glucan product). Subjects in the high-MW group consumed six grams of isolated β -glucan (7.5 grams of total barley β -glucan product). The treatments investigated in this trial were highly enriched barley fiber products, isolated β -glucan with gelling properties. The β -glucan products were produced from barley with a naturally high β -glucan content that was processed through milling and sieving. Percentage of β -glucan was assessed using an assay procedure by Megazyme, International. The two products were classified based on molecular weights (MW). Through processing, the barley β -glucans were altered to manufacture final products with high percentages of β -glucan. Both products were (1→3)(1→4)- β -D-glucan with varying proportions of β -glucan by weight. The low-MW product had a MW of 62,000 and was roughly 75% β -glucan by weight. The high-MW product had a MW of 139,000 and was approximately 85% β -glucan by weight.

Both were delivered as dietary supplements in the form of a powder. Subjects mixed their supplement with a beverage using a study-provided electric hand blender (Braun). Subjects were instructed to take the supplement via oral administration with their morning and evening meal for six weeks.

Diet Records

Three-day diet records were collected as baseline and 6 weeks. Subjects recorded two weekdays and one weekend day of their total food intake. All records were analyzed for total nutrient composition of each day's intake using the First DataBank Nutritionist Five software. Total calories, carbohydrate, fat, protein, cholesterol, saturated fat, monounsaturated fat, polyunsaturated fat, total fiber, soluble fiber, and insoluble fiber were analyzed for each subject for average intake. Analysis of fiber did not include the addition of β -glucan to the diet.

Blood Samples

At baseline and at 6 weeks, subjects were asked to fast overnight for a minimum of 10 hours and then have blood samples drawn at the General Clinical Research Center, University of Minnesota, Twin Cities. Blood was drawn for the following measurements: plasma total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, apolipoprotein A-1, apolipoprotein B, glucose, insulin, homocysteine, and CRP. Blood pressure and body weight were measured at baseline and after 6 weeks of intervention.

To assess tolerance of the treatment products, subjects completed symptoms forms at each visit. Each participant filled out symptom questionnaires comparing changes in gastrointestinal activity to their normal baseline behavior. The topics on the form included stool frequency, stool consistency, as well as the degree of intestinal bloating and flatulence. Stool frequency was simply a count per day. Measures of stool consistency were ranked on a scale between 1 and 10, 1 = diarrhea, 10 = hard stool/constipation. Scores were based on subjective rating scales. When measuring degree of bloating or flatulence, a subject would rank this symptom between 1 and 10, 1 = "minimal" and 10 = "excessive." Mealtime hunger ratings

were also analyzed subjectively. At each study visit, subjects recorded their typical hunger measures corresponding to each meal. Intensity of hunger was measured according to a rating scale ranging from 1-9, 1 = "no desire to eat" and 9 = "unbearable hunger-must eat immediately."

Data Analysis

Baseline values and changes from baseline at week 6 were compared by two-sample two-sided t-test. Within-group changes from baseline were assessed by paired t-test. Categorical values were compared by chi-square test. Changes in lipids at week 6 were also compared after adjusting for baseline values and other covariates. Differences were regarded as significant at $P < 0.5$, but exact P-values are presented. Statistical analyses were performed in SAS, version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Baseline characteristics of the 90 subjects who completed the six-week study are summarized in Table 1. The high-MW

Table 1. Baseline Demographic Comparisons

	High MW	Low MW	P-value
Participants (n)	45	45	
Women	78%	64%	.16
Age (years)	45.1 ± 14	44.1 ± 13	.71
Weight (kg)	73.6 ± 12	74.8 ± 15	.67
Body mass index	26.7 ± 4.2	26.0 ± 3.2	.38
Diastolic blood pressure (mmHg)	66.6 ± 8	67.2 ± 9	.76
Systolic blood pressure (mmHg)	114.0 ± 12	117.4 ± 12	.19
LDL-cholesterol (mmol/L)	146.7 ± 29	152.4 ± 29	.35
HDL-cholesterol (mmol/L)	56.3 ± 14	54.6 ± 12	.52
Total cholesterol (mmol/L)	229.7 ± 30	226.9 ± 32	.68
Ratio (Cholesterol:HDL)	4.3 ± 1	4.3 ± 1	.84
Triacylglycerol (mmol/L)	133.1 ± 67	99.1 ± 45	.005
C-reactive protein (mg/L)	0.42 ± 0.6	0.29 ± 0.3	.20
Homocysteine (uM/L)	8.0 ± 1.9	8.1 ± 2.1	.93
ApoA1 (g/L)	163.4 ± 24	159.3 ± 23	.42
ApoB (g/L)	122.1 ± 20	112.2 ± 18	.02
ApoB/ApoA1	0.77 ± 0.2	0.72 ± 0.2	.22
Insulin (pmol/L)	45.1 ± 2.8	45.1 ± 3.5	.94
Glucose (mmol/L)	5.2 ± 0.09	5.2 ± 0.06	.92
Kcalories	1928 ± 458	1895 ± 516	.75
Protein (g)	78.9 ± 24	74.5 ± 24	.39
Carbohydrate (g)	249 ± 72	254 ± 79	.76
Fat (g)	68.1 ± 27	65.3 ± 25	.61
Saturated fat (g)	20.9 ± 9.4	21.7 ± 9.7	.70
Monounsaturated fat (g)	22.6 ± 11.4	19.1 ± 8.8	.11
Polyunsaturated fat (g)	12.4 ± 6.1	11.5 ± 5	.46
Total fiber (g)	19.8 ± 9.3	18.2 ± 7.8	.39
Soluble fiber (g)	0.46 ± 0.4	0.47 ± 0.5	.95
Insoluble fiber (g)	2.1 ± 1.5	2.2 ± 2.2	.87

Values are mean ± standard deviation, or percent.

P-values are from a two-sided two-sample t-test comparing high MW to low MW groups.

group had higher mean triacylglycerol and apolipoprotein B; with respect to the other outcomes, the groups were similar. There were no differences in baseline dietary records or in reported GI symptoms.

Changes from baseline to the end of the study (week 6) are shown in Table 2. The only difference between treatments in lipid outcomes at week 6 was a reduction of the cholesterol/HDL ratio in the low-MW group and a small increase in the high-MW group. The low-MW group experienced significant reductions from baseline in LDL-cholesterol and CRP.

There were no statistically significant differences in fasting glucose or insulin between the groups at the start of the study (Table 1). There were no significant changes in fasting glucose and insulin concentrations within or between the treatment groups after treatment (Table 2).

There were no significant differences in systolic blood pressure or diastolic blood pressure between the groups at baseline and no significant differences in systolic blood pressure or diastolic blood pressure at 6 weeks of intervention.

Body weight (kg) was well matched between the groups at baseline. At 6 weeks the change in body weight was significant between the groups. By the end of the study, body weight decreased with high-MW barley β -glucan and increased with low-MW barley β -glucan (-0.41 ± 0.27 and 0.37 ± 0.16 , respectively; $P = 0.02$). The increase in body weight from

baseline to 6 weeks was also significant within the low-MW treatment group ($P < 0.05$).

The low MW group reduced their dietary intake of PUFA and fiber, compared to the high MW group. The low MW group significantly reduced their intake of Kcal, carbohydrates, fat, MUFA, PUFA, and fiber. There were no significant differences in mealtime hunger between the treatment groups at baseline. There were no significant changes in hunger ratings at breakfast or dinner after the intervention. However, comparisons of changes in hunger at lunch were significant. The change in lunch hunger from baseline to 6 weeks was significant between the treatment groups, decreasing slightly in the low-MW group and decreasing by a greater amount in the high-MW group (0.03 ± 0.21 and 0.90 ± 0.30 ; $P = 0.02$). This change was also significant within the high-MW group. Although lunch hunger was reduced in the low-MW group from baseline to 6 weeks, the result was not statistically significant.

There were no significant differences in gastrointestinal symptoms between the groups at baseline. There were no changes in gastrointestinal symptoms from baseline to 6 weeks.

Adjusting the comparisons of each lipid endpoint for its baseline value plus baseline triacylglycerol and weight change, did not alter the significance, magnitude, or direction of the lipid changes shown in Table 2.

Table 2. Comparison of Outcome Changes from Baseline

	High MW	Low MW	P-value
LDL-cholesterol (mmol/L)	1.6 ± 3.6	$-5.4^* \pm 2.6$.13
HDL-cholesterol (mmol/L)	-1 ± 0.9	0.8 ± 0.8	.11
Total cholesterol (mmol/L)	0.3 ± 4.2	-2.7 ± 3.1	.56
Ratio (Cholesterol:HDL)	0.13 ± 0.1	-0.10 ± 0.1	.03
Triacylglycerol (mmol/L)	-1 ± 7	12 ± 8	.23
C-reactive protein (mg/L)	-6.7 ± 4	$-11.0^* \pm 5$.48
Homocysteine (uM/L)	-0.06 ± 0.1	0.12 ± 0.2	.43
ApoA1 (g/L)	-0.81 ± 3	2.14 ± 3.8	.54
ApoB (g/L)	0.21 ± 2.9	0.1 ± 2.2	.97
ApoB/ApoA1	0.01 ± 0.02	-0.001 ± 0.02	.63
Glucose (mmol/L)	-0.21 ± 0.08	-0.08 ± 0.07	.20
Insulin (pmol/L)	-2.6 ± 2.9	3.4 ± 2.8	.15
Diastolic blood pressure (mmHg)	1.4 ± 1	0.2 ± 0.8	.37
Systolic blood pressure (mmHg)	2.4 ± 1.5	1.5 ± 1.2	.62
Body weight (kg)	-0.41 ± 0.3	$0.37^* \pm 0.2$.02
Kcalories	-1 ± 85	$-173^* \pm 69$.12
Protein (g)	-1.06 ± 3.9	-3.97 ± 2.7	.54
Carbohydrate (g)	0.77 ± 11.3	$-21.8^* \pm 10.4$.15
Fat (g)	1.1 ± 4.5	$-8.5^* \pm 3.3$.09
Saturated fat (g)	0.58 ± 1.4	-2.04 ± 1.3	.18
Monounsaturated fat (g)	-0.1 ± 1.9	$-3.15^* \pm 1.3$.19
Polyunsaturated fat (g)	1.52 ± 1.4	$-2.32^* \pm 0.9$.02
Total dietary fiber (g)	0.77 ± 1.4	$-2.82^* \pm 0.9$.03
Soluble fiber (g)	0.05 ± 0.1	-0.03 ± 0.1	.54
Insoluble fiber (g)	0.4 ± 0.5	-0.63 ± 0.3	.10

Values are mean \pm standard error.

P-values are from a two-sided two-sample t-test comparing high MW to low MW groups.

Significant within-group change from baseline is indicated by an asterisk (*).

DISCUSSION

We compared the effects of two concentrated barley β -glucans as an adjunct to a normal, self-selected diet in free-living, mildly hypercholesterolemic men and women. After six weeks of supplementation with barley β -glucans, small changes in blood lipids were found and the higher MW barley fiber significantly decreased body weight. The amounts of beta-glucan fed in this study were predicted to significantly lower serum cholesterol. The cholesterol-lowering activity of oats and barley is thought to be due to the β -glucan in the fractions of soluble fiber found within these grains. The β -glucan found in oats and barley appears structurally similar [15]. Oats contain an average of 4% β -glucan by weight [16] and barley contains 5–10% β -glucan by weight [17]. Assuming that β -glucan is the active hypocholesterolemic component, it is reasonable to expect soluble fiber from barley to have cholesterol-lowering capabilities similar to oat, if not greater.

High molecular weight fibers are more viscous and may reduce serum total cholesterol more effectively, but this relationship is not well established [4]. Molecular weights of barley typically range from 150,000 to 300,000 [17], although much larger molecular weights of barley beta-glucan have been reported [18]. This study examined the effects of two highly concentrated barley β -glucan treatments, similar in composition except for differences in molecular weight. The low-molecular-weight (low-MW) treatment product had a MW of 62,000 and was approximately 75% β -glucan by weight. The high-molecular-weight (high-MW) treatment product had a MW of 139,000 and was approximately 85% β -glucan by weight. Due to higher MW and increased viscosity, it was expected that the high-MW barley β -glucan treatment product would produce greater reductions in cholesterol concentrations than the low-MW barley β -glucan.

The high molecular weight of barley β -glucan contributes to high viscosity and leads to undesirable sensory traits. Thus, manufacturers are preparing lower molecular weight beta-glucans and there is a need to prove physiological effectiveness of these new fibers. Early reduced molecular weight β -glucan concentrates were likely prepared with the addition of exogenous β -glucanase or by manipulation of endogenous β -glucanase activity during production. Altering high molecular weight β -glucan through enzymatic hydrolysis either reduced or eliminated the cholesterol-lowering activity [19], leading researchers to theorize that molecular weight may play a role in the effectiveness of fiber as a hypocholesterolemic aid.

Few other studies have directly compared the same beta-glucan sources, just altered for molecular weight and viscosity. Frank et al [7] found no differences in lipid response when oat breads that contained either high or low molecular weight beta-glucans were compared. Keenan et al [5] found that 3 and 5 grams of concentrated barley beta-glucan lowered blood lipids with no statistically significant difference between the different molecular weight barleys. Clear definitions for low

and high molecular weight are not available for barley beta-glucan, making it difficult to compare results across studies. The beta-glucans fed in this study were both significantly lower molecular weight than native barley beta-glucans [18].

Other studies have shown that the addition of barley to the diet reduced serum total and LDL-cholesterol. In a study of hypercholesterolemic men and women by Behall et al [20], consumption of oat or barley products for 6 weeks reduced both serum total cholesterol and LDL-cholesterol, indicating that the source of the soluble fiber was not critical to reducing lipids [21]. Adult male subjects consumed barley or wheat products for a period of 4 weeks (averaging 25 grams of insoluble fiber and 13.4 grams of soluble fiber) [16]. These subjects had significant reductions in serum total cholesterol and LDL-cholesterol, without significant changes in triacylglycerol.

Cooking may also alter the effects of soluble fiber in lipid lowering. Kerckhoffs et al [22] found that beta-glucan from oats baked into bread and cookies had little effect on serum lipids in mildly hypercholesterolemic subjects. Frank et al [7] found no differences between oat breads with high or low molecular weight beta-glucan on blood lipids. Little research has been done to compare isolated fibers that are baked into foods to the same fiber consumed separately to determine if baking does alter physiological effectiveness of functional fibers. Studies with barley pasta enriched with beta-glucan suggest that processing does not diminish effectiveness [23]. The effect of beta-glucan-rich oat bran on serum lipids was related to apolipoprotein E phenotype, suggesting that careful screening and characterization of subject population is necessary in these types of studies.

Previous research suggests that consuming the recommended amounts of dietary fiber may reduce energy intake as a result of reduced hunger and thus lead to weight loss [24]. Epidemiological studies tend to show that fiber is associated with reduced long-term weight gain [25,26]. Clinical trials of soluble fibers find conflicting results. Kovacs et al [27] fed soluble guar gum to overweight males on an energy-restricted diet. These subjects had significant decreases in body weight and body fat percentages with no increase in hunger, although calories were restricted. Other studies report no effect of fiber supplementation on body weight or satiety, although these subjects were already at normal weight and were consuming self-selected diets [28].

Hunger at lunch was significantly reduced from baseline with consumption of high-MW barley β -glucan. Body weight (kg) decreased significantly from baseline to 6 weeks with consumption of high-MW barley β -glucan. This is interesting because there was no reported difference in nutrient intake from baseline to the end of the study. Also of interest is that the low-MW treatment group had reduced glucose concentrations and the high-MW treatment group did not have significant changes in glucose, but body weight and hunger decreased.

The mechanism explaining fiber's effect on hunger and body weight is unclear. Foods containing dietary fiber tend to

provide bulk to the diet [29], without providing as many calories, fat and added sugar. The combination of bulky diet, the increased chewing associated with these highly-dense foods and their gel-forming action all lead to stomach distension which may signal a person to stop eating and thus, decrease overall energy intake. This subsequent decrease in energy intake may help prevent obesity.

Many studies have examined the effects of oats on glycemic response, but few have studied the effects of consumption of viscous barley fiber [30]. It is assumed that barley will have effects on physiology similar to oats. Soluble fibers from oats or other viscous fibers generally improve glucose and insulin responses in normoglycemic and diabetic subjects [31]. A study by Tappy et al [32] reported that diabetics consuming soluble fiber from oats saw a significant reduction in postprandial glucose and insulin, compared to a low-fiber breakfast and this change appeared to be dose-dependent. Wood et al [33] described a significant reduction in postprandial insulinemia that appeared to be dependent on the amount of soluble fiber consumed. Other studies, however, have fed soluble fibers to normal and hypercholesterolemic subjects without significant changes in glucose and insulin concentrations [34]. It is possible that the amount of soluble fiber used in these studies was not adequate to reduce glycemic response.

Epidemiological studies have reported an inverse association between dietary fiber and blood pressure [35]. Data collected from clinical trials suggest a small, anti-hypertensive effect of fiber supplementation [36]. Other studies show no effect on blood pressure [37]. Hypotensive status of study subjects does not appear to be a determining factor in the effectiveness of fiber on blood pressure. In the current study, blood pressure did not change with barley β -glucan consumption. There were no significant changes in reported gastrointestinal symptoms. This is not too surprising since subjects consumed a small amount of additional functional fiber each day.

In conclusion, lunch hunger and body weight were reduced with consumption of high-MW barley β -glucan from baseline to 6 weeks with minor changes in other biomarkers. Because increasing the molecular weight increases viscosity, the high-MW product should have greater hypocholesterolemic effects than the low-MW product. Perhaps the molecular weight of an isolated β -glucan is not the primary determinant of hypocholesterolemic actions of fiber.

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Glucagel® may have a protective effect against a diet that elevates blood cholesterol in older men with raised LDL-cholesterol.

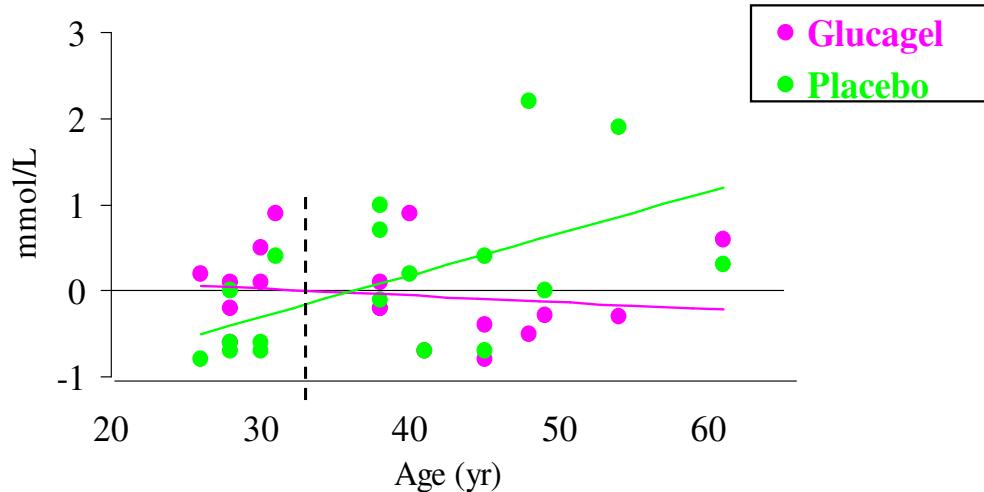
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Auckland Clinical Trial

- A controlled environment crossover single blind design
- Endpoints were blood lipid profile and energy intake
- Subjects were hyperlipidaemic males from 26 to 61

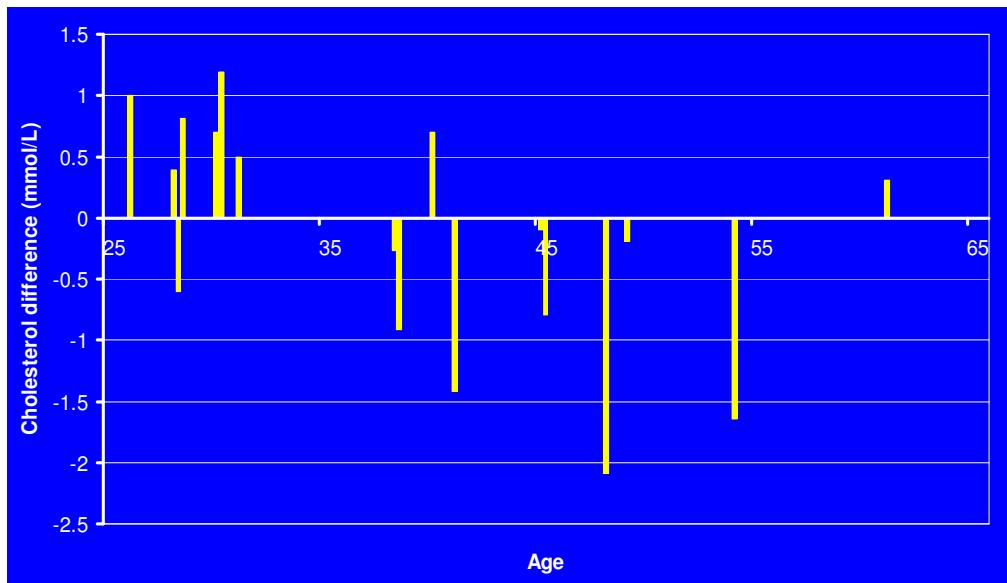
Effect of Age on Change in Total Cholesterol



Auckland Clinical Trial Results

- Non-significant 3.5% reduction in total cholesterol
- The placebo caused blood cholesterol to rise proportional to age, and Glucagel™ protected against that.
- However, subjects >37 years had a significant 11% reduction in total cholesterol

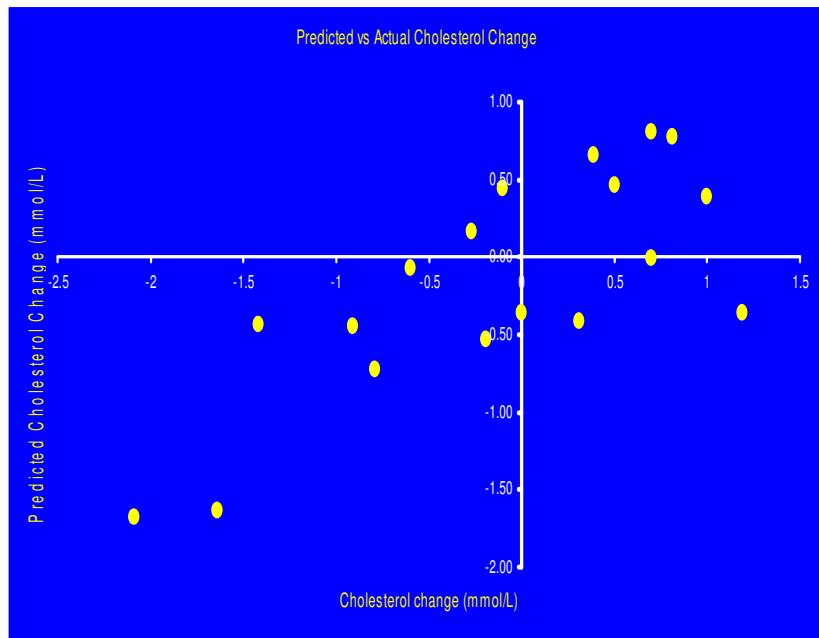
Effect of age on change in total cholesterol (Test – placebo)



Auckland Clinical Trial Results

- Non-significant 3.5% reduction in total cholesterol
- The placebo caused blood cholesterol to rise proportional to age, and Glucagel™ protected against that.
- However, subjects >37 years had a significant 11% reduction in total cholesterol

Predicted vs actual cholesterol change





Randomized controlled crossover study of the effect of a highly β -glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men^{1–3}

Geraldine F Keogh, Garth JS Cooper, Tom B Mulvey, Brian H McArdle, Graeme D Coles, John A Monro, and Sally D Poppitt

ABSTRACT

Background: Soluble-fiber β -glucan derived from oats can reduce cardiovascular disease (CVD) risk through reductions in total and LDL cholesterol. Barley-derived β -glucan may also improve serum cholesterol, but large quantities are required for clinical significance.

Objective: This trial investigated whether a β -glucan-enriched form of barley can favorably modify cholesterol and other markers of CVD and diabetes risk.

Design: Eighteen mildly hyperlipidemic ($\bar{x} \pm SD$; 4.0 ± 0.6 mmol LDL cholesterol/L) men with a mean ($\pm SD$) body mass index (kg/m^2) of 27.4 ± 4.6 were randomly assigned in this single-blind, 2×4 -wk trial to either the treatment arm [$8.1–11.9$ g β -glucan/d (scaled to body weight)] or the control arm (isoenergetic dose of $6.5–9.2$ g glucose/d). After a washout period of 4 wk, dietary regimens were crossed over. The trial took place in a long-stay metabolic facility, and all foods were provided (38% of energy from fat). Fasted blood samples were collected on days 0, 1, 7, 14, 21, 28, and 29 in both study arms. An oral-glucose-tolerance test was carried out on days 0 and 29.

Results: There was no significant change (Δ) in total ($\Delta = -0.08$ mmol/L, -1.3%), LDL ($\Delta = -0.15$ mmol/L, -3.8%), or HDL ($\Delta = 0$ mmol/L) cholesterol or in triacylglycerol ($\Delta = 0.18$ mmol/L), fasting glucose ($\Delta = -0.05$ mmol/L), or postprandial glucose when analyzed between treatments ($P > 0.05$; ANOVA).

Conclusion: The effect of β -glucan-enriched barley on lipid profile was highly variable between subjects, and there was no evidence of a clinically significant improvement in CVD risk across this group of mildly hyperlipidemic men. *Am J Clin Nutr* 2003;78:711–8.

KEY WORDS Serum cholesterol, soluble dietary fiber, β -glucan, barley, randomized controlled trial, hyperlipidemic men

INTRODUCTION

The protective effects of dietary fiber against cardiovascular disease (CVD), mediated through a reduction in serum lipids, was first reported >40 y ago by Keys (1); later research led to the dietary fiber hypothesis proposed by Burkitt (2) and Trowell (3) that a high intake of starchy carbohydrates and fiber is protective against cardiovascular disease. More than 140 intervention trials

have since been carried out with the use of fiber supplements, fiber-enriched foods, and high-fiber whole foods (4–8) to investigate the relation between fiber intake and CVD risk factors. More than 80% of these trials showed a hypocholesterolemic effect of an increased intake of soluble fiber from guar gum and cereals such as oats and psyllium.

Within recent years, the US Food and Drug Administration has endorsed the relation between an increase in soluble fiber and a decrease in serum total cholesterol by ratifying health claims for oats (9) and for psyllium fiber (10). The active component in oats has been identified as the linear mixed-link (1→3)(1→4)- β -D-glucan (β -glucan) (11), which reduces serum total cholesterol by ≈ 5 –10% (5) and which in oats is present at close to 4% (by wt) (12). Barley contains 5–10% (by wt) β -glucan (12) and so may be expected to have similar cholesterol-lowering effects, yet there are few published trials on barley cereal. The trials carried out used a variety of barley flour, bran, flakes, and brewer's spent yeast as the source of β -glucan, and most (13–20) although not all (21) showed barley β -glucan to be hypocholesterolemic. In addition, animal (22, 23), epidemiologic (24, 25), and human intervention (26–28) studies have shown that a diet high in soluble fiber may also improve glucose and insulin control and hence reduce the risk of type 2 diabetes (29–31). The high viscosity of β -glucan may be particularly effective at reducing postprandial glycemia (30), and several trials using oat or barley products (27, 29–37) reported significant reductions in glycemic response.

Barley, unlike oats, is not a readily accepted food source, and it is unlikely that sufficient amounts could be incorporated into

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the diet to achieve the recommended intake of 3 g β -glucan/d (9) without enrichment of the product. Viable options for dietary inclusion must be encapsulation or high enrichment in a manner similar to that used to incorporate oat gum into commercial food products. No trials of highly β -glucan-enriched barley were previously conducted. The aim of this study was to assess whether a highly β -glucan-enriched barley (75% by wt) would result in a clinically significant improvement in CVD risk in a group of men with mild hypercholesterolemia.

SUBJECTS AND METHODS

Subjects

Eighteen male volunteers were recruited into the study by means of an advertisement for interested participants. Subjects aged 18–65 y were recruited on the basis of a mildly elevated concentration of LDL cholesterol (>3.5 mmol/L), no current drug treatment for hyperlipidemia, and no history of CVD. No subjects were currently being treated for hypertension, overweight or obesity, metabolic disorders including diabetes mellitus, or depression. All had normal liver and thyroid functions. All volunteer subjects provided written informed consent. Ethical approval for this study was obtained from the University of Auckland and the Auckland North Health Authority ethics committees.

Protocol

This study was a randomized crossover intervention trial in which participants were blinded to treatment. Subjects were required to be resident at the University of Auckland Human Nutrition & Metabolic Unit throughout both dietary periods, and compliance was ensured by the provision of all foods and beverages during the intervention. Entry into the barley β -glucan treatment arm or the control arm of the trial was by random assignment using stratification to ensure that each arm was balanced. Each of the 2 intervention periods was 4 wk long, and they were separated by a minimum washout period of 4 wk, during which all volunteers returned home and resumed their normal diet. After the washout period, subjects crossed over to the other arm of the study. Blood and urine samples were routinely collected throughout the intervention. Blood samples were collected from fasted subjects by venipuncture on the morning of days 0 and 1 (preintervention baseline) and days 7, 14, 21, 28, and 29. An oral-glucose-tolerance test was also carried out on days 0 and 29 according to standard World Health Organization protocols (38). A liquid glucose polymer (Polycal; Nutricia-Bornem, Antwerp, Belgium), equivalent to 75 g anhydrous glucose and accepted for use by the World Health Organization, was used for the standard glucose load. 24-h urine samples to assess dietary compliance by nitrogen balance were collected on days 10 and 20 on both arms of the intervention. Body weight was measured daily while subjects were fasted and after voiding of the bladder. Blood samples were analyzed for total, LDL, and HDL cholesterol; triacylglycerol; fasting plasma glucose; and postprandial plasma glucose.

Treatment

The barley β -glucan fed in this trial was given as a highly enriched barley fiber product, a gelling form of β -glucan

(Glucagel; Graceline Ltd, Christchurch, New Zealand), produced from high β -glucan content barley that was milled and sieved to separate the starch and cell-wall material. A 2-step extraction process was carried out to produce the β -glucan-enriched product: 1) water extraction at 50–60 °C and 2) a freeze-and-thaw extraction from which the β -glucan was recovered. The final product was a (1→3)(1→4)- β -D-glucan (β -glucan), comprising 75% (by wt) β -glucan that was insoluble in cold water and that formed a weak gelling agent. Active treatment comprised a daily supplement of 0.67 g barley β -glucan/MJ of the total diet and control comprised an isoenergetic amount of monosaccharide glucose of 0.54 g/MJ of the total diet. The dose of both treatment and control was scaled to body size for all subjects. The β -glucan treatment and glucose control supplements were incorporated into snacks and meals consumed throughout the day; cooked into bread, waffles, and muffins for breakfast; cooked into bread for sandwiches at lunch; cooked into savory dishes such as spaghetti Bolognese and chicken curry and into desserts for evening meals; and cooked into cakes and cookies for between-meal snacks.

Background diet

The background diet was designed to be identical on both arms of the intervention to ensure that the only nutrient that differed between treatments was β -glucan. The diet was controlled for macronutrient and micronutrient composition through careful measurement of all food ingredients, each of which was weighed to the nearest gram during diet preparation. The energy and macronutrient contents of the diet were calculated with the use of the dietary program, FOODWORKS (version 2.05; Xyris Software, Brisbane, Australia), which included data from the New Zealand Food Composition Database, to be 38% of energy from fat, 13% of energy from protein, and 49% of energy from carbohydrate, and this content was typical of a Westernized diet. Duplicate samples of the entire 7-d diet rotation were collected from each of 4 randomly chosen subjects on one occasion during the β -glucan-enriched barley treatment and on one occasion during the control treatment. Each of the 7-d samples was individually homogenized, and an aliquot was frozen for later chemical analysis. The content of dietary fiber was verified by direct chemical analyses from these duplicate diets by using the methods of Englyst et al (39).

Subjects were fed to energy balance, based on a multiple of predicted basal metabolic rate (40), and diets were reviewed by members of the research team on a daily basis to ensure that a constant body weight was maintained during each intervention period. A combination of change in body weight, reported activity, and hunger levels was used to assess total daily energy requirements. A 7-d dietary rotation was used so that every week the entire diet was repeated. Subjects were provided with breakfast, lunch, dinner, and between-meal snacks. Breakfast and dinner were eaten under supervision at the Nutrition Unit, and lunch and snacks were packed and participants were able to take them to college or their place of work as they chose. Decaffeinated, sugar-free sodas and decaffeinated, sugar-free tea and coffee were freely available. Subjects were asked to eat all of the foods provided for the trial and no others. Alcohol was prohibited throughout the intervention. The subjects were self-selected and well motivated. Independent dietary compliance was assessed from 24-h urinary nitrogen balance data, in which urinary losses of nitrogen were directly compared with dietary protein intake (where g protein = 6.25 × g





TABLE 1

Clinical characteristics at screening of 18 men who completed both arms of the crossover intervention¹

Clinical variable	
Age (y)	38.8 ± 10.1
Body weight (kg)	86.3 ± 15.8
BMI (kg/m ²)	27.4 ± 4.6
Waist (cm)	96.1 ± 11.7
SBP (mm Hg)	128 ± 12.0
DBP (mm Hg)	86 ± 9.9
Total cholesterol (mmol/L)	5.9 ± 0.7
LDL cholesterol (mmol/L)	4.2 ± 0.7
HDL cholesterol (mmol/L)	0.9 ± 0.2
Triacylglycerol (mmol/L)	1.8 ± 0.9
Plasma glucose (mmol/L)	5.3 ± 0.5

¹ $\bar{x} \pm SD$. SBP, systolic blood pressure; DBP, diastolic blood pressure. All variables measured in subjects in the fasted state.

nitrogen). Para-aminobenzoic acid supplementation was used in an attempt to verify complete 24-h urine collections, according to the method of Bingham (41).

Statistical analysis

Body weight and metabolic outcomes (total, LDL, and HDL cholesterol; total:HDL; triacylglycerol; and glucose) were analyzed by using linear mixed-models analysis of variance (PROC MIXED, version 8.0; SAS Institute Inc, Cary, NC) and corrected for autocorrelation of errors over time. The dietary treatment, the arm of the trial (stratum), the intervention period, and the study day within period were explicitly modeled as fixed factors, as was the treatment × day interaction that addressed whether the trajectory over time during the intervention period differed between treatments (diet × time). Subjects within strata were treated as random, as were their interactions with day and intervention period. Repeat baseline measures before intervention (days 0 and 1) were combined into a single mean value to reduce variability at baseline. Repeat data collected at the end of the intervention (days 28 and 29) were not combined. Variable intervals between blood collections were also included in the analyses so that the unequal numbers of days between measurements were modeled as an autoregressive order 1 process with constant day-to-day correlation. Dietary composition measured by chemical analysis was analyzed

between treatments by using paired Student's *t* test. All biochemical assays were analyzed in triplicate, and the values are presented as means ± SEMs. Statistical significance was based on 95% CIs (*P* < 0.05).

RESULTS

Eighteen male subjects, recruited on the basis of mildly elevated LDL cholesterol, entered the trial and completed both arms of the intervention (Table 1). There were no subjects who withdrew or who were excluded for noncompliance. The subjects were aged 26–61 y, and they tended to be overweight [mean body mass index (in kg/m²): 27.4 ± 4.6; range: 22–39]. At baseline, mean total and LDL cholesterol concentrations were 5.8 ± 0.8 and 4.0 ± 0.6 mmol/L, respectively, both of which were above the ideal range for healthy men. HDL cholesterol, triacylglycerol, and glucose concentrations were all within the normal range, and there was no evidence of significant hypertension.

The average dose of barley β-glucan added to the diet on a daily basis during the period of active treatment was 9.9 ± 0.9 g/d (range: 8.1–11.9 g/d; Table 2). This dose was matched isoenergetically with an average dose of 7.7 ± 0.8 g glucose/d (range: 6.5–9.2) during the control arm. When measured by direct chemical analyses from the duplicate diets collected throughout the trial, the total fiber content of the diet, including the barley β-glucan supplement, was estimated to be 28.7 ± 2.6 g/d in the control period and 35.8 ± 4.8 g/d in the β-glucan period (*P* < 0.001; Table 3); the mean increase was 7.1 g/d. Soluble fiber was shown to increase significantly from 14.0 ± 1.1 to 20.7 ± 4.8 g/d between the control and β-glucan arms of the trial (*P* < 0.001). This was an estimated increase of 6.7 g/d, which showed that the analytic methods used for fiber analyses did not detect all of the β-glucan added into the diet, possibly because of poor solubility during 80% ethanol precipitation if the β-glucan were depolymerized, but they were somewhat informative. Insoluble fiber was kept almost constant between diets, at an average of 15.2 ± 1.9 and 15.8 ± 3.4 g/d on the control and β-glucan arms of the trial, respectively. Body weight was kept stable throughout both arms of the intervention through manipulation of energy intake on a daily basis in response both to changes in body weight and to the reported hunger of the subjects. There was no significant difference between body weight at baseline or weight change throughout the 4 wk intervention periods between the 2 treatments (*P* > 0.05). When subjects were on the

TABLE 2

Composition of the barley β-glucan and glucose control treatments

Composition	β-Glucan treatment		Glucose control treatment	
	Per 100 g	Amount ¹	Per 100 g	Amount ²
Enriched barley fiber product (g) ³	100	13.1	0	0
Soluble-fiber β-glucan (g) ⁴	75	9.9 ± 0.9 ⁵	0	0
Total energy (kJ)	957	125 ± 13	1570	125 ± 13
Protein (g)	13	1.7 ± 0.2	0	0
Fat (g)	0	0	0	0
Total sugars (g)	7	0.9 ± 0.1	100	7.7 ± 0.8
Water (g)	5	0.7 ± 0.07	0	0

¹ Given to subjects; average dietary intake of 14.7 MJ/d.

² Given to subjects; average dietary intake of 14.4 MJ/d.

³ 75% soluble-fiber β-glucan.

⁴ Metabolizable energy content of soluble-fiber β-glucan based on 8.4 kJ/g (42).

⁵ $\bar{x} \pm SD$.

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TABLE 3

Composition of the diet during the treatment (barley β -glucan supplement) and control (glucose) arms of the trial as calculated and as measured by direct chemical analyses of a 7-d duplicate diet collected during the trial from a subset of 4 subjects¹

	β -Glucan treatment	Glucose control treatment	Difference
EI, calculated (MJ/d) ²	14.7 ± 0.1 ³	14.4 ± 0.1	0.3
Protein (% of energy)			
Calculated ²	13	13	0
Measured ⁴	12.1 ± 0.4	12.3 ± 0.5	-0.2
Carbohydrate (% of energy)			
Calculated ²	49	49	0
Measured ⁴	53.9 ± 2.3	54.5 ± 1.0	-0.6
Soluble fiber (g/d)			
Calculated ²	NA	NA	9.9 ⁵
Measured ^{4,6}	20.7 ± 4.8 ⁶	14.0 ± 1.1	6.7 ⁷
Insoluble fiber (g/d)			
Calculated ²	NA ⁵	NA ⁵	0 ⁵
Measured ^{4,6}	15.8 ± 3.4 ⁶	15.2 ± 1.9	0.6
Total fiber (g/d)			
Calculated ²	37.0	27.0	10.0
Measured ^{4,6}	35.8 ± 4.8 ⁶	28.7 ± 2.6	7.1 ⁷
Fat (% of energy)			
Calculated ²	38	38	0
Measured ⁴	34.0 ± 2.0	33.2 ± 1.4	0.8
SFA, measured (% of energy) ⁴	15.2 ± 2.4	14.0 ± 0.9	1.2
MUFA, measured (% of energy) ⁴	12.5 ± 0.9	12.3 ± 0.3	0.2
PUFA, measured (% of energy) ⁴	6.3 ± 1.7	6.9 ± 0.4	-0.6
Cholesterol, measured (mg/d) ⁴	236 ± 43	237 ± 47	-1

¹ EI, energy intake; NA, not available; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

² Calculated from FOODWORKS.

³ $\bar{x} \pm$ SD.

⁴ Chemical analyses of duplicate diets for 4 subjects, adjusted for an average intake of 14.7 MJ/d on the treatment arm and 14.4 MJ/d on the control arm.

⁵ Represents added fiber.

⁶ Measured soluble-fiber and total-fiber contents of the diet were significantly higher during β -glucan supplementation (paired *t* test), *P* < 0.001.

⁷ Calculated by the method of Englyst et al (39).

barley β -glucan treatment, mean body weight was 86.5 ± 3.6 and 86.8 ± 3.6 kg at baseline and at the end of the intervention, respectively; when subjects were on the control arm, mean body weight was 87.0 ± 3.5 and 87.1 ± 3.5 kg at baseline and at the end of the intervention, respectively. No subject lost or gained > 1 kg body wt during the intervention periods.

Blood samples were collected on 14 occasions from each of the 18 subjects during the 4-wk active and control treatment arms. There was no significant change in lipid profile between baseline and the end of the intervention on either the active treatment (time, *P* > 0.05; Figure 1) or control diet (time, *P* > 0.05), nor were there significant between-treatment effects of diet over time on total, LDL, and HDL cholesterol concentrations; total:HDL (diet × time, *P* > 0.05; Figure 1); or triacylglycerol (diet × time, *P* > 0.05; Figure 2). Total cholesterol changed by -0.09 and -0.02 mmol/L between baseline (days 0 and 1 combined) and the end of the intervention (day 29) on β -glucan and control diet, respectively. This represented a between-treatment differential of 1.3%. LDL cholesterol changed by -0.21 and -0.05 mmol/L

between baseline and the end of the intervention on the β -glucan and the control diet, respectively. This represented a between-treatment differential of 3.8%. Throughout the barley β -glucan treatment, total and LDL cholesterol concentrations were consistently lower than when subjects were on the control arm, and there were also pronounced week-to-week fluctuations in circulating lipids during the active treatment. Both between-subject (0.68 mmol/L, 11.6%) and within-subject (0.36 mmol/L, 6.1%) variability in total cholesterol was high. When individual time points were analyzed within the linear mixed model as daily pairwise comparisons, there was no significant between-treatment effect of diet at day 7 or day 14 for any of the measured lipids—total, LDL, and HDL cholesterol concentrations; total:HDL; or triacylglycerol (*P* > 0.05). There was a significant between-treatment effect at day 21 for total cholesterol (*P* < 0.01) but not for LDL or HDL cholesterol, total:HDL, or triacylglycerol (*P* > 0.05), and this effect on total cholesterol was no longer detectable on the days after day 21. The least-squares mean ± SEM differences for total cholesterol between the treatment and control (after correcting for the other factors) were 0.042 ± 0.126 on day 14, 0.367 ± 0.126 on day 21, 0.076 ± 0.126 on day 28, and 0.05 ± 0.126 on day 29, with 2 outlier data points on control diet responsible for the effect. Whereas these changes may indicate some activity of the soluble fiber with respect to cholesterol lowering, there was neither consistency of effect nor significant difference between treatments when they were analyzed over the entire 4-wk intervention (*P* > 0.05).

Fasting plasma glucose tended to decrease over 4 wk on both treatments, but these decreases were not significant by the end of either intervention period (time, *P* > 0.05). There were no significant between-treatment diet × time effects on circulating fasting plasma glucose (diet × time, *P* > 0.05; Figure 2). When given an oral-glucose-tolerance test challenge, average postprandial plasma glucose changed in a predictable manner on both treatments, increasing to a peak at 60 min ($t_{60\text{min}}$) and returning to near baseline by 120 min ($t_{120\text{min}}$; Figure 3). Nine subjects were sufficiently insulin sensitive that a rise in glucose at $t_{60\text{min}}$ was prevented for ≥ 1 of the 4 tests. Two subjects had flat curves on all 4 occasions. With the use of fasting plasma glucose concentrations > 5.5 mmol/L as an indicator, 6 subjects were shown to be mildly glucose intolerant on ≥ 1 of the 4 tests. Three subjects showed impaired glucose tolerance on all 4 tests. No subjects were shown to be diabetic. When analyzed as a group, there were no significant changes in the area under the 120-min glucose curve or in the glucose concentration at $t_{120\text{min}}$ between baseline and day 29 on either the β -glucan or control diets (*P* > 0.05), nor were there significant changes with analysis between treatments (*P* > 0.05).

DISCUSSION

This randomized crossover trial was unable to provide evidence of a significant improvement in CVD risk or type 2 diabetes risk in a group of mildly hypercholesterolemic, middle-aged men fed a highly enriched form of barley-derived β -glucan as part of a typical 38% fat diet. Total cholesterol decreased by only 1.3% and LDL cholesterol by 3.8% over the 4-wk intervention period, which indicated a very modest improvement, if any, in the risk profile.

The 10 g/d dose given to subjects in this trial was more than 3 times the intake recommended by the Food and Drug Administration for efficacious action of soluble-fiber β -glucan (9), an intake level established from >40 intervention studies feeding oat products



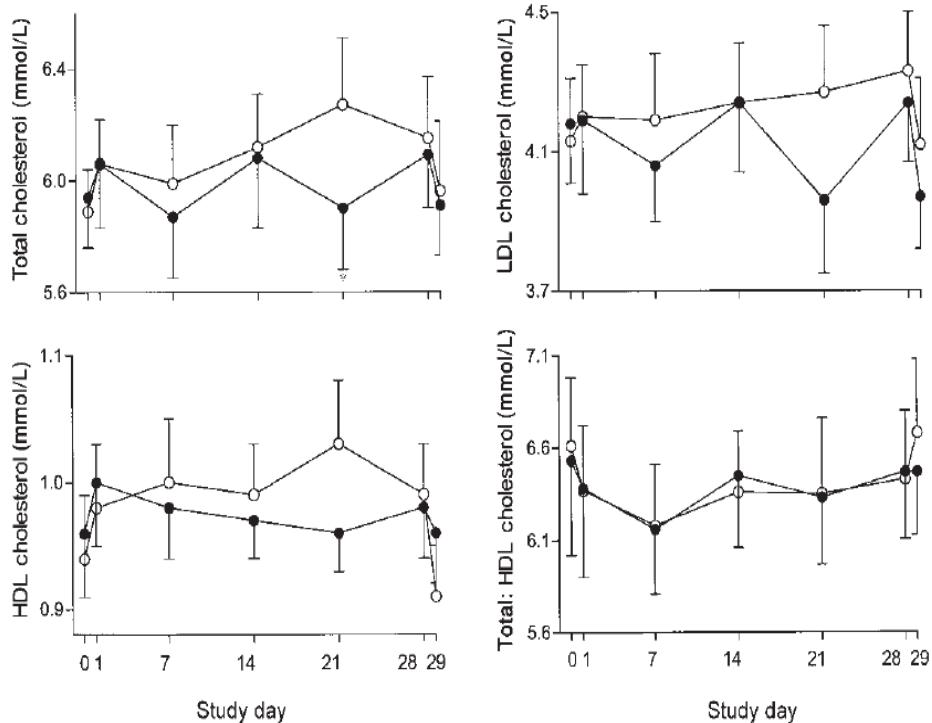


FIGURE 1. Mean (\pm SEM) total, LDL, and HDL cholesterol concentrations and the ratio of total to HDL cholesterol (total:HDL) during the 4-wk barley β -glucan (●) and control (○) treatments in 18 mildly hyperlipidemic men. There was no significant main effect of treatment (diet; ANOVA) for total ($P = 0.25$), LDL ($P = 0.37$), or HDL ($P = 0.34$) cholesterol or total:HDL ($P = 0.88$) or treatment \times time interaction (diet \times time; ANOVA) for total ($P = 0.24$), LDL ($P = 0.44$), or HDL ($P = 0.37$) cholesterol or total:HDL ($P = 0.92$). *Significantly different from control treatment, $P < 0.01$.



(43–52). Most of these trials, which fed oat cereal at doses of 3–145 g/d to both healthy and hyperlipidemic subjects for periods of 2–12 wk, showed 5–10% reductions in total cholesterol, which have been established as both statistically and clinically significant. The current study was based on this earlier body of evidence and powered to detect a 5% decrease in the primary outcome, total cholesterol. The variability in lipid profiles in the group of hyperlipidemic subjects recruited into this trial was greater than predicted: 0.68 mmol/L (11.6%) between subjects and 0.36 mmol/L (6.1%) within subjects. This variability would have masked small improvements in circulating total cholesterol of <5%. It is clear, however, that the β -glucan-enriched barley product used in this trial was unable to match the established efficacy of oat-soluble fiber when fed as oat fiber supplements, fiber-enriched ingredients, or mixed fibers (43–52) or of barley-soluble fiber given as mixed barley diet, barley bran flour, barley oil, brewer's spent grain, and barley β -glucans per se (13–15, 17–20, 53, 54). There have been far fewer trials investigating efficacy of barley β -glucan on CVD or type 2 diabetes risk factors or potential mechanisms of action (55), and no trials at all of β -glucan-enriched barley.

Debate exists as to the mechanism by which a soluble fiber such as β -glucan exerts its effect. Possible mechanisms include 1) increased viscosity in the gastrointestinal tract (56–58), delay in

cholesterol absorption, and increased conversion of cholesterol into bile acids (59, 60) through increased fecal bile acid excretion after binding, although there is little evidence that barley-soluble fiber binds bile acids (61); 2) inhibition of cholesterol synthesis through short-chain fatty acid production (62, 63); and 3) thickening of the unstirred layer of gut lumen, a consequence of an immune response leading to the secretion of materials including glycosaminoglycans, proteoglycans, and glycoproteins that collectively form a viscous mucin (G Coles, unpublished data, 2000). In comparison, insoluble fiber has little effect on serum cholesterol but improves the function of the large intestine by means of rapid intestinal transit time and gastric emptying (64, 65).

The relatively large amount of oat cereal that must be consumed (40 g oat bran or 60 g oatmeal) to achieve an intake of 3 g β -glucan led to the development of enriched forms of oat β -glucan. Seven trials investigated the efficacy of oat β -glucan at concentrations of 10–80% (44, 66–71). Only 4 of the trials reported significant cholesterol lowering (44, 67, 69, 71), which raised the issues of whether the process of enrichment may directly affect bioactivity and whether efficacy may be compromised during processing (72).

The study that we have reported is the first trial to investigate the efficacy of a β -glucan-enriched barley. Unlike oats, 3 g

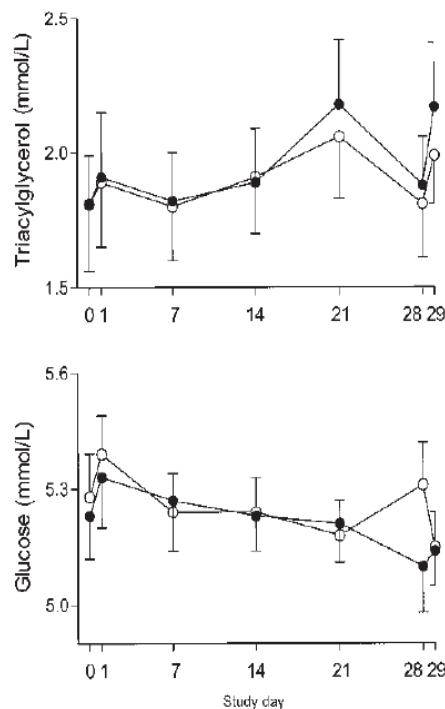


FIGURE 2. Mean (\pm SEM) triacylglycerol and fasting plasma glucose concentrations during the 4-wk barley β -glucan (●) and control (○) treatments in 18 mildly hyperlipidemic men. There was no significant main effect of treatment (diet; ANOVA) for triacylglycerol ($P = 0.61$) or fasting plasma glucose ($P = 0.32$) or treatment \times time interaction (diet \times time; ANOVA) for triacylglycerol ($P = 0.95$) or fasting plasma glucose ($P = 0.36$).



barley β -glucan/d cannot be incorporated into a typical diet but must be highly enriched and consumed either as a supplementary food or nutraceutical product, such as psyllium fiber, or in a commercial food preparation, such as oat gum. In this trial, there was no evidence of a consistent improvement in serum lipids, despite the high dose of enriched β -glucan incorporated into the diet. The only significant between-treatment effect was that for total cholesterol at day 21 of intervention, and this single result may represent a type I error, exacerbated by 2 outliers. To investigate this further, within-treatment differences in total cholesterol between baseline and day 21 for active treatment were analyzed by pairwise t test, but they did not change significantly ($\Delta = 0.0944$, $t = 0.69$, $P = 0.5012$); hence the difference between treatment and control was not due to the effect of active treatment on cholesterol.

There are a number of possible reasons for reduced efficacy: 1) unfavorable structural changes during commercial purification, such as depolymerization of the linear structure (72), that result in decreased molecular weight and reduced viscosity in the gastrointestinal tract; 2) mild extraction conditions (50–60°C), which

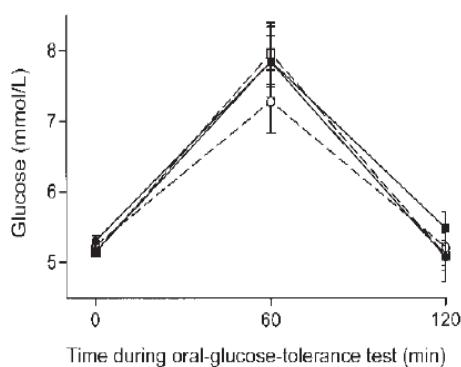


FIGURE 3. Mean (\pm SEM) change in venous glucose concentrations during an oral-glucose-tolerance test at baseline (●, solid line) and at the end of the 4-wk barley β -glucan treatment (■, solid line) and at baseline (○, dashed line) and at the end of the 4-wk control treatment (□, dashed line) in 18 mildly hyperlipidemic men. There were no significant differences between treatment and control arms (diet \times time; ANOVA), $P = 0.34$.

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may not deactivate endogenous β -glucanases and may also lead to increased depolymerization (73, 74); 3) cooking processes that in vitro digestion systems showed may reduce peak molecular weight (75); and 4) freezing and storage, which were shown to reduce the extractability (75) but not the molecular weight (76) of oat β -glucan in the intestine. The quantity of β -glucan ingested accounts only in part for hypocholesterolemic effects, because the viscosity and molecular weight of soluble fiber in the gastrointestinal tract are critical (57, 58). A higher molecular weight may be associated with a higher viscosity and greater cholesterol reduction. A combination of these factors may help to explain the unexpectedly poor response in this trial.

There was no evidence of an improvement in glucose control, which may be unsurprising because no subjects were diabetic and few had impaired glucose tolerance. Many, although not all, of the animal and human trials that showed improvements in glucose control when a variety of soluble fibers were introduced into the diet were performed in prediabetic persons (22–37). Of the 10 trials that investigated responses to oats or barley (27, 29–37), all but one (33) reported significant improvement in glycemic response; however, only 5 of these trials tested the effect of β -glucan per se (30, 31, 34, 36, 37). Dietary fiber acts on glucose absorption and the rate of gastric emptying, which are determined largely by viscosity of fiber in solution (77). Reduction in postprandial glycemia has been attributed to the high viscosity of β -glucan (30).

In conclusion, this current trial—which investigated the efficacy of a highly β -glucan-enriched barley product—did not show clinically significant improvements in lipid or glucose control, and thus there was no evidence of improvement in CVD or type 2 diabetes risk in this group of young to middle-aged, mildly hypercholesterolemic men. Because β -glucan was previously shown to be highly efficacious at doses as low as 3 g/d, we suggest that this lack of effect may be, at least in part, a consequence of structural changes in β -glucan that result from the commercial processing of the barley into a highly enriched β -glucan product or from the



freezing, storage, or baking of the product during the intervention period. Highly enriched products of either barley or oat origin warrant further investigation to establish efficacy.

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GFK was the senior author, co-principal investigator, and trial coordinator. GJSC was the head of the research group, fundraiser, protocol designer, and clinician. TBM was the senior laboratory analyst (metabolic). BHM was the biostatistician. CC was responsible for the development of enriched barley β -glucan (Glucagel). JAM was the senior laboratory analyst (foods). SDP was the co-principal investigator, fundraiser, and director of the metabolic unit and had responsibility for protocol design and manuscript preparation. GDC is employed by Graceline, the manufacturer of Glucagel. None of the other authors had any conflicts of interest.

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ORIGINAL ARTICLE

Beta-glucan- or rice bran-enriched foods: a comparative crossover clinical trial on lipidic pattern in mildly hypercholesterolemic men

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Background/ Objectives: There has been growing interest in using dietary intervention to improve the lipid profile. This work aims at analyzing the effects and the comparison of the enrichment of a diet with beta-glucans or rice bran in mildly hypercholesterolemic men.

Subjects/Methods: The subjects initially consumed a 3-week Step 1 American Heart Association diet with rice bran-enriched foods. After this adaptation period, volunteers were randomly assigned to follow a crossover, controlled trial that consisted of two treatment periods with beta-glucan- or rice bran-enriched foods, each of 4 weeks, with a 3-week wash-out period, like the adaptation period, between trials. Fasted blood samples were collected on days 0, 21, 49, 70 and 98 in both study arms for measuring low-density lipoprotein (LDL)-cholesterol (primary outcome), total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, apolipoprotein (apo) A-I, apo B and glucose levels.

Results: Twenty-four men (mean age: 50.3 ± 5.3 , mean body mass index: 24.9 ± 1.9) completed the 14-week trial. Subjects in the 3-week adaptation period experienced significant reductions in the mean change of LDL cholesterol, total cholesterol, total cholesterol/HDL cholesterol, LDL cholesterol/HDL cholesterol, apo A-I, apo A-I/apo B and glucose. During the intervention diet periods, a difference was found between treatment groups for the mean change in LDL (0.21 (95% confidence interval (CI): 0.02 – 0.40), $P=0.033$) and total cholesterol (0.34 (95% CI: 0.20 – 0.47), $P<0.001$). Other parameters evaluated were not significantly affected by the diet consumed.

Conclusions: The results of the present crossover clinical trial showed that beta-glucan-enriched foods are more effective in lowering serum LDL levels, compared with rice bran-enriched foods.

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Introduction

Lowering serum cholesterol reduces the risk of coronary heart disease (National Cholesterol Educational Program, Adult Treatment Panel III, 2002). The protective effects of

dietary fiber against cardiovascular disease (CVD), mediated through a reduction in serum lipids, were first reported >40 years ago by Keys *et al.* (1960); later research led to the dietary fiber hypothesis proposed by Burkitt *et al.* (1974) and Trowell (1975) that states that a high intake of starchy carbohydrates and fiber is protective against CVD. Many trials showed a hypocholesterolemic effect of an increased intake of fiber from cereals, such as barley, rice and oats; the active component in barley has been identified as beta-glucans, which reduces serum total cholesterol by 5–10% (according to Anderson, 1987; McIntosh *et al.*, 1991; Shimizu *et al.*, 2008; Talati *et al.*, 2009) and by 20% (according to Behall *et al.*, 2004), although not all studies

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(Keogh *et al.*, 2003) showed barley beta-glucans to be hypocholesterolemic.

Although all the possible mechanisms explaining the cholesterol-lowering effect of beta-glucans are not well known, the most likely explanation is that water-soluble fibers lower the re-absorption of bile acids (Kirby *et al.*, 1981; Lia *et al.*, 1995). As a result, the hepatic conversion of cholesterol into bile acids increases and the hepatic pools of free cholesterol decrease. Consequently, hepatic low-density lipoprotein (LDL)-cholesterol receptors become upregulated to re-establish hepatic cholesterol stores, thus promoting a decrease of serum LDL cholesterol (Reihner *et al.*, 1990). Moreover, a recent study showed that the reduced intestinal fatty acid uptake observed with beta-glucans is associated with the inhibition of genes regulating intestinal uptake and with synthesis of lipids (Drozdowski *et al.*, 2010).

As regards rice, active components with cholesterol-lowering effect have been identified in bran and are, over insoluble fiber, novel compounds such as desmethyl tocotrienol and didesmethyl tocotrienol (Qureshi *et al.*, 2000), gamma-oryzanol (Sugano *et al.*, 1999) and polyphenolic ferulic acid (Wilson *et al.*, 2007).

In recent years, the US Food and Drug Administration has endorsed the relationship between an increase in soluble fiber and a decrease in serum total cholesterol by ratifying health claims for barley (FDA, 2005). Overall, the question as to whether various types of fibers exert different effects, and if so to what extent, is not yet conclusive (Brown *et al.*, 1999); moreover, results from different clinical trials vary depending on whether dietary fiber is given to volunteers as a natural ingredient in food sources or as an isolated food supplement.

Given this background, this study aimed (I) at testing the effects of beta-glucan-enriched food on lipid pattern in mildly hypercholesterolemic men and (II) at comparing, in a crossover clinical trial, the effects of a soluble fiber (beta-glucans from barley) and an insoluble fiber (bran from rice) on lipid levels in this population.

Subjects and methods

Subjects

Subjects were recruited from Pavia by advertising in national newspapers and were screened through a procedure involving a clinical evaluation, an interview and an estimation of plasma total and LDL cholesterol levels.

Twenty-four men aged 18–60 years, body mass index 19–30 kg/m², with mild hypercholesterolemia (5.4–7.0 mmol/l) and no history of CVD, volunteered for the trial. The men were not taking any medication that was likely to affect lipid metabolism and they were free of overt liver, renal, metabolic disease such as diabetes and thyroid disease. Screening excluded men who smoked or drank more than two standard alcoholic beverages per day (20 g per day of alcohol). The experimental protocol was approved by the Ethics Committee of the University of Pavia and volunteers gave their written informed consent.

Before beginning the study, energy and nutrient intakes of the volunteers were assessed using the food record method for 3 days. Once the data were collected, the food intake registered was converted into energy and nutrients using the Rational Diet (Milan, Italy).

Diets were fed in a crossover manner. The intervention consisted of an adaptation period (3 weeks) and two diet periods (beta-glucan- and rice bran-enriched foods, 4 weeks each), with a wash-out period (3 weeks) in between (Figure 1).

At adaptation and during the wash-out period, subjects consumed a Step 1 American Heart Association diet (American Heart Association, 1988) consisting of a 7-day rotating menu enriched with rice bran fiber (on average 30 g per day). Each week, volunteers were supplied with foods and instructed to use them within a personalized normocaloric and balanced diet prepared by a dietitian on the basis of a specific single subject's requirement of energy and nutrients. As regards beta-glucan-enriched foods, every day each subject consumed 100 g of pasta with beta-glucans, together with tomato sauce with beta-glucans (100 g), 200 g of vegetable

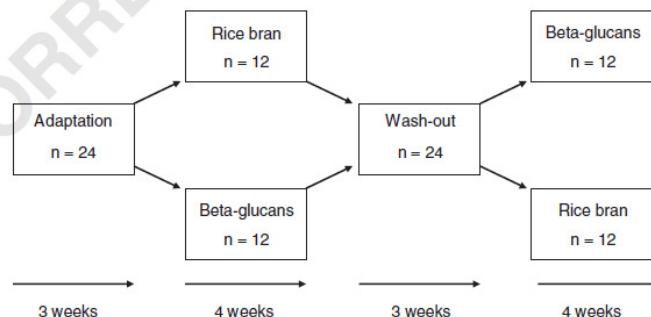


Figure 1 Summary of the treatment periods and the study design.

Table 1 Nutritional composition of 100 g of foods enriched with beta-glucans or rice bran

	Rice pasta enriched with beta-glucans	Whole pasta	Bread rice enriched with beta-glucans	Bread rice enriched with rice bran	Rice cakes with beta- glucans	Rice cakes with rice bran ^a	Tomato sauce with beta-glucans	Tomato sauce with rice bran	Vegetable soup with beta-glucans	Vegetable soup with rice bran
Energy (kJ)	1459	1429	1142	1159	1515	1652	292	330	150	197
Protein (g)	7.0	12.5	9.8	9.3	9.6	8.8	2.3	2.3	2.8	2.3
Fat (g)	1.9	2.0	4.8	5.3	1.6	3.9	4.1	5.0	0.7	1.6
Saturated fatty acids (g)	0.8	0.5	1.15	1.27	0.7	1.04	0.98	1.15	0.23	0.73
Carbohydrate (g)	74.7	67.2	45.4	45.1	76.0	77.7	4.2	3.9	3.3	4.6
Complex (g)	70.4	63.7	40.0	42.88	75.7	76.68	0.62	1.06	1.64	3.64
Simple (g)	4.3	3.5	5.4	2.22	0.3	1.02	3.58	2.84	1.66	0.96
Dietary fiber (g)	3.5	7.3	3.3	4.8	4.0	4.6	3.7	4.9	2.6	2.5
Insoluble (g)	2.1	7.1	2.0	3.3	3.0	4.1	2.1	3.9	1.5	2.1
Soluble (g)	1.4	0.2	1.3	1.5	1.0	0.5	1.6	1.0	1.1	0.4
Beta-glucans (g)	1.7	—	1.2	—	1.4	—	1.5	—	0.9	—

^aMean (s.d.).

soup with beta-glucans, 95 g of bread with beta-glucans (four slices) and 55 g of rice cakes with beta-glucans (seven rice cakes). As regards rice bran-enriched foods, every day each subject consumed 100 g of whole pasta together with tomato sauce with rice bran (100 g), 200 g of vegetable soup with rice bran, 95 g of bread with rice bran (four slices) and 55 g of rice cakes with rice bran (seven rice cakes).

Intervention foods

Palatable, beta-glucan- or rice bran-enriched foods were developed and manufactured by Riso Scotti (Pavia, Italy).

The rice pasta with beta-glucans was produced using 91% rice flour, 2% rice germ and 7% Barley Balance (PolyCell Technologies, Crookston, MI, USA), which is type of beta-glucan-enriched barley flour. Barley Balance is a dry, processed, high-molecular-weight beta-glucan concentrate that contains about 30% beta-glucans (method: AOAC 995.16, 18th edn, 2005). Control rice pasta was produced using 100% whole durum wheat instead of rice flour, rice germ and Barley Balance.

The rice cakes with beta-glucans were produced using rice and water, except for replacing 7% of rice with Barley Balance (6%) and rice germ (1%). Control rice cakes were prepared replacing 100% Barley Balance with Orybran (Riso Scotti), a type of rice bran.

The bread with beta-glucans was produced on a laboratory scale. To obtain 1 kg of wheat flour type '0', 0.55 kg of rice flour, 0.05 kg of rice germ, 0.15 kg of Barley Balance, 1.5 kg of water, 0.1 kg of rice oil, 0.08 kg of 0.06 dextrose, 0.035 g of salt and 0.055 yeast were used. The control bread was produced in the same manner, except for replacing 100% Barley Balance with Orybran.

The sauces with beta-glucans, packed in single pouches, were produced by mixing, for each pouch, 71 g of water, 31 g

of tomatoes, 6 g of rice oil, 6 g of Barley Balance, 6 g of mixed vegetables and 0.5 g of salt. The control sauces were produced replacing 100% Barley Balance with Orybran.

The cream soups with beta-glucans, packed in a single pouch, were produced by mixing, for each pouch, 98 g of water, 95 g of mixed vegetables, 6 g of Barley Balance and 0.5 g of salt. The control cream soups were produced replacing 100% Barley Balance with Orybran.

The chemical composition of enriched foods is presented in Table 1.

Nutritional status

Every week, body weight was assessed and body mass index was calculated. Skinfold thicknesses, assessed following a standardized technique (Frisancho, 1984), and sagittal abdominal and waist girth were measured on days 0, 21, 49, 70 and 98.

Health-related quality of life

The studied subjects were tested using the Short-Form 36-Item Health Survey (SF-36) (Ware et al., 1993) in order to evaluate their quality of life.

The Wexner score for constipation was assessed (Agachan et al., 1996).

Analyses

Fasted blood samples were collected between 0745 and 1000 hours on days 0, 21, 49, 70 and 98 in both study arms for measuring direct LDL-cholesterol, total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, apolipoprotein (apo) A-I, apo B and glucose levels. These parameters were measured enzymatically using an autoanalyzer (Hitachi, Tokyo, Japan).

Compliance and palatability

Sensory acceptance of the enriched foods and treatment-related side effects were assessed at each study visit.

Statistical analysis

Sample size. The difference in levels in LDL-cholesterol after 4 weeks of treatment (primary end point) was used to compute the sample size of the study. Computations were based on data from McIntosh *et al.* (1991). A sample size of 24 will have 80% power to detect a difference of 0.33 mmol/l in means (corresponding to final LDL-cholesterol levels of 4.87 mmol/l in control and 4.54 mmol/l in treated patients), assuming a common standard deviation (s.d.) of differences of 0.54, using a paired Student's *t*-test with a 0.050 two-sided significance level. After accounting for a 10% dropout rate, we planned to enroll 26 patients per group. Query advisor 4 (Statistical Solutions, Cork, Ireland) was used.

Randomization and masking. A randomly permuted block randomization list for the two possible sequences was generated. Masking was guaranteed by identical packages for both treatments, labeled with A or B according to the randomization arm.

Analysis of the primary and secondary end points. Descriptive statistics were computed for each treatment and end point (mean and s.d. for continuous variables and counts and % for categorical variables). Mean changes from screening to baseline and from baseline to the final values of the considered outcome variables were computed for each arm, together with their 95% confidence intervals (95% CI), and assessed with the paired Student's *t*-test. For both primary and secondary end points, the final levels of LDL-cholesterol (and other biological parameters) were compared between treatments with a multilevel mixed-effects linear regression (with a random effect for patients and unstructured covariance), including also baseline values and period as covariates. The treatment effect was presented as the mean difference between final values (95% CI), adjusted for baseline values and period effect.

A two-sided *P*-value <0.05 was considered statistically significant. Stata 11 (StataCorp., College Station, TX, USA) was used for computation. *Post hoc* comparisons with treatments were tested at the 0.025 level (Bonferroni correction).

Results

Dietary intake and body weight

The nutritional composition of the diets consumed daily by the volunteers at screening and during the different periods of the trial is shown in Table 2. Body mass index remained stable (at screening: 24.92 ± 1.93 ; at the end of the study: 24.82 ± 2.01).

Cardiovascular risk variables

All the 24 men (mean age: 50.33 ± 5.34) completed the 14-week trial and their characteristics at the commencement and at the end of the 3-week adaptation period of the study are shown in Table 3.

Subjects in the 3-week adaptation period experienced significant reductions in the mean change of lipid and glucose parameters (Table 3): the average percentage reduction in LDL, total cholesterol, apo A-I and glucose was 13, 8, 14 and 3%, respectively.

The effects of intervention diets on cardiometabolic risk variables are summarized in Table 4. During the intervention diet periods, a difference of 0.21 mmol/l was found between the treatment groups for the mean change of the primary end point, which is the LDL-cholesterol (*P*<0.033); the decrease was greater in the beta-glucans group than in the rice bran arm (*P*=0.012 and 0.66, respectively): the average percentage reduction in LDL-cholesterol was 8.6% in the beta-glucans arm and only 1.1% in the rice bran arm.

Further, a difference was found between the treatment groups for the mean change of total cholesterol (*P*<0.001); the decrease in total cholesterol was significant only in the beta-glucans group and not in the rice bran arm (*P*=0.001 and 0.25, respectively): the average percentage reduction in total cholesterol was 5.0% in the beta-glucans arm and the average percentage increase in total cholesterol in the rice bran arm was 1.3%.

Serum lipid profile and glucose were not significantly affected by the diet consumed in both groups; hence, no significant intergroup difference was observed for these parameters.

Health-related quality of life

As regards the SF-36 score, the results showed that treatment with beta-glucans did not have a different effect from treatment with rice bran (Table 4).

The mean changes in the scores of the Wexner scale were not significantly different in both groups, although the final score was lower in the latter (Table 4).

Compliance and palatability

Treatment was well tolerated by all subjects, with excellent compliance. The fact that there were no study dropouts further indicates the tolerability of the study treatments. No adverse events were reported.

Discussion

The effect of dietary fiber on cholesterol metabolism has been studied extensively (Keys *et al.*, 1960; Burkitt *et al.*, 1974; Trowell, 1975), even if results between studies on the effects of different kinds of fibers on LDL-cholesterol concentrations are variable (Anderson, 1987; McIntosh *et al.*, 1991; Talati *et al.*, 2009). This variability may be due to several

Table 2 Nutritional intakes of volunteers' previous enrolment in the crossover clinical trial and nutritional composition of total diet and of foods enriched with beta-glucans or rice bran and of other nutrients consumed daily by the volunteers during the different periods of the crossover clinical trial

	Pretreatment period ^a	Periods 1 and 3 total diet ^a	Beta-glucans period ^a		Rice bran period ^a	
			Total diet	Foods enriched with beta-glucans	Total diet	Foods enriched with rice bran
Energy (kJ)	11058.73 (780.87)	11181.87 (754.56)	10972.3 (391.63)	3972.88	11166.69 (688.47)	4167.58
Protein (g)	96.67 (18.17)	79.41 (6.72)	85.81 (6.89)	29.49	88.95 (6.55)	33.07
(% energy)	14.72 (3.11)	11.89 (0.74)	12.95 (0.79)	—	13.38 (0.71)	—
Fat (g)	81.77 (16.49)	73.91 (5.08)	78.29 (3.39)	12.81	80.60 (3.53)	17.37
(% energy)	27.81 (5.17)	24.92 (1.27)	26.65 (1.73)	—	27.32 (1.35)	—
Saturated fatty acids (%)	9.58 (2.92)	6.09 (1.05)	6.22 (1.56)	—	6.55 (1.65)	—
Carbohydrate (g)	404.16 (62.88)	447.32 (35.27)	406.24 (31.38)	170.74	397.96 (34.04)	166.21
(%)	57.28 (7.61)	62.74 (1.31)	57.44 (1.53)	—	56.03 (1.59)	—
Complex (g)	326.04 (57.28)	357.62 (35.98)	302.44 (33.20)	154.29	305.73 (41.93)	155.29
(%)	46.18 (6.97)	50.11 (2.08)	42.72 (2.76)	—	42.16 (2.59)	—
Simple (g)	78.01 (20.27)	89.70 (9.44)	103.79 (8.48)	16.44	98.16 (8.60)	10.92
(%)	11.10 (2.93)	12.63 (1.55)	14.72 (1.35)	—	13.87 (1.35)	—
Dietary fiber (g)	19.73 (3.03)	26.74 (3.16)	39.33 (3.33)	17.74	45.70 (3.49)	24.29
Soluble (g)	7.01 (1.10)	9.12 (0.91)	13.74 (0.93)	6.98	10.36 (0.95)	3.69
Insoluble (g)	12.69 (2.21)	17.62 (2.44)	24.89 (2.15)	10.76	34.63 (2.24)	20.60
Beta-glucans (g)	—	—	5.99	5.99	—	—
Cholesterol (mg)	287 (89)	275 (93)	279 (99)	—	281 (93)	—

^aMean (s.d.); Nutritional evaluation from Carnovale E, Marletta L: 'Tabelle di composizione degli alimenti', Istituto Nazionale della Nutrizione, Roma, 1997 and from the results of the laboratory assessment, according to the following methods: Proteins: Protein determination through acid-catalyzed mineralization, with alkaline ammoniacal nitrogen. The ammonia produced is determined through titrimetric measuring. The value of ammonia obtained is multiplied for the factor 6.25 (ISO 937-1978 (E), AOAC Official Method 'Nitrogen in Beer' 950.53, AOAC Official Method 'Nitrogen in Liquid eggs' 932.08, AOAC Official Method 'Nitrogen in milk' 991.20, AOAC Official Method 'Nitrogen in wines' 920.70). Fats: Determination by gravimetric, with acid hydrolysis of the sample and subsequent extraction with petroleum ether in Soxhlet (ISTISAN 1996/34, pp 41-43). Fatty acids: The methyl esters obtained by transmethylation of fatty material are separated in capillary gas chromatography column (Method NGD C2—1989; Method NGC C41/42—1976, Regulation (CEE) No. 2568/91; Method CO/T.20/Doc. No. 24—2001, Regulation (CE) No. 796/2002). Carbohydrates: Value obtained by difference to 100, with known values of water, ash, fat, protein and dietary fibers content in the food. Simple sugars: The sugars are extracted with hot water, clarified with the Carez reagent and saponified. The revelation of the trimethylsilyl ethers is carried out in gas chromatography (GC-FID) on a capillary column. Dietary fibers: Gravimetric determination—with enzymatic precipitation of soluble fiber with hot ethanol (AOAC 985.29 and 17th 2003).

Table 3 Cardiovascular risk variables at screening and after adaptation period

Parameter	At screening ^a	After adaptation period ^a	Mean change (95% CI)	P-value
LDL-cholesterol (mmol/l)	4.17 (0.56)	3.64 (0.86)	-0.53 (-0.79 to -0.26)	<0.001
Total cholesterol (mmol/l)	6.44 (0.55)	5.90 (0.68)	-0.54 (-0.75 to -0.33)	<0.001
HDL-cholesterol (mmol/l)	1.52 (0.42)	1.61 (0.49)	0.09 (-0.07 to 0.24)	0.28
Total cholesterol/HDL-cholesterol	4.58 (1.45)	4.03 (1.32)	-0.55 (-0.88 to -0.22)	0.002
LDL-cholesterol/HDL-cholesterol	3.00 (1.06)	2.55 (1.09)	-0.45 (-0.75 to -0.15)	0.005
Apolipoprotein A1 (g/l)	1.31 (0.31)	1.15 (0.23)	-0.16 (-0.23 to -0.09)	<0.001
Apolipoprotein B (g/l)	1.27 (0.20)	1.31 (0.23)	0.04 (-0.04 to 0.12)	0.33
Apolipoprotein A1/apolipoprotein B	1.06 (0.33)	0.91 (0.27)	-0.15 (-0.21 to -0.09)	<0.001
Triglycerides (mmol/l)	1.63 (0.99)	1.42 (0.82)	-0.21 (-0.50 to 0.09)	0.16
Glucose (mmol/l)	5.21 (0.36)	5.04 (0.38)	-0.17 (-0.30 to -0.05)	0.009

Abbreviations: CI, confidence interval; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

^aMean (s.d.).

factors, such as fiber intake, baseline serum cholesterol concentrations, mode of administration, food matrix, and solubility or molecular weight of the fiber. This study was dedicated to comparatively studying, by a crossover clinical

trial, the effects of two different kinds of dietary fibers on lipidemic pattern in mildly hypercholesterolemic subjects. To our knowledge, this is the first time that water-soluble fiber beta-glucans and non-soluble fiber rice bran have been

Table 4 Comparison of treatments on cardiovascular risk variables, the mental component summary and physical component summary of SF-36 score and Wexner score for constipation at baseline and at the end of intervention

Parameter	Treatment	Baseline ^a	Final, after 4 weeks of intervention periods ^a	Mean change (95% CI)	P-value for change	Treatment effect ^b	Treatment comparison P-value	
Q3	LDL-cholesterol (mmol/l)	Beta-glucans	3.84 (0.64)	3.51 (0.66)	-0.33 (-0.58 to -0.08)	0.012	-0.21 (-0.40 to -0.02)	0.033
	Rice bran	3.68 (0.87)	3.63 (0.63)	-0.04 (-0.29 to 0.19)	0.66			
	Total cholesterol (mmol/l)	Beta-glucans	6.14 (0.53)	5.83 (0.46)	-0.30 (-0.47 to -0.14)	0.001	-0.34 (-0.47 to -0.20)	<0.001
	Rice bran	5.99 (0.72)	6.07 (0.57)	0.08 (-0.06 to 0.23)	0.25			
	HDL-cholesterol (mmol/l)	Beta-glucans	1.65 (0.41)	1.70 (0.49)	0.05 (-0.13 to 0.23)	0.59	0.01 (-0.10 to 0.11)	0.91
	Rice bran	1.65 (0.46)	1.68 (0.49)	0.03 (-0.09 to 0.16)	0.58			
	Total cholesterol/HDL-cholesterol	Beta-glucans	3.94 (1.01)	3.76 (1.26)	-0.18 (-0.57 to 0.21)	0.35	-0.18 (-0.38 to 0.01)	0.07
	Rice bran	3.96 (1.32)	3.96 (1.46)	0.00 (-0.37 to 0.37)	1.00			
	LDL-cholesterol/HDL-cholesterol	Beta-glucans	2.51 (0.84)	2.33 (1.05)	-0.18 (-0.54 to 0.17)	0.30	-0.11 (-0.26 to 0.04)	0.17
	Rice bran	2.44 (1.03)	2.38 (1.07)	-0.07 (-0.36 to 0.23)	0.64			
	Apolipoprotein A1 (g/l)	Beta-glucans	1.15 (0.22)	1.13 (0.21)	-0.02 (-0.08 to 0.05)	0.61	-0.06 (-0.13 to 0.01)	0.10
	Rice bran	1.12 (0.21)	1.15 (0.24)	0.04 (-0.04 to 0.11)	0.31			
	Apolipoprotein B (g/l)	Beta-glucans	1.23 (0.17)	1.10 (0.12)	-0.14 (-0.09 to -0.18)	<0.001	-0.04 (-0.09 to 0.01)	0.12
	Rice bran	1.18 (0.21)	1.11 (0.11)	-0.07 (-0.01 to -0.14)	0.03			
	Apolipoprotein A1/apolipoprotein B	Beta-glucans	0.95 (0.23)	1.06 (0.29)	0.11 (0.18 to 0.04)	0.004	0.02 (-0.06 to 0.10)	0.58
	Rice bran	0.98 (0.28)	1.06 (0.30)	0.08 (0.13 to 0.03)	0.005			
	Triglycerides (mmol/l)	Beta-glucans	1.41 (0.72)	1.36 (0.61)	-0.05 (-0.27 to 0.18)	0.67	-0.19 (-0.48 to 0.10)	0.20
	Rice bran	1.38 (0.84)	1.55 (0.85)	0.17 (-0.16 to 0.50)	0.29			
	Glucose (mmol/l)	Beta-glucans	4.97 (0.48)	4.82 (0.56)	-0.15 (-0.33 to 0.03)	0.10	-0.08 (-0.29 to 0.13)	0.44
	Rice bran	4.88 (0.36)	4.81 (0.46)	-0.08 (-0.27 to 0.11)	0.42			
	SF-36 physical component summary	Beta-glucans	52.35 (8.13)	53.32 (7.11)	0.97 (-1.06 to 3.00)	0.33	0.89 (-0.17 to 1.96)	0.10
	Rice bran	52.35 (8.13)	54.25 (7.20)	1.90 (-0.17 to -3.62)	0.032			
	SF-36 mental component summary	Beta-glucans	53.87 (10.88)	56.47 (8.83)	2.60 (-0.27 to -4.94)	0.03	-0.31 (-1.39 to 0.77)	0.57
	Rice bran	53.87 (10.88)	56.17 (8.88)	2.30 (-0.33 to -4.93)	0.08			
	Wexner score for constipation	Beta-glucans	2.46 (2.93)	2.29 (2.90)	-0.17 (-0.55 to 0.22)	0.38	0.55 (-0.08 to 1.17)	0.09
	Rice bran	2.65 (2.35)	1.91 (2.48)	-0.74 (-0.20 to -1.28)	0.01			

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; SF-36, Short-Form 36-Item Health Survey.

^aMean (s.d.).

^bMean difference between final values (95% CI) adjusted for baseline values of each period and for period effect.

compared with regard to their efficacy on lipid pattern in a crossover study. The studied fiber sources, namely, beta-glucans from barley and bran from rice, were selected from among cereals because of their particular hypocholesterolemic properties, the mechanisms of action of which on lipidic pattern are very different. Beta-glucans are water-soluble dietary fibers, having gel-forming properties that cause effects on lipid pattern (Marlett *et al.*, 1994; Drozdzowski *et al.*, 2010), whereas rice bran is a water-insoluble fiber with active components having a cholesterol-lowering effect (Qureshi *et al.*, 2000; Wilson *et al.*, 2007).

The first result of this trial is that the subjects after the 3-week adaptation period with rice bran-enriched foods experienced significant reductions in blood LDL-cholesterol, total cholesterol, apo A-I, total/HDL-cholesterol and glucose, in agreement with previous large studies (Keys *et al.*, 1960; Burkitt *et al.*, 1974; Trowell, 1975; Cheng *et al.*, 2010). The mean fiber intake of the volunteers before the start of the study (12 ± 2 g per day) was lower than the recommended daily intake (30 g per day), in agreement with previous studies on the adherence of the general Italian population to the recommendations for correct nutritional behavior (Avellone *et al.*, 1997; Rivellesse *et al.*, 2008). The increase in the intake of fiber, including water-insoluble fiber, such as rice bran, caused a significant cholesterol-lowering effect.

These results show that therapeutic dietetic changes remain an essential modality in clinical management of hypercholesterolemia (Grundy *et al.*, 2004).

Moreover, the comparison of the treatment effects clearly indicated that beta-glucans fiber exhibited the most marked influence on cholesterol: in the study, participants receiving beta-glucan-enriched foods had statistically significant reductions in LDL-cholesterol (-0.33 mmol/l) and total cholesterol (-0.31 mmol/l), compared with participants receiving rice bran-enriched foods. In reality, the therapy of mild hypercholesterolemia is lacking. The use of statins, the 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors that are the mainstay in the pharmacological management of dyslipidemia, has been reserved in patients with almost one other risk of CVD. Moreover, the unwanted side effects of statins are numerous, frequent and dangerous (Kostapanos *et al.*, 2010). On the contrary, the consumption of foods enriched in beta-glucans has no known side effects, and, moreover, permits a higher intake per serving with a minimum decrease in palatability (Jenkins *et al.*, 2002). The food products used in this study were developed taking into consideration the problem of compliance. Rice bread and pasta, sauces and soups enriched with beta-glucans are ready to use and are hence easy to eat. Moreover, these foods are well known to contain adequate amounts of beta-glucans

and rice bran because the analysis for the amount of fibers has been carried out in finite products. Finally, another possible advantage of beta-glucan-enriched foods compared with drug therapy is lower costs.

A limitation of the study is that it was conducted exclusively in a group of men. The choice of including only men in the study accrued from two considerations: (1) hypercholesterolemia is a disease more commonly found in male subjects than in female subjects; and (2) many confounding factors related to hormonal status (cycle time, pre-, peri- or post-menopausal phase) may be present in women. Further studies are needed to collect evidence in women.

As regards health-related quality of life, there were no significant differences for the SF-36 scores; the results of this study showed that general health parameters in each group did not differ from each other at baseline and did not change appreciably during the course of the study. A significant change in the SF-36 was not expected; it was included in this study to rule out any adverse effects on general health functioning in either group.

In conclusion, health practitioners should feel comfortable recommending barley beta-glucan-enriched foods to their patients with mild hypercholesterolemia to help reduce total and LDL-cholesterol concentrations. It is well established that an elevated LDL-cholesterol concentration is a risk factor for CVD (National Cholesterol Educational Program, Adult Treatment Panel III, 2002). Studies have shown that, for each milligram per deciliter (0.0026 mmol/l) reduction in a patient's LDL-cholesterol level, their relative risk of having a coronary heart disease event is decreased by 1%; therefore, this reduction in LDL cholesterol observed with food enriched with beta-glucans is likely to be clinically significant as well (Grundy et al., 2004). Moreover, such changes in cardiovascular risk factors, when applied to the whole population, have significant potential for reducing CVD (Kottke et al., 1985).

Conflict of interest

The authors declare no conflict of interest.

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The effects of concentrated barley β -glucan on blood lipids in a population of hypercholesterolaemic men and women

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Barley, like oats, is a rich source of the soluble fibre β -glucan, which has been shown to significantly lower LDL-cholesterol (LDL-C). However, barley foods have been less widely studied. Therefore, we evaluated the LDL-C-lowering effect of a concentrated barley β -glucan (BBG) extract as a vehicle to deliver this potential health benefit of barley. In a 10-week blinded controlled study, subjects were randomized to one of four treatment groups or control. Treatment groups included either high molecular weight (HMW) or low molecular weight (LMW) BBG at both 3 and 5 g doses. Treatment was delivered twice per day with meals in the form of two functional food products: a ready-to-eat cereal and a reduced-calorie fruit juice beverage. Levels of total cholesterol, LDL-C, HDL-cholesterol (HDL-C), and TAG were determined at baseline and after 6 weeks of treatment. The study group comprised 155 subjects. All treatments were well tolerated and after 6 weeks of treatment the mean LDL-C levels fell by 15% in the 5 g HMW group, 13% in the 5 g LMW group and 9% in both the 3 g/d groups, versus baseline. Similar results were observed for total cholesterol. HDL-C levels were unchanged by treatment. Concentrated BBG significantly improves LDL-C and total cholesterol among moderately dyslipidaemic subjects. Food products containing concentrated BBG should be considered an effective option for improving blood lipids.

Soluble fibre: Barley: LDL-cholesterol: CVD

CVD is the leading cause of morbidity and mortality for both men and women in the USA with over 1·4 million deaths and 865 000 myocardial infarctions each year (American Heart Association, 2005). The National Cholesterol Education Program's Adult Treatment Panel III (ATP III) has developed guidelines for reducing the risk of CVD which strongly urge lifestyle modification, including dietary changes, as the foundation and initial intervention for persons at risk for CVD (National Cholesterol Education Program, 2001). An important component of the lifestyle modification is a 'heart-healthy' diet, which specifically includes a recommendation for consumption of at least 5–10 g viscous soluble fibre (VSF) per day. As much as 10–25 g/d can provide additional LDL-lowering effects in some individuals. The current average intake of VSF in the USA is well below that at about 3–4 g/d (Bazzano *et al.*, 2003).

The ATP III guidelines emphasize attainment of a healthy level of LDL-C as the primary goal in CVD risk reduction. Clinical trials using VSF treatments have shown the potential for a 10–15% reduction in LDL-C when it is added to a 'heart-healthy' diet (Bell *et al.*, 1990; Behall *et al.*, 2004a, b). VSF is found naturally in some grains, especially oats and barley, in select fruits, such as apples, guava and

pears, and in most legumes (e.g. peas and pinto beans). It can also be consumed as a dietary supplement (e.g. psyllium). Despite recommendations for increased intakes of VSF in the diet, most individuals do not meet the recommended levels due, in part, to poor palatability of some fibres and the need to consume a relatively large amount of naturally high-fibre foods in order to achieve the desired level.

In an effort to increase consumption of VSF, concentrated extracts of β -glucan VSF have been added to foods and have been effective in modifying CVD risk (Behall *et al.*, 1997). Recently, a process has been developed for extracting the β -glucan from barley to achieve a barley β -glucan (BBG) concentrate with weight-average molecular weight in the range of 50–400 kDa. This represents a reduction in molecular weight from native (high molecular weight (HMW)) BBG, with weight-average molecular weight of 1000 kDa. This reduction in molecular weight improves BBG sensory properties and performance in foods. Food scientists have successfully incorporated it into foods (e.g. cereals, juices and baked goods) to produce palatable food products which are high in VSF.

The present paper reports the results of a clinical trial of concentrated BBG extract in human subjects. The paper

Abbreviations: ATP III, National Cholesterol Education Program's Adult Treatment Panel III; BBG, barley β -glucan; HDL-C, HDL-cholesterol; HMW, high molecular weight; LDL-C, LDL-cholesterol; LMW, low molecular weight; VSF, viscous soluble fibre; TAG, triglycerides; CVD, Cardiovascular Disease; CHD, Coronary Heart Disease.

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focuses on the blood lipid results of this intervention. Additional manuscripts are in review or preparation that will report results on insulin sensitivity, adipocytokines, and other CVD risk factors. The aim of the present study was to evaluate the efficacy of a diet augmented with food products (cereal and juice beverage) that were enriched with BBG to increase their VSF content. The study population included subjects at moderate CVD risk who would be considered candidates for the ATP III therapeutic lifestyle changes. The primary variable of interest was the change in LDL-C using two different doses (3 and 5 g) of both low molecular weight (LMW) and HMW forms of BBG. Of particular interest was the percentage of subjects who attained their personal risk-adjusted LDL-C goal using this daily therapy.

Methods

Subjects

The study group comprised men ($n = 75$) and women ($n = 80$) aged 25–73 years who met the National Cholesterol Education Program ATP III criteria for diet therapy due to elevated LDL-C. From September 2003 to October 2004, subjects were recruited from the University of Minnesota-Twin Cities and the greater Twin Cities area. The study was approved by the University of Minnesota Institutional Review Board, and all subjects gave informed consent. Inclusion criteria were: LDL-C between 1300 and 1900 mg/l; TAG < 400 mg/l; fasting glucose < 1260 mg/l. Individuals were excluded if they had diabetes, cancer, secondary hyperlipidaemia, CVD or other chronic medical conditions; TAG > 4000 mg/l; BMI ≥ 40 ; or a large or unexplained weight change within the previous 6 months. In addition, individuals were excluded if they were taking lipid-altering medications or dietary supplements (2 months prior to screening) which might affect blood lipids; consumed greater than two alcoholic beverages per day on a regular basis; were allergic to aspirin, grain products or any ingredients used in the treatment foods; were following a special diet; or had smoked within the past year. Pregnant and lactating women were also excluded.

Study design

This randomized, double-blind, controlled, five-arm parallel group trial consisted of a 4-week diet stabilization phase followed by a 6-week treatment period. Individuals meeting all inclusion criteria as determined at an initial screening visit were eligible to enter diet stabilization (Fig. 1). These participants attended a group education class in which they were given dietary instruction to consume a diet low in saturated fat and trans-fats (< 10% of kJ/d) and to discontinue any lipid-altering dietary supplements. Participants who still met all inclusion criteria after the diet period were randomly allocated using a block randomization scheme to receive one of five treatments: low-dose (3 g) LMW BBG, high-dose (5 g) LMW BBG, low-dose HMW BBG, high-dose HMW BBG or control. Subjects were instructed to continue following the low saturated and trans-fat diet and to maintain other lifestyle habits throughout the study. Subjects returned to the clinic for evaluation of side-effects and compliance after 3 and 6 weeks of treatment. Blood pressure, blood lipids,

blood apo and other CVD risk markers were evaluated at baseline and at the end of treatment.

Treatment

Two food products were chosen as vehicles to deliver the BBG (Barliv™ barley β -glucan concentrate; Cargill Health and Food Technologies, Wayzata, MN, USA): ready-to-eat cornflakes breakfast cereal and a low-energy tropical juice beverage containing 5% fruit juice. The foods were formulated such that their nutritional profiles were consistent with FDA heart health claim requirements. Prior to the study, an informal screening exercise was conducted to confirm the sensory acceptability of the treatment foods.

The cereal and juice were packaged in single-serving packages (one cup of cereal or juice per serving) and subjects received a 3-week supply of treatment at baseline and after 3 weeks of treatment. They were instructed to consume two packages of juice beverage and one package of cereal with meals each day (Table 1). Subjects were instructed to save all used and unused cereal and juice containers. These were collected and counted at weeks 3 and 6 as a measure of compliance.

Clinical and laboratory measurements

All visits were conducted at the University of Minnesota General Clinical Research Center. At the screening visit a general medical history was obtained; blood pressure, height and weight were measured; and blood samples were collected to assess fasting chemistry and lipid values. Fasting lipids and lipoproteins were reassessed after the diet stabilization period. Scheduled visits during the treatment period were at baseline and weeks 3 and 6. At all treatment visits, blood pressure and weight were measured and side-effects were assessed. At baseline and week 6, blood was drawn to assess total cholesterol, HDL-C, LDL-C, and TAG.

All blood draws and clinical measurements were performed by University of Minnesota General Clinical Research Center medical staff. Weight and height measurements were obtained with subjects wearing indoor clothing and no shoes. Blood pressure measurements were obtained with an automatic Colin® blood pressure monitor (Press-mate® BP/8800C; Medical Instruments Corp., San Antonio, TX) after subjects had rested in a seated position for at least 5 min. Measurements were repeated four times at 1 min intervals, and the mean of the last three readings was used in analyses. All blood samples were obtained using standard venepuncture techniques after subjects had fasted for 12 h. All laboratory analysis was done using standard automated technology at the Quest Diagnostics® Laboratory (Wood Dale, IL) branch laboratory (certified and accredited laboratory by the Clinical Laboratory Improvement Amendment of 1988 and the College of American Pathologists) or at the University of Minnesota. Specifically, total cholesterol, LDL-C and TAG concentrations were determined using enzymatic methods with Olympus reagents, with automated spectrophotometry performed on Olympus AU5400®. HDL-C was determined directly using Roche reagents on the Olympus AU5400®.

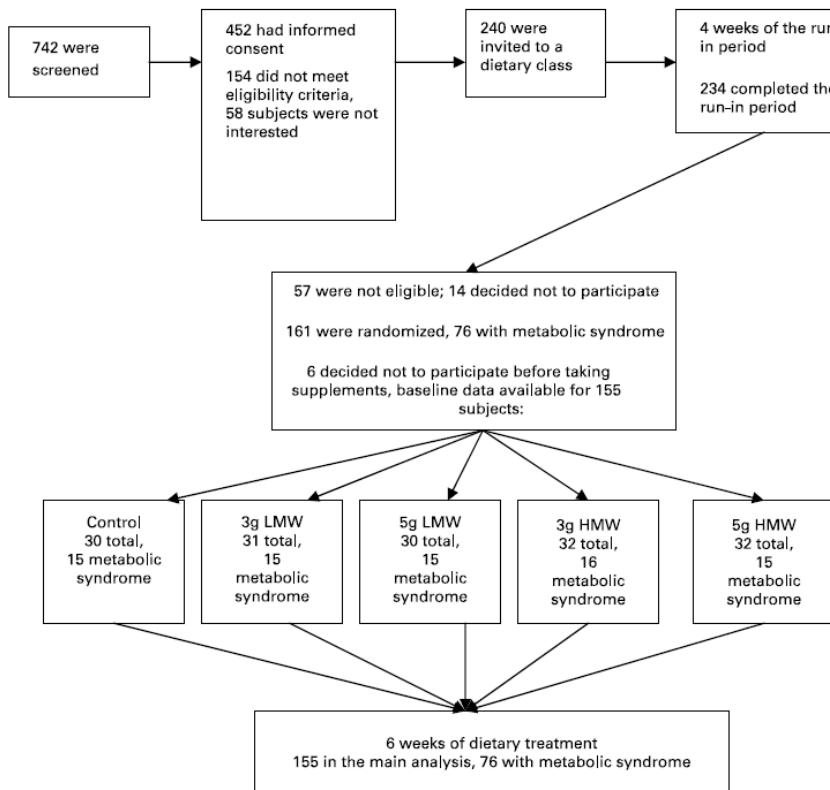


Fig. 1. Flow diagram of study eligibility for concentrated barley β -glucan extract trial. HMW, high molecular weight; LMW, low molecular weight.

Dietary data were collected during the treatment period to monitor diet compliance and consistency. Each subject completed a 3 d food record during the first and last week of treatment and returned them at weeks 3 and 6. Research staff reviewed the records for completeness and clarity during the study visits. Food records were analysed for energy, macronutrient and micronutrient intake using Nutrition Data System for Research software version 5.0_35 (NDS-R; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA).

Study-related side-effects were assessed by a thirteen-questionnaire side-effect questionnaire completed at baseline and subsequent treatment visits. Participants were asked to check the category that best represented their symptoms over the last month at baseline or since their last study visit at each subsequent visit. The categorical options for each symptom were 'Not at all', 'Somewhat', 'Moderately', 'Very much' or 'Extremely'. Frequency counts were used in the analyses and were categorized in two ways: (1) dichotomized as 'Any' v. 'No' side-effects or (2) the top two categories were collapsed and were used to indicate the presence of

side-effects. Analyses were conducted using both methods of determining side-effects.

Statistical analysis

Differences in baseline demographic and clinical variables among the treatment groups were compared using ANOVA for continuous variables and the χ^2 test for categorical variables. The treatment effect was based on the measurement and comparison of the mean levels of lipids and lipoproteins among treatment groups using ANOVA. The GENMOD procedure of SAS version 8 (SAS Institute Inc., Cary, NC, USA) was used to perform the analyses. In addition, a χ^2 test was performed comparing all side-effect counts (frequencies) at baseline, mid-study and post-study visits. Regression analysis using a general linear model was used to determine the differences in side-effects over time and between the treatment groups and the control group. Test of independent proportions was used to compare the percentage of subjects who attained their LDL-C goal in the treatment groups versus the control

Table 1. Treatment schedule by group

Treatment group	Juice†		Cereal‡		Total BBG consumed (g)
	Servings consumed	BBG consumed (g)	Servings consumed	BBG consumed (g)	
Control (0 BBG/d)	Two cups/d at 0 g BBG/serving	0	One cup/d at 0 g BBG/serving	0	0
High dose HMW (5 g HMW BBG/d)	Two cups/d at 1.0 g BBG/serving	2.0	One cup/d at 3.0 g BBG/serving	3.0	5.0
High dose LMW (5 g LMW BBG/d)	Two cups/d at 1.0 g BBG/serving	2.0	One cup/d at 3.0 g BBG/serving	3.0	5.0
Low dose HMW (3 g HMW BBG/d)	Two cups/d at 0.75 g BBG/serving	1.5	One cup/d at 1.5 g BBG/serving	1.5	3.0
Low dose LMW (3 g LMW BBG/d)	Two cups/d at 0.75 g BBG/serving	1.5	One cup/d at 1.5 g BBG/serving	1.5	3.0

BBG, barley β -glucan; HMW, high molecular weight; LMW, low molecular weight.

† Subjects consumed two juice drinks per day: one with breakfast and the other with their largest meal.

‡ Subjects consumed one cereal per day as part of their breakfast and in lieu of their usual cereal.

group. Statistical significance adjustments were made using Dunnett's test for multiple comparisons.

Results

All baseline variables were similar among the treatment groups (Tables 2 and 3). The mean age overall was 55 years (age range 25–73 years). The ratio of men to women was similar in each treatment arm. The mean BMI between the groups was similar, with each group being borderline obese by National Institutes of Health and WHO standards. The proportion of subjects in each group that had a positive family history of CHD (as defined by the ATP III guidelines) was similar between the treatment groups. Each treatment group was block stratified on metabolic syndrome status resulting in an even distribution of metabolic and non-metabolic syndrome subjects in each group. Metabolic syndrome status was determined according to the ATP III guidelines (elevated TAG, low HDL-C, elevated blood pressure or blood pressure medication, elevated glucose and/or elevated waist girth) and meeting at least three of the five criteria. All study subjects were determined to be generally

healthy at baseline and without history of CHD; 38 % had two or more CHD risk factors while 62 % had 0–1 CHD risk factors. For all study participants the mean baseline levels for blood lipids and lipoproteins were as follows (in mg/l): LDL-C, 1540 (range 1100–2200); total cholesterol, 2350 (range 1840–3270); HDL-C, 500 (range 270–1040); TAG, 1600 (range 440–4680).

The mean changes in total cholesterol, LDL-C, TAG and TC/HDL-C for the different treatment groups are shown in Table 3. After 6 weeks of treatment, total cholesterol dropped significantly in all treatment groups compared to control. Specifically, total cholesterol was reduced by 12% in the 5 g HMW group, a decrease that was slightly more than the other treatment groups: 5 g LMW group, 11% reduction; 3 g HMW group (−190 mg/l), 8% reduction; 3 g LMW group, 7% reduction. LDL-C levels were significantly reduced from baseline in all treatment groups compared to control. The 5 g HMW group experienced a 15% drop in LDL-C where LDL-C was reduced by 13% in the 5 g LMW group, 9% in the 3 g HMW group and 9% in the 3 g LMW group.

Table 2. Subject characteristics at baseline by treatment group and overall totals†

Variable	Control (n = 30)		5 g, HMW (n = 32)		5 g, LMW (n = 30)		3 g, MW (n = 32)		3 g, LMW (n = 31)		Total (n = 155)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	53.7	12.5	58.6	10.6	52.8	11.9	53.9	10.2	55	10.1	54.8	11.1
BMI	30.8	4.0	28.9	6.7	28.9	5.3	29.6	5.9	28.1	4.3	28.8	5.3
Body weight (kg)	82.8	15.1	81.7	19.8	80.7	16.8	86.4	19.4	80.7	14.9	82.5	17.2
n	n	%	n	%	n	%	n	%	n	%	n	%
CHD family history	6	20	11	34.4	9	30	10	31.3	9	29	45	29
Metabolic syndrome‡	15	50	15	46.9	15	50	16	50	15	48.4	76	49
Gender												
Male	17	56.7	15	46.9	11	36.7	16	50.0	16	51.6	75	48.4
Female	13	43.3	17	53.1	19	63.3	16	50.0	15	48.4	80	51.6
Race												
Caucasian	30	100	29	90.6	28	93.3	30	93.8	29	93.5	146	94.2

HMW, high molecular weight; LMW, low molecular weight.

† For details of treatment groups, see Table 1. χ^2 tests of association between groups were performed for gender and ANOVA. F tests were performed for age and BMI.P values were not significant ($P > 0.09$).

‡ Each group was block stratified on metabolic syndrome status as defined by the National Cholesterol Education Program's Adult Treatment Panel III guidelines.

Table 3. Blood lipids results at baseline (Pre) and after 6 weeks of treatment (Post) by treatment group†

Variable	Control (n = 30)		5 g, HMW (n = 32)		5 g, LMW (n = 30)		3 g, HMW (n = 32)		3 g, LMW (n = 31)	
	Mean	SD								
TC										
Pre	234.0	22.7	235.1	25.3	238.0	27.6	233.6	22.8	235.9	23.0
Post	231.3 ^a	26.9	205.9 ^b	25.1	211.6 ^b	20.2	214.5 ^b	21.6	218.8 ^b	20.1
TAG										
Pre	153.9	75.4	158.3	79.2	166.7	91.7	164.7	88.7	154.9	61.7
Post	158.8 ^a	64.7	133.7 ^b	47.4	145.7 ^a	62.7	152.5 ^a	55.8	142.2 ^a	49.2
HDL-C										
Pre	50.5	14.4	50.8	14.2	50.4	13.7	47.9	10.7	49.6	14.8
Post	49.9	13.8	51.9	12.7	49.7	12.8	47.4	11.2	50.8	15.8
TC/HDL-C										
Pre	4.9	1.2	4.9	1.3	5.0	1.4	5.1	1.2	5.0	1.2
Post	5.0 ^a	1.4	4.2 ^b	1.0	4.5 ^b	1.2	4.8 ^a	1.1	4.6 ^b	1.3
LDL-C										
Pre	152.7	13.9	154.5	16.5	154.6	19.9	152.8	18.1	153.9	15.1
Post	150.9 ^a	24.3	132.0 ^b	11.4	134.3 ^b	12.8	138.8 ^b	20.3	140.5 ^b	15.1

HDL-C, HDL-cholesterol; HMW, high molecular weight; LDL-C, LDL-cholesterol; LMW, low molecular weight; TC, total cholesterol.

^{a,b} Mean values within a row with unlike superscript letters were significantly different (with adjustments for multiple comparisons; P<0.05).Mean values were significantly different from those of the baseline (paired Student's *t*-tests); *P<0.05.

† For details of treatment groups, see Table 1. ANOVA F tests were done for each variable. No significant differences were found between groups at baseline (P>0.60).

Fasted TAG levels were reduced from baseline in all treatment groups except the 3 g HMW group (Table 3), while the control group experienced a modest increase. However, after adjusting for multiple comparisons only the 5 g HMW group experienced a significant drop in TAG levels compared to control. Fasted TAG level was reduced by 16% in the 5 g HMW group. There were no significant changes from baseline in any of the treatment groups regarding HDL-C.

Table 3 shows the decrease in the total cholesterol/HDL-C ratio in all the treatment groups at the final study visit. The ratio of total cholesterol/HDL-C was significantly changed by treatment in all the treatment groups except the 3 g HMW group. The 5 g HMW group experienced a 15% drop in the total cholesterol/HDL-C ratio while this ratio was reduced by 10% in the 5 g LMW group and 9% in the 3 g LMW group. The 3 g HMW group also experienced a reduction in the total cholesterol/HDL-C ratio from baseline but this change was not significantly different from the control group after adjusting for multiple comparisons.

Diet was unchanged throughout the study in both the treatment groups and the control group. All treatment groups (but not the control group) attained the ATP III guidelines goal of ≥10 g VSF/d when the dose of the treatment fibre was

added to the background dietary soluble fibre intake. Body weight was unchanged over the duration of the study in all study groups.

The treatment was well tolerated by most subjects, with excellent compliance (average treatment compliance by group: control, 96%; 5 g HMW, 95%; 5 g LMW, 97%; 3 g HMW, 94%; 3 g LMW, 97%). The fact that there were no study dropouts further indicates the tolerability of the study treatments. Moreover, adverse events were monitored at all study visits and none were reported. Treatment-related side-effects were also assessed at each study visit. There were no differences in the frequency of side-effects at baseline between any of the study treatment groups or the control group. Additionally, there was no change in the frequency of side-effects from baseline to the mid-study visit or to the final study visit in any of the treatment groups when compared to the control group except for the frequency of intestinal gas. In all groups except the control group the frequency of intestinal gas increased over the first 3 weeks of the study and persisted over the final 3 weeks of treatment. However, the change in frequency of intestinal gas only reached statistical significance in the 5 g HMW group (at week 3 and week 6 of treatment) when all treatment groups were compared to the control group (P<0.05).

Table 4. LDL-cholesterol goal attainment at baseline and week 6 by number of CHD risk factors†

Treatment group	Zero or one CHD risk factors at baseline	Zero or one CHD risk factors at week 6	Two or more CHD risk factors at baseline	Two or more CHD risk factors at week 6
Total (n = 154)	66/95	80/95	2/59	20/59
Control (n = 30)	19/22	15/22	0/8	0/8
5 g HMW (n = 32)	12/17	15/17	1/15	8/15
5 g LMW (n = 29)‡	15/21	20/21	0/8	4/8
3 g HMW (n = 32)	9/18	15/18	1/14	7/14
3 g LMW (n = 31)	11/17	15/17	0/14	1/14
Any BBG treatment (n = 124)	47/73	65/73	2/51	20/51

BBG, barley β-glucan; HMW, high molecular weight; LDL-C, LMW, low molecular weight.

† For details of treatment groups, see Table 1. CHD risk factors as defined by National Cholesterol Education Program's Adult Treatment Panel III guidelines.

‡ One subject was left out of analysis (5 g LMW group) because we were unable to get all risk factor data.

The National Cholesterol Education Program ATP III guidelines were applied to each study participant to determine his or her LDL-C goal of therapy based on level of CHD risk (Table 4). A greater percentage of individuals in the treatment groups attained their LDL-C goal compared to the control group. At study conclusion 89% of those with zero or one CHD risk factors who received any study treatment had attained their LDL-C treatment goal compared to 68% in the control group. Similarly, among the subjects with two or more CHD risk factors, 39% (20/51) who received any of the study treatments attained their LDL-C goal compared to 0% (0/8) in the control group ($P<0.05$).

Discussion

The aim of the present study was to assess the impact of BBG-enriched foods on CVD risk factors, specifically LDL-C and other blood lipid levels, in human subjects with moderate dyslipidaemia. The present study demonstrated that both HMW and LMW BBG, when added at either 3 or 5 g/d, reduced the primary study variable, LDL-C, with significant reductions at both the 3 g and 5 g daily dose. Reductions were 9% for the 3 g dose and 15% or 13% for the 5 g dose (HMW and LMW, respectively). Additionally, total cholesterol was significantly reduced among all treatment groups, while the ratio TC/HDL was more significantly reduced among the 5 g/d groups. The present findings demonstrate that the efficacy of a BBG-enriched diet in modifying blood lipid CVD risk factors is at least comparable to previous clinical trials of VSF-enriched diets. As important, the LMW BBG which has even greater therapeutic potential because of its improved sensory properties and performance in foods demonstrated comparable efficacy to the HMW BBG in blood lipid improvement.

An important study outcome that is a corollary to the LDL-C reduction is the number of subjects who were able to attain their personal LDL-C goal as established by the ATP III guidelines. The ATP III guidelines use a system of assessing core CVD risk factors to establish the LDL level or cut point at which an individual can consider their efforts at risk reduction successful (National Cholesterol Education Program, 2002). If a person does not reach their goal with lifestyle changes, then they will generally need to progress to more aggressive interventions such as pharmacotherapy. It is an additional important measure of the efficacy of the BBG intervention that 69% of the subjects in the treatment groups were able to attain their LDL-C goal as opposed to 50% of the control group on a 'heart-healthy' diet alone. Of particular note is the fact that all treatment groups, both the 3 g and 5 g LMW and HMW groups, showed a substantial increase in persons reaching their ATP III goal for LDL-C. The study subjects were only moderately dyslipidaemic; 40% of the subjects in the treatment groups and 63% of the control group had already achieved their LDL-C goal on the run-in diet. Nevertheless, LDL-C is a continuous risk variable and additional improvement in LDL-C levels with the BBG intervention further enhanced their CVD risk reduction and maintenance of healthy lipid levels.

Overall compliance with study treatments and the lack of significant study-related side-effects demonstrated excellent acceptance and tolerance of BBG. As is common with an increase in fibre intake, subjects on active treatment did

report an initial increase in intestinal gas, but for most subjects this side-effect did not increase over the duration of the study. Three-day food records obtained at baseline and at the end of the study indicated that subjects were generally compliant with overall diet recommendations and there were no significant changes in energy consumption or specific nutrient intake over the 6-week period. Of note, all subjects within the four treatment groups attained the ATP III goal of consumption of 10–25 g VSF/d when the treatment dose of BBG was added to their background VSF consumption on the 'heart-healthy' diet.

To date, most of the human studies investigating the hypocholesterolaemic effects of β -glucan have utilized diets rich in oat and oat products. However, human clinical trials have been conducted using barley foods as the source of β -glucan as well. (McIntosh *et al.* 1991) conducted one of the first trials comparing diets rich in barley versus wheat in a cross-over design. Compared to the wheat period, the barley diet period resulted in a 6% lower total cholesterol level and a 7% lower LDL-C level. In 2004, Behall *et al.* reported that adding 6 g soluble fibre from barley per day for 5 weeks in addition to a Step 1 diet resulted in a 24% reduction in LDL-C (Behall *et al.*, 2004b). However, not all studies investigating the cholesterol-altering effects of barley have reported a treatment effect. (Keogh *et al.* 2003) reported that adding β -glucan-enriched barley to the diets of hypercholesterolaemic men containing 38% of kJ from fat did not significantly reduce total or LDL-C levels.

To date, there have been even fewer studies investigating the cholesterol-altering effects of extracted β -glucan. There have been a few studies showing the benefit of oat β -glucan extract in CVD risk reduction (Behall *et al.*, 1997). However, there has only been one previous study reporting a dietary intervention using β -glucan extracted from oats with molecular weight modification (Frank *et al.*, 2004); the study used 6 g/d oat β -glucan extract (both LMW and HMW) for 3 weeks and failed to show a significant effect on blood lipids, specifically LDL-C. Compared to the findings in the present trial, the results of (Frank *et al.* 2004) would suggest that the extent of the molecular weight reduction of the β -glucan fibre could significantly alter its hypocholesterolaemic action. Additionally, it is apparent from a review of the literature that not all soluble fibre forms and sources have comparable effects on CVD risk factors (Truswell, 1995).

Experts contend that the LDL-C-lowering effects of high-VSF foods, such as oats and barley, are due to the action of VSF in the gastrointestinal tract. VSF has been shown to increase the elimination of bile salts, and secondarily, bacterial fermentation products (SCFA) have been shown to suppress hepatic cholesterol biosynthesis (Marlett *et al.*, 1994). There is a substantial body of knowledge supporting these mechanisms of action, and this persuaded the (US Food & Drug Administration 1993) to grant the first health claim for reduced risk of heart disease in 1993 to foods rich in soluble fibre from oats.

Lowering the molecular weight of β -glucan does improve sensory properties and performance in foods, but it can also reduce the viscosity of the fibre, thus the tradeoff can be a decrease in efficacy. This appears to be the reason that some previous studies of other LMW β -glucans in animals and man had reduced efficacy (Yamada *et al.*, 1999; Frank *et al.*,

2004). In addition, some feel that any β -glucan extract, even a concentrated source, loses some of the important components, such as polyphenolics and antioxidants, present in whole-grain products and thereby may be less effective in overall CVD risk reduction. (Jacobs & Gallaher 2004) have reviewed a number of prospective trials and have concluded that consumption of whole-grain products reduces CVD risk. The present study of foods enriched with extracted BBG demonstrates their efficacy in reducing LDL-C, a major surrogate marker for CVD, and gives support to the position that extracted VSF can significantly reduce CVD risk.

A study of longer duration may be helpful to show maintenance of the benefit. Further, in order to generalize the results to a broader, more diverse population, it may be helpful to study certain subgroups and other population groups over the age of 65.

Conclusion

The present study demonstrates the efficacy and excellent tolerance of a dietary intervention using BBG-enriched foods to reduce CVD risk, specifically LDL-C. All subjects in BBG treatment groups were able to reach the ATP III dietary goal of consumption of 10–25 g/VSF d. An important finding in the present study was that LMW BBG had comparable efficacy gram for gram when compared to native HMW BBG. This is clinically important because the improved sensory properties and performance in foods of LMW BBG make it a more viable food ingredient for broader applications. An additional important outcome of the present study was that a greater number of BBG-treated subjects versus control attained their ATP III goal for LDL-C. The findings of the present study have clear clinical benefits in CVD risk reduction and significant healthcare cost benefits due to reduced need for pharmacotherapy if the results can be sustained long term.

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Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women^{1–3}

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ABSTRACT

Background: Barley has high amounts of soluble fiber but is not extensively consumed in the US diet.

Objective: This study investigated whether consumption of barley would reduce cardiovascular disease risk factors comparably with that of other sources of soluble fiber.

Design: Mildly hypercholesterolemic subjects (9 postmenopausal women, 9 premenopausal women, and 7 men) consumed controlled American Heart Association Step 1 diets for 17 wk. After a 2-wk adaptation period, whole-grain foods containing 0, 3, or 6 g β -glucan/d from barley were included in the Step 1 diet menus. Diets were consumed for 5 wk each and were fed in a Latin-square design. Fasting blood samples were collected twice weekly.

Results: Total cholesterol was significantly lower when the diet contained 3 or 6 g β -glucan/d from barley than when it contained no β -glucan; the greatest change occurred in the men and postmenopausal women. HDL and triacylglycerol concentrations did not differ with the 3 amounts of dietary β -glucan. Large LDL and small VLDL fractions and mean LDL particle size significantly decreased when whole grains were incorporated into the 3 diets. Large LDL and large and intermediate HDL fractions were significantly higher, mean LDL particle size was significantly greater, and intermediate VLDL fractions were significantly lower in the postmenopausal women than in the other 2 groups. A group-by-diet interaction effect was observed on LDL fractions and small LDL particle size.

Conclusion: The addition of barley to a healthy diet may be effective in lowering total and LDL cholesterol in both men and women. *Am J Clin Nutr* 2004;80:1185–93.

KEY WORDS Barley, β -glucans, whole grains, cholesterol, triacylglycerols, lipoprotein fractions

INTRODUCTION

Cardiovascular disease (CVD) continues to be the number one cause of death in the United States despite numerous efforts to reduce its prevalence. Consumption of diets high in whole grains has been reported to have health benefits, such as a reduced risk of CVD (1, 2). These benefits have been attributed to the effects of the fiber content of whole-grain foods on risk factors, primarily on cholesterol concentration (3, 4). Other, more general, physiologic benefits of the consumption of whole grains include reduced transit time for foods, which may reduce the risk of colon cancer (5, 6), and reduced absorption of nutrients (7, 8), which may reduce glucose and insulin responses and thus the risk of obesity (9).

Epidemiologic studies often combined several fiber food sources (mixed grains and cereals, fruit, and vegetables with or without legumes), which made it difficult to determine the specific beneficial dietary component. Many of the studies in humans added either fiber supplements or fiber-containing foods to self-selected diets. Numerous studies showed that whole grains containing a high amount of soluble fiber, such as oats, are more effective in lowering blood cholesterol than are grains containing predominantly insoluble fibers, such as wheat or rice (10–13). The US Food and Drug Administration (14) allows the health claim statement that, depending on the β -glucan content, consumption of soluble fiber from oats or psyllium in a diet low in saturated fat and cholesterol may reduce the risk of CVD. Most clinical studies evaluating the effects of soluble fibers have used oats or psyllium even though barley contains at least as much β -glucan as they do (15). The purpose of this study was to examine the effects on CVD risk factors of the consumption of various amounts of β -glucan from barley, a grain not frequently consumed by Americans, in a controlled whole-grain diet in mildly hypercholesterolemic men and women. Mildly hypercholesterolemic men alone were evaluated in a previous study (16).

SUBJECTS AND METHODS

Subjects

Mildly hypercholesterolemic [total cholesterol: 5.18–6.2 mmol/L (200–240 mg/dL)], normotensive men and women who had been weight stable for 6 mo and who were not taking medication known to affect lipid metabolism or blood pressure were recruited for this study. Men were included in this study to confirm previous results because the diet was modified from the previous study. The study design was approved by the Johns Hopkins School of Public Health Institutional Review Board, and it conformed to US Government regulations governing human research. Written informed consent was obtained from each subject after an oral explanation of the study.

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TABLE 1
Prestudy characteristics of study participants¹

	Men (n = 7)	Premenopausal (n = 9)	Women (n = 9)
Age (y)	43 ± 5 ^a	47 ± 4 ^a	50 ± 3 ^a
Height (cm)	176.0 ± 3.7 ^a	160.8 ± 2.8 ^b	164.3 ± 1.3 ^b
Weight (kg)	81.0 ± 4.1 ^a	89.5 ± 7.8 ^a	80.3 ± 7.3 ^a
BMI (kg/m ²)	26 ± 1 ^a	34 ± 3 ^b	30 ± 3 ^{a,b}
Percentage body fat (%)	23 ± 2 ^a	38 ± 3 ^b	35 ± 3 ^b
Cholesterol (mmol/L) ²	5.58 ± 0.24 ^a	5.63 ± 0.22 ^a	6.12 ± 0.21 ^a
LDL cholesterol (mmol/L)	3.75 ± 0.21 ^a	3.71 ± 0.19 ^a	3.81 ± 0.19 ^a
HDL cholesterol (mmol/L)	1.08 ± 0.11 ^a	1.32 ± 0.10 ^{a,b}	1.74 ± 0.10 ^b
Total:HDL	5.30 ± 0.44 ^a	4.47 ± 0.39 ^{a,b}	3.53 ± 0.39 ^b
Triacylglycerols (mmol/L) ³	1.60 ± 0.42 ^a	1.58 ± 0.37 ^a	1.54 ± 0.37 ^a

¹ All values are $\bar{x} \pm$ SEM. Values in a row with different superscript letters are significantly different, $P < 0.05$.

² To convert units to mg/dL, multiply by 38.67.

³ To convert units to mg/dL, multiply by 88.57.

A general clinical screening of fasting blood and urine samples was used to select subjects with mildly elevated cholesterol who had no other medical conditions and who were taking no medications that would affect lipid or glucose metabolism. Heights and weights were measured, and duplicate blood pressure readings were obtained. Subjects completed a health history questionnaire. Before subjects were accepted as participants, physicians from Johns Hopkins University School of Public Health evaluated the health history and clinical screening values for underlying disease before subjects were accepted as participants, and the physicians provided medical supervision throughout the study.

Twenty-seven subjects with mildly elevated plasma cholesterol concentrations were selected for the study. Two subjects withdrew during the study for reasons not related to the study. Prestudy characteristics of the 7 men, 9 premenopausal women, and 9 postmenopausal women who completed the study are listed in Table 1.

Diets and Procedures

Subjects initially were placed on an American Heart Association Step 1 diet (17) with a 7-d rotating menu for 2 wk as an adaptation period to the study regimen, dietary changes, and fiber content (Table 2). Initial estimates of the subjects' energy needs were made during this period. Energy intakes were adjusted proportionately in 300-kcal increments to maintain initial body weights. Breakfast and dinner were consumed Monday through Friday in the Human Study Facility. Lunch and an evening snack were packaged for off-site consumption. Weekend meals were frozen or packed in ice (or both) for home consumption. All foods were weighed to 0.5 g. Subjects were weighed daily Monday through Friday, and body weights were verified by Human Study Facility personnel. Subjects agreed to consume only the study food given to them and to consume all food items given to them. Water, selected spices, noncaloric beverages, and noncaloric sweeteners were allowed ad libitum, and the subjects recorded

TABLE 2
Nutrient content of diets¹

	Diet			
	Step 1	Low- β -glucan	Medium- β -glucan	High- β -glucan
Energy (kcal)	2812	2788	2777	2766
Protein (g)	110	113	113	112
Fat (g)	96	96	96	96
Saturated (g)	25	27	27	27
Cholesterol (mg)	291	297	297	297
Carbohydrate (g)	388	384	385	386
Dietary fiber (g)	27	27	31	34
Soluble fiber (g) ²	2.1	2.3	5.6	8.8

¹ Values were calculated by using NUTRITIONIST software (version 5.0; First Data Bank, San Bruno, CA). Some whole-grain foods were included in the American Heart Association Step 1 diet. At an energy level of 2800 kcal, the diets designated low-, medium-, and high- β -glucan were designed to contain approximately the same amounts of total dietary fiber but different amounts of soluble fiber added from barley: 0, 3, and 6 g β -glucan/d, respectively. Foods containing whole wheat and brown rice (low- β -glucan diet), barley (high- β -glucan diet), or a 50:50 mix (medium- β -glucan diet) included pancakes, spice cookie bars, no-bake cookies, hot cereal, granola, steamed grain, tabbouleh, and muffins.

² Total and soluble fiber contents were determined by Covance Laboratories Inc (Madison, WI), and β -glucan content of the barley was determined by the US Department of Agriculture (Western Region, Albany, CA).

TABLE 3
Sample menus

Breakfast	Lunch	Dinner	Evening snack
Control			
Plain pancakes	Turkey breast	Chicken breast	Ginger snaps
Pancake syrup	Swiss cheese	Gravy	
Breakfast patties	Lettuce	Egg noodles	
Margarine	Cucumber	Cole slaw	
Cranberry juice	Italian dressing	Green beans	
Low-fat (2% fat) milk (low-lactose milk)	Peaches (light syrup) Popcorn cakes Lemonade	Tomato juice Chocolate cake	
Test			
Test pancakes ¹	Turkey breast	Chicken breast	Spice cookie bar ¹
Pancake syrup	Swiss cheese	Gravy	
Breakfast patties	Lettuce	Steamed rice or barley ¹	
Margarine	Cucumber	Cole slaw	
Cranberry juice	Italian dressing	Green beans	
Low-fat (2% fat) milk (low-lactose milk)	Peaches (light syrup) Tabbouleh ¹ Lemonade	Tomato juice Chocolate cake	

¹ Made with whole-wheat flour, whole-wheat flakes, or brown rice; barley flour, flakes, or pearls; or a 50:50 mixture of wheat and barley or rice and barley.

the consumption of these items daily. No discretionary salt was allowed.

After the 2-wk adaptation period, whole-grain foods containing soluble fiber from barley were included in the Step 1 diet. Diets were fed in a Latin-square design for 5 wk each. The 3 diets (low-, medium-, and high- β -glucan) were designed to contain approximately the same amount of total dietary fiber but different amounts of β -glucan (0, 3, and 6 g added β -glucan/2800 kcal, respectively). In the experimental menus, a test food was substituted into the Step 1 menu at breakfast, lunch, dinner, and evening snack (Table 3). Wheat and rice test foods (pancakes, spice cookie bar, no-bake cookies, hot cereal, granola, steamed grain, tabbouleh, and muffins) were made with whole-wheat flour, wheat flakes, and brown rice. The basic diet without the test foods and the diet containing wheat and rice test foods were designed to contain little added soluble fiber. Diets including barley flakes, barley flour, or pearled barley in the test foods (replacing the wheat or rice) contained ≥ 6 g β -glucan/2800 kcal as part of the total dietary fiber. Diets including test foods made with half barley and half whole wheat or brown rice contained 3 g β -glucan/2800 kcal in the total dietary fiber (Table 2). The β -glucan content of the barley used to prepare the experimental foods was determined enzymatically by the National Barley Foods Council (16) and the US Department of Agriculture (Western Region, Albany CA) with the use of American Association of Cereal Chemists method 32-23. Total and soluble fiber content of the diets were determined by using the Association of Official Analytical Chemists method 991.43, which was performed at Covance Laboratories Inc (Madison WI). Whole-wheat flour and brown rice were purchased from a local grocery store. Wheat flakes were purchased in one lot from Barry Farm Enterprises (Wapakoneta, OH). Barley flakes, barley flour, and pearled barley were produced from one lot of barley and donated by the National Barley Foods Council (Spokane, WA).

Statistical analysis

Two blood samples (separated by 1 d) were collected after an overnight fast of ≥ 12 h before controlled feeding began and weekly during each period. Plasma was separated and stored at -80°C until all samples were collected. Triacylglycerol and total cholesterol concentrations were measured enzymatically with the use of an automated spectrophotometric system (Baker Instruments Corp, Allentown, PA). HDL-cholesterol concentrations were measured after other fractions were precipitated with the use of dextran sulfate and manganese chloride (18). VLDL and LDL concentrations were calculated (19). Lipid subclass fractions were measured during the last week of each period with the use of nuclear magnetic resonance spectroscopy (LipoScience, Raleigh, NC; 20). Data were statistically analyzed by analysis of variance by using a mixed-model procedure (PC/SAS, version 8.2; SAS Institute, Cary, NC). Subjects acted as their own control subjects. Data were examined for normal distribution. Triacylglycerol concentrations were log transformed for statistical evaluation. Data reported are least-squares means (\pm SEM). Significance was defined as $P < 0.05$. When effects were significant, mean comparisons were done with the use of Šidák-adjusted P values so that the experimentwise error was 0.05.

RESULTS

Some subjects noted some gastrointestinal discomfort during the equilibration period. The major complaints were that there was too much food and that subjects had a very full feeling after eating. Compared with the equilibration period, complaints about bloating and flatulence increased during all experimental diets; the greatest number of complaints occurred during the high- β -glucan diet.

Average body weights varied by <1 kg from the initial weight (overall average: 85.6 ± 4.5 kg) to the end of the Step 1 equilibration period (85.0 ± 4.5 kg). Subjects' average weight after

TABLE 4

Fasting lipid concentrations determined enzymatically after the equilibration and experimental dietary periods¹

	Diet				<i>p</i> ²		
	Step 1 ³	Low- β -glucan	Medium- β -glucan	High- β -glucan	Diet effect	Group effect	Diet \times group interaction
Cholesterol (mmol/L)							
All subjects	5.65 ± 0.13 ^a	5.44 ± 0.13 ^a	5.17 ± 0.13 ^b	5.12 ± 0.33 ^b	<0.0001	0.090	0.437
Premenopausal women	5.39 ± 0.22	5.19 ± 0.21	5.10 ± 0.21	5.16 ± 0.22			
Postmenopausal women	6.09 ± 0.22	5.87 ± 0.22	5.54 ± 0.22	5.44 ± 0.22			
Men	5.48 ± 0.24	5.25 ± 0.25	4.88 ± 0.25	4.77 ± 0.25			
LDL cholesterol (mmol/L)							
All subjects	3.93 ± 0.13 ^a	3.82 ± 0.13 ^a	3.57 ± 0.13 ^b	3.50 ± 0.13 ^b	<0.0001	0.750	0.367
Premenopausal women	3.75 ± 0.21	3.64 ± 0.21	3.60 ± 0.21	3.56 ± 0.21			
Postmenopausal women	4.08 ± 0.21	4.02 ± 0.22	3.68 ± 0.21	3.55 ± 0.22			
Men	3.97 ± 0.24	3.79 ± 0.24	3.44 ± 0.24	3.37 ± 0.24			
HDL cholesterol (mmol/L)							
All subjects	1.34 ± 0.06 ^b	1.22 ± 0.06 ^a	1.22 ± 0.06 ^a	1.22 ± 0.06 ^a	<0.0001	<0.0004	0.169
Premenopausal women	1.23 ± 0.10	1.13 ± 0.10	1.12 ± 0.10	1.19 ± 0.10			
Postmenopausal women	1.70 ± 0.10	1.53 ± 0.10	1.54 ± 0.10	1.53 ± 0.10			
Men	1.08 ± 0.12	1.00 ± 0.12	0.99 ± 0.12	0.94 ± 0.12			
Total:HDL (mmol/L)							
All subjects	4.55 ± 0.24 ^a	4.83 ± 0.24 ^b	4.62 ± 0.24 ^{a,b}	4.56 ± 0.24 ^a	<0.016	<0.035	0.977
Premenopausal women	4.64 ± 0.39	4.92 ± 0.39	4.78 ± 0.39	4.66 ± 0.39			
Postmenopausal women	3.71 ± 0.39	4.00 ± 0.39	3.74 ± 0.39	3.71 ± 0.39			
Men	5.30 ± 0.44	5.60 ± 0.45	5.33 ± 0.45	5.31 ± 0.45			
Triacylglycerol (mmol/L)							
All subjects	1.92 ± 0.22	2.02 ± 0.22	1.90 ± 0.22	2.03 ± 0.23	0.858	0.568	0.784
Premenopausal women	2.06 ± 0.37	2.10 ± 0.37	1.86 ± 0.37	2.03 ± 0.38			
Postmenopausal women	1.53 ± 0.37	1.66 ± 0.38	1.63 ± 0.37	1.78 ± 0.38			
Men	2.14 ± 0.42	2.30 ± 0.43	2.22 ± 0.43	1.59 ± 0.37			

¹ All values are $\bar{x} \pm \text{SEM}$. $n = 27$ (all subjects), 9 (premenopausal women), 9 (postmenopausal women), and 7 (men). Values in a row with different superscript letters are significantly different, $P < 0.05$ (\bar{S} idák mean separation).

² ANOVA.

³ American Heart Association Step 1 diet; low- β -glucan diet, 0 g added soluble fiber; medium- β -glucan diet, 3 g added soluble fiber/2800 kcal; high- β -glucan diet, 6 g added soluble fiber/2800 kcal. To convert cholesterol and triacylglycerol units to mg/dL, multiply by 38.67 and 88.57, respectively.

consuming all 3 whole-grain diets (low-: 84.3 ± 5.1 kg; medium-: 84.2 ± 5.1 kg; high- β -glucan: 84.2 ± 5.1 kg) was less than initial weights or weights after the Step 1 equilibration diet. The weights of the subjects did not differ significantly during the 3 experimental diets ($P = 0.73$). Energy intake during the Step 1 diet averaged 2600 kcal/d, and that during the experimental diets averaged 2725 kcal/d; this increase in intake was intended to correct the small weight loss observed during the Step 1 diet and to maintain a constant weight in subjects during the experimental diets.

Total plasma cholesterol concentrations were significantly affected by the diet consumed ($P < 0.0001$, Table 4) and by the length of time (in wk, $P < 0.001$; Figure 1) the diets were consumed. No significant interaction between diet and time was observed ($P = 0.92$). Cholesterol concentrations on average did not significantly decrease until week 4 of each period. Compared with prestudy concentrations (Table 1), overall total cholesterol was 0.5% lower after consumption of the Step 1 diet and 4%, 9%, and 10% lower, respectively, after the low-, medium-, and high- β -glucan diets. Total cholesterol concentrations after the medium- and high- β -glucan diets were significantly lower than those after the low-diet. The reductions observed by group (men, premenopausal women, or postmenopausal women) were not significantly different, and no diet-by-group interaction was observed.

Calculated LDL-cholesterol concentrations followed the same significant (Table 4, diet, $P < 0.0001$; Figure 1, wk, $P < 0.001$) pattern of reduction as that of total cholesterol. Compared with prestudy concentrations, LDL cholesterol was 3.6% lower after the Step 1 diet and significantly ($P < 0.001$) lower (8.0%, 13.8%, and 17.4%, respectively) after the low-, medium-, and high- β -glucan diets. LDL-cholesterol concentrations after the medium- and high- β -glucan diets were significantly lower than those after the Step 1 or low- β -glucan diet. No significant difference was observed among men, premenopausal women, or postmenopausal women, and no diet-by-group interaction was observed.

HDL-cholesterol concentrations were significantly affected by the diet consumed ($P < 0.001$; Table 4). Compared with prestudy concentrations, HDL-cholesterol concentrations were significantly lower ($P < 0.05$) after all 3 test diets but did not differ significantly among the 3 diets. Postmenopausal women had significantly ($P < 0.01$) higher HDL cholesterol (1.56 ± 0.10) than did men (1.00 ± 0.11) or premenopausal women (1.17 ± 0.10). Total:HDL cholesterol was significantly ($P < 0.001$) affected by the diet consumed. The ratio was highest after the low- β -glucan diet. The postmenopausal women had significantly lower total:HDL cholesterol (3.74 ± 0.38) than did the

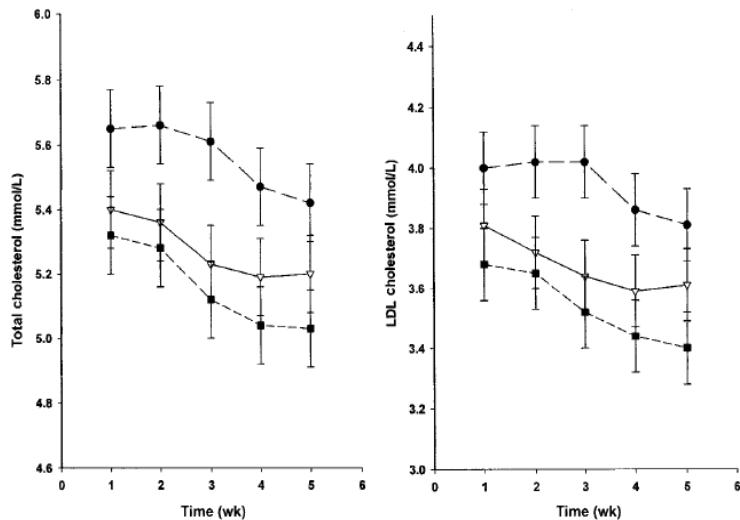


FIGURE 1. Mean weekly total and LDL cholesterol by diet: low- β -glucan diet, ●; medium- β -glucan diet, ▽; high- β -glucan diet, ■. Total and LDL cholesterol were significantly different by diet ($P < 0.0001$) and week within a diet ($P < 0.0001$), but there was no interaction between diet and week (total cholesterol: $P = 0.660$; LDL cholesterol: $P = 0.402$). Mean total and LDL-cholesterol concentrations for weeks 1 and 2 are significantly different from those for weeks 4 and 5, $P < 0.05$ (Šidák mean separation). Error bars represent SEMs.

men (5.36 ± 0.43 ; $P < 0.05$) or the premenopausal women (4.69 ± 0.38 ; $P < 0.26$). No group-by-diet interaction was observed for either the HDL-cholesterol concentrations or total: HDL cholesterol.

Overall triacylglycerol concentrations increased from the pre-study concentrations (Tables 1 and 4), but the differences were not significant. No differences were observed between the experimental diets. No significant difference was observed among men, premenopausal women, or postmenopausal women, and no diet-by-group interaction was observed. Log transformation of the triacylglycerol data did not change the results of the statistical comparisons.

Lipid fraction concentrations by diet are shown in Table 5. Concentrations of intermediate and large particle fractions of VLDL cholesterol; small and intermediate fractions of LDL cholesterol; and small, intermediate, and large fractions of HDL cholesterol after the Step 1 diet and the 3 experimental diets did not differ significantly. Large fractions of LDL cholesterol after the low-, medium-, and high- β -glucan diets were significantly lower than those after the Step 1 diet, and there were no significant differences between the 3 experimental diets.

Lipid fraction concentrations by group are presented in Table 6. The concentration of intermediate VLDL cholesterol fractions and small LDL cholesterol fractions was significantly lower and

TABLE 5
Fasting lipid fractions and particle number by diet, determined by nuclear magnetic resonance spectroscopy at the end of each controlled dietary period¹

	Diet				P for diet effect
	Step 1 ²	Low- β -glucan	Medium- β -glucan	High- β -glucan	
VLDL cholesterol (mmol/L)					
Large	0.036 ± 0.016	0.059 ± 0.016	0.048 ± 0.016	0.054 ± 0.016	0.389
Intermediate	0.497 ± 0.097	0.597 ± 0.098	0.605 ± 0.097	0.558 ± 0.099	0.108
Small	0.228 ± 0.032^a	$0.184 \pm 0.033^{a,b}$	0.174 ± 0.032^b	$0.189 \pm 0.033^{a,b}$	<0.029
LDL cholesterol (mmol/L)					
Large	2.10 ± 0.27^a	1.46 ± 0.28^b	1.62 ± 0.28^b	1.50 ± 0.28^b	<0.002
Intermediate	1.22 ± 0.21	1.56 ± 0.22	1.56 ± 0.22	1.21 ± 0.21	0.368
Small	0.44 ± 0.23	0.54 ± 0.22	0.67 ± 0.22	0.76 ± 0.22	0.108
Particle number (nmol/L)	1530 ± 74	1539 ± 75	1501 ± 74	1497 ± 75	0.258
HDL cholesterol (mmol/L)					
Large	0.510 ± 0.061	0.520 ± 0.061	0.491 ± 0.062	0.501 ± 0.062	0.691
Intermediate	0.202 ± 0.028	0.145 ± 0.029	0.145 ± 0.028	0.170 ± 0.029	0.094
Small	0.535 ± 0.021	0.568 ± 0.022	0.551 ± 0.021	0.543 ± 0.022	0.260

¹ All values are $\bar{x} \pm$ SEM. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Šidák mean separation).

² American Heart Association Step 1 diet; low- β -glucan diet, 0 g added soluble fiber; medium- β -glucan diet, 3 g soluble fiber/2800 kcal; high- β -glucan diet, 6 g soluble fiber/2800 kcal.

TABLE 6
Fasting lipid fractions by group determined by nuclear magnetic resonance spectroscopy¹

	Men (n = 7)	Women		P	
		Premenopausal (n = 9)	Postmenopausal (n = 9)	Group effect	Diet × group interaction
VLDL cholesterol (mmol/L)					
Large	0.060 ± 0.033	0.040 ± 0.026	0.084 ± 0.026	0.384	0.312
Intermediate	0.697 ± 0.159 ^a	0.627 ± 0.116 ^a	0.280 ± 0.117 ^b	<0.012	0.842
Small	0.222 ± 0.052	0.221 ± 0.038	0.169 ± 0.038	0.431	0.347
LDL cholesterol (mmol/L)					
Large	1.094 ± 0.465 ^a	1.746 ± 0.332 ^b	2.483 ± 0.333 ^c	<0.018	0.119
Intermediate	1.758 ± 0.316	1.152 ± 0.245	1.003 ± 0.247	0.172	0.969
Small	0.551 ± 0.363 ^{a,b}	0.870 ± 0.263 ^a	0.196 ± 0.265 ^b	<0.023	0.659
Particle number (nmol/L)	1497 ± 126	1564 ± 89	1439 ± 90	0.356	0.333
HDL cholesterol (mmol/L)					
Large	0.350 ± 0.110 ^a	0.541 ± 0.074 ^{a,b}	0.643 ± 0.074 ^b	<0.036	0.990
Intermediate	0.076 ± 0.046 ^a	0.180 ± 0.034 ^{a,b}	0.242 ± 0.034 ^b	<0.014	0.538
Small	0.563 ± 0.032	0.560 ± 0.025	0.541 ± 0.025	0.763	0.565

¹ All values are $\bar{x} \pm \text{SEM}$. Values in a row with different superscript letters are significantly different, $P < 0.05$ (Šidák mean separation).

the concentrations of large LDL cholesterol fractions and large and intermediate HDL cholesterol fractions were significantly higher in the postmenopausal women than in the men or the premenopausal women. The numbers of VLDL, LDL, and HDL particles did not significantly vary with diet, group, or diet-by-group interaction.

Mean VLDL particle size (Table 7) showed a significant diet by group interaction. However, few *a priori* comparisons were significant, and no consistent pattern was evident by group or diet in the significant pairs. Mean LDL particle size was smaller after all 3 test diets than after the Step 1 diet. Mean LDL particle size was significantly larger in the postmenopausal women than in the other groups. A significant diet-by-group interaction was observed (Table 7), which appeared to be driven by the higher

values after the Step 1 diet. Concentrations of LDL particle size in the men were lowest on the high- β -glucan diet; the premenopausal and postmenopausal women had no significant differences between the experimental diets.

DISCUSSION

Most research studies using food as the soluble fiber source have fed oats or oat products (1, 7, 11, 12, 21–28). Significantly lower total cholesterol (1, 21–23) and LDL-cholesterol (1, 21–23) concentrations were reported after the consumption of oat bran than after that of wheat bran or rice bran added to the self-selected diets of hypercholesterolemic subjects. Generally, no significant change was reported in triacylglycerol (1, 21, 24)

TABLE 7
Mean particle size in 7 men and 9 premenopausal and 9 postmenopausal women determined by nuclear magnetic resonance spectroscopy¹

	Diet				P		
	Step 1 ²	Low- β -glucan	Medium- β -glucan	High- β -glucan	Diet effect	Group effect	Diet × group interaction
VLDL cholesterol (nm)							
Men	44.4 ± 3.9 ^a	51.0 ± 4.1 ^b	47.6 ± 4.1 ^{a,b}	49.0 ± 4.1 ^{a,b}	0.328	0.146	<0.048
Women							
Premenopausal	47.4 ± 3.3	46.4 ± 3.3	46.1 ± 3.3	47.2 ± 3.3			
Postmenopausal	53.8 ± 3.3 ^{a,b}	59.6 ± 3.3 ^a	52.4 ± 3.2 ^{a,b}	49.8 ± 3.2 ^b			
LDL cholesterol (nm)							
Men	20.7 ± 0.3 ^{a,x}	20.6 ± 0.3 ^{a,x}	20.6 ± 0.3 ^{a,x}	20.2 ± 0.3 ^b	<0.002	<0.005	<0.007
Women							
Premenopausal	21.1 ± 0.2 ^{a,x,y}	20.6 ± 0.2 ^{b,x}	20.7 ± 0.2 ^{b,x}	20.9 ± 0.2 ^{a,b}			
Postmenopausal	21.5 ± 0.2 ^{a,y}	21.3 ± 0.2 ^{b,y}	21.4 ± 0.2 ^{a,b,y}	21.3 ± 0.2 ^b			
HDL cholesterol (nm)							
Men	8.49 ± 0.13	9.61 ± 0.13	8.52 ± 0.13	8.51 ± 0.13	0.096	<0.014	0.947
Women							
Premenopausal	8.72 ± 0.09	8.67 ± 0.09	8.66 ± 0.09	8.71 ± 0.09			
Postmenopausal	8.88 ± 0.09	8.92 ± 0.10	8.86 ± 0.09	8.88 ± 0.10			

¹ All values are $\bar{x} \pm \text{SEM}$. Values in a row (a or b) or in a column (x or y) with different superscript letters are significantly different, $P < 0.05$ (Šidák mean separation).

² American Heart Association Step 1 diet; low- β -glucan diet, 0 g added soluble fiber; medium- β -glucan diet, 3 g soluble fiber/2800 kcal; high- β -glucan diet, 6 g soluble fiber/2800 kcal.

or HDL-cholesterol (1, 22–24) concentrations in these subjects when oatmeal or oat bran was included in the diet. The lipids of normolipemic subjects usually do not decrease with the addition of soluble fiber to their diet (1, 25, 29, 30).

Total and LDL cholesterol were significantly reduced in mildly hypercholesterolemic women after consumption of a modified Step 1 diet containing oats, but not wheat, for 6 wk (31). Mildly hypercholesterolemic men and women consuming a self-selected American Heart Association Step 2 diet averaging 8 g more soluble fiber per day than the control diet had significantly lower total cholesterol, total:HDL cholesterol, and LDL:HDL cholesterol (10). Our results with the use of a controlled Step 1 diet with 3 or 6 g β -glucan/d were similar, even though different soluble fibers were used.

Some studies reported the β -glucan (primarily from oats) content of the diets fed to the subjects (1, 3, 10, 11, 26–28, 32, 33). Similar to our results with barley, total and LDL cholesterol of hypercholesterolemic subjects decreased significantly after consumption of 3–11 g oat β -glucan/d for ≥ 4 wk, whereas it did not decrease after consumption of the placebo diet (3, 11, 26, 32, 33). The greatest percentage decrease in total and LDL cholesterol occurred after the higher β -glucan intake (14.5%). Uusitupa et al (33) reported that the significant reductions in LDL cholesterol observed after 4 wk were not sustained; after 8 wk, LDL-cholesterol concentrations had increased and were only 4% lower than initial concentrations. No significant decreases in total (27, 28, 34) or LDL (27, 28, 34) cholesterol after diets containing 1.9, 3.0, or 11.2 g β -glucan/d were reported. Törrönen et al (28) suggested that the lack of effect in their study could have been due to poor solubility of the β -glucan that resulted in low viscosity in the intestine. The food matrix (liquid or baked) used to incorporate the oat β -glucan into the diet also affects total:LDL and total:HDL cholesterol; both ratios were significantly lower after consumption of orange juice but not of bread and cookies containing ≈ 5.9 g β -glucan/d than after consumption of the control wheat fiber (35). Brown et al (36) performed a meta-analysis of 67 controlled dietary studies and calculated that, for each gram of soluble fiber from oats, psyllium, or pectin, total cholesterol and LDL-cholesterol concentrations decreased by ≈ 1.55 mg/dL (0.04 mmol/L). The meta-analysis showed no significant change in triacylglycerols and HDL cholesterol. The observed changes appeared to be independent of study design, treatment length, and dietary fat content.

A few studies reported barley as the source of β -glucan in the diet. Similar to our results, the addition of β -glucan from barley to the diet of mildly hypercholesterolemic men and women resulted in total and LDL-cholesterol concentrations lower than those before the study or after consumption of a control grain (15, 16, 29, 30, 37). Blood lipid concentrations of the men and women who began the study with normal cholesterol concentrations did not change (29, 30). No significant difference between oats and barley was observed, which is an indication that β -glucan and not the source was critical in lipid reduction (37). Similar to our results, the addition of the barley bran flour and barley oil (13) or whole-grain barley (16) to a Step 1 diet of hypercholesterolemic subjects resulted in a significant decrease in total and LDL cholesterol; the greatest decrease occurred after the diet containing 6 g added β -glucan/d (16). The men and the postmenopausal women reported here had lower blood lipids that resembled the pattern previously reported for men (16); premenopausal women were the most resistant to changes in blood lipids with a change

in diet. Li et al (38), however, reported significant decreases in total, LDL-cholesterol, and triacylglycerol concentrations in women (average age: 20 y) after they consumed ≈ 3.6 g β -glucan/d extracted from barley. In contrast to other studies feeding barley, Keogh et al (39) reported no significant change in total, LDL, or HDL cholesterol or triacylglycerol concentrations after mildly hyperlipidemic men consumed 8–11.9 g β -glucan/d extracted from barley. The authors concluded that structural changes might have occurred in the β -glucan during extraction or handling.

The increased risk for coronary artery disease has been associated with a predominance of small, dense LDL particles. This is characterized by elevated triacylglycerol and lower HDL cholesterol concentrations (subclass pattern B). Sex differences were reported in lipoprotein subclass distribution patterns (40–43). Women generally have higher HDL cholesterol concentrations, larger LDL and HDL particle sizes, and lower triacylglycerol concentrations. Postmenopausal women were reported to have significantly higher total, VLDL, and LDL cholesterol and triacylglycerol concentrations; lower HDL cholesterol concentrations; smaller HDL particle size; and a strong correlation between LDL and HDL particle size (41). In contrast to the observation by Li et al (41), the postmenopausal women in the current study had the highest HDL concentrations and mean HDL particle size but no difference in triacylglycerol concentrations from those of the men. Mildly hypercholesterolemic men who consumed up to 6 g barley-derived β -glucan/d (16) and overweight men who consumed 5.5 g oats-derived β -glucan/d (44) were reported to have significantly lower LDL-cholesterol concentrations and significantly fewer LDL particle numbers than they had before the study. Although the amount of β -glucan consumed in the current study was similar to the amounts in those other studies, the change in LDL particle numbers in the current study was not significant. Davy et al (44) suggested that the decrease in small LDL-cholesterol concentrations and LDL particle numbers might contribute to the beneficial effect of oat fiber on CVD. Freedman et al (45) reported that men with relatively high concentrations of either small HDL or large VLDL particles were 3–4 times more likely to have extensive coronary artery disease than were men with concentrations below average. The postmenopausal women in our study had the highest concentrations of large LDL particles, the largest mean LDL particle size, and the smallest concentration of small LDL particles, which suggests that the men or the premenopausal women were at greater risk of CVD. However, the LDL particle size of all of our subjects remained <25 nm regardless of the diet consumed, which indicated their continued risk of coronary artery disease.

A combination of factors and mechanisms appears to contribute to the reduction in lipids observed after the consumption of barley. Mechanisms suggested for the reduction in cholesterol after increased consumption of soluble fiber include increased excretion of bile acids or neutral sterols, increased catabolism of LDL cholesterol, and reduced absorption of fat (46–48). Increased viscosity of the gastric and intestinal contents can delay gastric emptying, decrease nutrient absorption, and interfere with micelle formation. Soluble fibers were shown to be fermented in the colon (46–48) and thus to give rise to short-chain fatty acids that can be absorbed and may inhibit hepatic cholesterol synthesis. In addition to the soluble fiber, barley contains a wide range of phytochemicals, some of which are being investigated for their effect on metabolism.

Consumption of barley-containing foods and the associated soluble fiber significantly improved several CVD risk factors. These results show the potential to moderate several health risk factors through changes in food and nutrient intake without changing energy intake. The highest β -glucan intake resulted in the greatest reduction in total and LDL-cholesterol concentrations and total:HDL cholesterol, especially in postmenopausal women and men. These results indicate that dietary changes including greater consumption of whole grains including barley, higher β -glucan intake, and lower fat intake can reduce risk factors associated with CVD.



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KMB was responsible for the conception and design of the study, data interpretation, and manuscript preparation. DS was responsible for data analysis and interpretation and assisted in study design and manuscript preparation. JH was responsible for the conception and design of the study and for data interpretation and assisted in manuscript preparation. All of the authors were employed by the US Department of Agriculture at the time this research was carried out.

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Original Research

Lipids Significantly Reduced by Diets Containing Barley in Moderately Hypercholesterolemic Men

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ABSTRACT

Objective: To determine whether barley, as the soluble fiber source, would beneficially change cardiovascular risk factors. Soluble fiber from oats has been recognized as beneficial in decreasing blood cholesterol levels. Although barley contains high amounts of soluble fiber, it is not consumed as extensively as oats.

Methods: Eighteen moderately hypercholesterolemic men (28–62 y) consumed a controlled equilibration diet (Step 1, 30% fat, 55% carbohydrate, 15% protein, < 300 mg cholesterol) for 2 weeks followed by the diet with about 20% of energy replaced with brown rice/whole wheat, 1/2barley & 1/2brown rice/whole wheat or barley (< 0.4 g, 3 g and 6 g added soluble fiber/2800 kcal, respectively) for 5 weeks in a Latin square design. Fasting blood was drawn twice weekly. Total cholesterol, HDL cholesterol, and triacylglycerols were measured enzymatically and lipid fractions were measured by nuclear magnetic resonance spectroscopy.

Results: Compared with prestudy concentrations, total cholesterol (14%, 17%, and 20%, respectively) and LDL cholesterol (17%, 17%, and 24%, respectively) were significantly lower ($p < 0.0001$) after the low, medium, and high-soluble fiber diets. Triacylglycerol was 6%, 10%, and 16% lower ($p = 0.09$) whereas HDL cholesterol (9%, 7%, and 18%) was higher ($p < 0.001$) after the experimental diets. Total cholesterol and LDL cholesterol after the high-soluble fiber diet were significantly lower than concentrations after the low- or medium-soluble fiber diets. Mean LDL particle number significantly decreased ($p < 0.007$) and the large LDL cholesterol fraction showed a trend toward lower concentrations ($p = 0.06$).

Conclusion: Increasing soluble fiber through consumption of barley in a healthy diet can reduce cardiovascular risk factors.

Key words: barley, beta-glucans, whole grains, cholesterol, triacylglycerols

► INTRODUCTION

Consumption of diets high in whole grains has been reported to have beneficial health effects such as a reduced risk of cancer [1], cardiovascular disease [2,3], and noninsulin-dependent diabetes mellitus [4,5]. These results have been attributed to the effects of the fiber content of whole-grain foods on risk factors for these diseases, including blood glucose [6], insulin [7], and cholesterol [8,9]. Other more general beneficial physiological effects of consumption of whole grains include reduced transit time for foods, which may reduce risk of colon cancer [10,11], and reduced absorption of nutrients [12,13], which may reduce glucose and insulin responses and risk of obesity [14].

The U.S. Food and Drug Administration allows three health claims related to grain intakes [15]. One claim is that low-fat diets rich in fiber-containing grain products, fruits, and vegetables may reduce risk of some types of cancer. A second claim allows the statement that consumption of soluble fiber from oats or psyllium in a diet low in saturated fat and cholesterol may reduce the risk of heart disease. A specific claim for whole-grain foods allows the statement that low-fat diets rich in whole-grain foods and other plant foods may reduce the risk of heart disease and certain cancers.

Numerous studies have demonstrated that whole grains that are high in soluble fiber, such as oats, are more effective in lowering blood cholesterol than are grains in which fibers are predominantly insoluble, such as wheat or rice [16–19]. Epidemiological studies often combined several food sources that contain fiber (such as all cereals plus grains, all fruits plus all vegetables), making it difficult to determine the specific beneficial dietary component. Clinical studies testing the effects of soluble fibers have used oats or psyllium even though barley contains as much or more soluble fiber [20]. Many of the studies in humans have added either fiber supplements or fiber-containing foods to self-selected diets. The purpose of this study was to examine the effects of the consumption of various amounts of soluble fiber from barley in a controlled whole-grain diet on risk factors for coronary heart disease in moderately hypercholesterolemic men.

► MATERIALS AND METHODS

Subjects

The study was approved by the Johns Hopkins School of Public Health Institutional Review Board. Mildly hypercholesterolemic men were recruited for this study based on their being weight stable for 6 months before the study and not taking medication known to affect lipid metabolism or blood pressure. Written informed consent was obtained from each subject after an oral explanation of the study.

Blood and urine were collected before the study for a general clinical screening to select men with moderately elevated cholesterol but no other conditions that would affect lipid

or glucose metabolism. Heights and weights were measured and duplicate blood pressure readings were taken. Subjects filled out a health history questionnaire. Physicians from Johns Hopkins University School of Public Health evaluated the health history and clinical screening values for underlying disease before subjects were accepted for study participation and provided medical supervision throughout the study.

Twenty-one men with moderately elevated plasma cholesterol concentrations were selected for the study. Two subjects withdrew during the first week; one had an international business-related trip and one withdrew because he was not willing to comply with the regimen of the study. Another subject withdrew during the adaptation period after an automobile accident made transportation to the center for meals difficult. Prestudy characteristics of the 18 men completing at least one experimental diet are listed in [Table 1](#). One subject completed 2 and two subjects completed 3 of the 4 periods. Two of the 18 subjects lost significant weight at the beginning so that weights during all three whole-grain diets were less than initial weights or weight after the Step 1 equilibration diet. Prestudy characteristics of the 16 men who were weight stable are listed separately in [Table 1](#).

Table 1. Prestudy Characteristics of Men Participating in the Study*

	All subjects (n = 18)	Weight-stable subjects (n = 16)
Age (years)	45.6 ±2.5†	46.9 ±2.5
Weight (pounds)	201.9 ±10.5	189.6 ±6.5
(Kg)	91.7 ±4.8	96.1 ±3.0
Height (inches)	70.6 ±0.6	70.6 ±0.6
(Centimeters)	179.3 ±1.5	179.3 ±1.5
Body mass index‡	28.5 ±1.4	26.7 ±0.7
Plasma triacylglycerols (mg/dL)	190.8 ±18.3	186.9 ±16.8
Plasma cholesterol (mg/dL)	235.7 ±7.6	235.2 ±8.4
LDL cholesterol (mg/dL)	154.9 ±7.8	154.2 ±8.2
HDL cholesterol (mg/dL)	42.7 ±1.6	43.6 ±1.6
Total/HDL ratio	5.7 ±0.3	5.5 ±0.4

* Two subjects lost significant weight at the beginning of the study. Their weight during all three whole-grain diets were less than initial weights or weights after the Step 1 equilibration diet.

† Mean ± SEM.

To convert cholesterol and triacylglycerols to mmol/L, multiply by 0.0258 and 0.01114, respectively.

‡ Body mass index (kg/m²).

Diets and Procedures

Subjects initially consumed a Step 1 American Heart Association diet [21] with a 7-day rotating menu for 2 weeks to allow them to adjust to the regimen and fiber content and to establish energy needs ([Table 2](#)). Breakfast and dinner were consumed in the Human Study Facility Monday through Friday. Lunch and an evening snack were packaged for off-site consumption. Weekend meals were frozen and/or packed in ice for home consumption. All foods were weighed to 0.5 g. Men were weighed daily Monday through Fridays and body weights were verified by Human Study Facility personnel. Energy levels were adjusted proportionately in 300-kcal increments to maintain initial body weights. Men agreed to consume only the study food given to them and to consume all of it. The only exceptions to this were water, noncaloric beverages, and noncaloric sweeteners; their consumption was recorded daily. No discretionary salt was allowed.

Table 2. Nutrient Content of Diets

	Step 1	Low	Medium	High
Energy (kcal)	2812	2839	2828	2817
Protein (g)	110	107	107	106
Fat (g)	96	98	98	98
Saturated (g)	25	24	24	24
Cholesterol (mg)	291	262	262	262
Carbohydrate (g)	388	400	401	402
Dietary fiber (g)	27	27	30	33
Soluble (barley) (g)	0	<0.4	3	6

At the end of the 2-week adaptation period, subjects consumed the American Heart Association Step 1 diet modified to contain either low levels of soluble fiber or 3 g or 6 g of soluble fiber from barley per 2800 kcal/day. Diets were fed in a Latin square design for 5 weeks each. The three diets were designed to contain approximately the same amount of total dietary fiber but different amounts of soluble fiber. The experimental menus had a test food substituted into the Step 1 menu at breakfast, lunch, dinner, and the evening snack ([Table 3](#)). Test foods (pancakes, spice cake, no-bake cookies, hot cereal, toasted flakes, steamed pilaf, muffins) were made with whole-wheat flour, wheat flakes, and brown rice; the diet was designed to contain little added soluble fiber. Diets containing 6 g added soluble fiber used barley flakes, barley flour, or pearly barley in the test foods, replacing the wheat or rice. Test foods in diets containing 3 g added soluble fiber from barley per 2800 kcal were made with half barley and half whole wheat or brown rice ([Table 4](#)). Whole-wheat flour and brown rice were purchased from a local grocery store. Wheat flakes were purchased in one lot from Barry Farm Enterprises (Wapakoneta, OH).

Barley flakes, flour, and pearled barley were produced from one lot of barley and donated by the National Barley Foods Council (Spokane, WA).

Table 3. Sample Menus

Breakfast	Lunch	Dinner	Evening snack
Control			
Grapefruit juice	Tuna salad	Chicken/gravy	Apple
English muffin	Pita bread	Green beans	Peanut butter
Scrambled eggs	Carrots	Rice pilaf	Rice cakes
Margarine/jelly	Cookies	Tossed salad	
	Lemonade	Strawberries/cake	
Test			
Grapefruit juice	Tuna salad	Chicken/gravy	Test cookies
Test hot cereal	Pita bread	Green beans	Lemonade
Lactose-free milk	Carrots	Test pilaf	
English muffin	Test cake	Tossed salad	
Margarine/jelly			

Table 4. Nutrient Content of Barley, Whole Wheat Flour, and Brown Rice (100 g)

	Barley	Whole wheat flour	Brown rice
Energy (kcal)	352	339	370
Protein (g)	9.9	13.7	7.94
Fat (g)	1.2	1.9	2.9
Saturated (g)	0.2	0.3	0.6
Carbohydrate (g)	77.7	72.6	77.2
Dietary fiber (g)	15.6	12.2	3.5
Soluble fiber (g)	5.0	1.1	0.3

Analysis and Statistics

Two fasting blood samples (separated by 1 day) were collected before controlled feeding began and weekly during each period after an overnight fast of at least 12 hours. Plasma was separated and stored at -80°C until all samples were collected. Triacylglycerol and total cholesterol concentrations were determined enzymatically with an automated spectrophotometric system (Baker Instruments Corp, Allentown, PA). High-density lipoprotein (HDL) cholesterol was determined after other fractions were precipitated with dextran sulphate and manganese chloride [22]. Very-low-density lipoprotein (VLDL) and low-density-lipoprotein (LDL) concentrations were calculated [23]. Lipid subclass fractions were measured during the last week of each period by nuclear magnetic resonance spectroscopy [24]. Data were statistically analyzed by analysis of variance using a mixed model procedure (PCSAS, Version 8.2, SAS Institute, Cary, NC). Each subject served as his own control. Data were examined for normal distribution. Triacylglycerol concentrations were log transformed for statistical evaluation. Differences of least squares means were determined for significant factors. Data reported are least squares means \pm SEM. Statistical significance was defined as $p < 0.05$.

► RESULTS

Some gastrointestinal discomfort was noted by some of the subjects during the equilibration period when a diet higher in fiber than was typical was consumed. The major complaint was that there was too much food and that subjects had a very full feeling after eating. More subjects complained about bloating and flatulence with the high-soluble fiber diet.

Average body weights varied by a little more than 1 kg from the initial weight to the end of the Step 1 equilibration period, a difference that was statistically significant ($p < 0.038$). Subjects' average weight after consuming all three whole-grain diets was less than initial weights or weights after the Step 1 equilibration diet. Two of the 18 subjects were responsible for most of the weight change. The weights of the remaining subjects did not significantly change throughout the study ($p < 0.192$).

Total plasma cholesterol concentrations with and without subjects who lost weight was significantly affected by the diet consumed ($p < 0.0001$, [Table 5](#)) and by the length of time (by week, $p < 0.001$; data not shown) the diet was consumed. No interaction between diet and week was observed ($p = 0.912$). The cholesterol concentrations on average did not significantly decrease until the fourth week of each period. Compared with prestudy concentrations ([Table 1](#)), total cholesterol was 4% lower ($p < 0.026$) after consumption of the Step 1 diet. Total cholesterol was significantly lower than prestudy concentrations (14%, 17%, and 20%, respectively; $p < 0.001$) after subjects consumed the low-, medium-, and high-soluble fiber diets. Total cholesterol concentrations after the high soluble fiber diet were significantly lower than those after the low- or medium-soluble fiber diets. Including the two subjects who lost weight did not change the level of statistical significance nor the differences between the diets. Means including the subjects who lost weight ([Table 5](#)) did not appreciably differ from those of the weight stable group.

Calculated LDL cholesterol concentrations followed the same significant ($p < 0.0001$) pattern of reduction that was observed for total cholesterol. Compared with prestudy concentrations, LDL cholesterol was 4% lower after the Step 1 diet and significantly lower (17%, 17%, and 24%, respectively; $p < 0.001$) after subjects consumed the low-, medium-, and high-soluble fiber diets. LDL cholesterol concentrations after the high-soluble fiber diet were significantly lower than those after the low- or medium soluble fiber diets. The level of statistical significance, the differences between the diets and relative mean concentrations were unchanged when the two subjects who lost weight were included ([Table 5](#)).

Table 5. Fasting Lipid Concentrations (mg/dL) Determined Enzymatically after the Equilibration and Experimental Dietary Periods

	Step 1	Low	Medium	High	Diet effect
Weight-stable subjects (n = 16)					
Cholesterol	225.0 \pm 8.4 ^a	204.1 \pm 8.7 ^b	202.1 \pm 8.4 ^b	186.2 \pm 8.3 ^c	$p < 0.0001$
HDL cholesterol	38.2 \pm 1.6 ^a	41.5 \pm 1.7 ^b	39.7 \pm 1.6 ^b	41.0 \pm 1.6 ^b	$p < 0.003$
LDL cholesterol	152.3 \pm 8.3 ^a	130.4 \pm 8.6 ^b	130.0 \pm 8.3 ^b	116.1 \pm 8.22 ^c	$p < 0.0001$
Total/HDL ratio	6.1 \pm 0.36 ^a	5.2 \pm 0.4 ^{bc}	5.4 \pm 0.3 ^b	4.9 \pm 0.4 ^c	$p < 0.0001$
Triacylglycerol	171.6 \pm 16.8 ^a	167.9 \pm 17.4 ^{ab}	161.7 \pm 16.8 ^{ab}	151.7 \pm 16.6 ^b	$p = 0.0525$
All subjects (n = 18)					
Cholesterol	223.6 \pm 7.6 ^a	201.6 \pm 7.8 ^b	199.4 \pm 7.6 ^b	186.5 \pm 7.5 ^c	$p < 0.0001$
HDL cholesterol	37.5 \pm 1.6 ^b	40.8 \pm 1.7 ^a	38.9 \pm 1.6 ^{ab}	39.9 \pm 1.6 ^a	$p < 0.001$
LDL cholesterol	149.3 \pm 7.8 ^a	126.2 \pm 8.0 ^b	126.2 \pm 7.8 ^b	115.5 \pm 7.8 ^c	$p < 0.0001$
Total/HDL ratio	6.2 \pm 0.3 ^a	5.2 \pm 0.3 ^b	5.4 \pm 0.3 ^b	5.0 \pm 0.3 ^b	$p < 0.0001$
Triacylglycerol	184.2 \pm 18.3 ^a	178.4 \pm 18.6 ^{ab}	171.7 \pm 18.3 ^b	161.2 \pm 18.2 ^b	$p = 0.0278$

* Means within a row with different superscripts are significantly different.

Conversions of English to Metric units: cholesterol mg/dL \times 0.0258 = mmol/L; triacylglycerols mg/dL \times 0.01114 = mmol/L.

HDL cholesterol was significantly affected by the diet consumed ($p < 0.001$, all subjects; $p < 0.001$, weight-stable subjects) ([Table 5](#)). Compared with prestudy concentrations, HDL cholesterol was significantly lower ($p < 0.003$) after the Step 1 diet and the medium-soluble fiber diets. When the subjects who lost weight were included, only the concentrations after the Step 1 diet were significantly lower than prestudy concentrations. HDL cholesterol concentrations after the low-, medium- and high-soluble fiber diets were not significantly different in either subject grouping. The ratio of total cholesterol to HDL cholesterol was significantly affected by the diet consumed ($p < 0.001$). The ratio was

significantly lower ($p < 0.001$) after the three fiber diets compared with prestudy or Step 1 concentrations. The ratio was lowest after the high-soluble fiber diet, significantly lower than that after the medium diet. The ratios after the three experimental diets were not significantly different when data from the subjects who lost weight were included.

Overall triacylglycerol concentrations tended to decline ($p = 0.0525$, weight-stable subjects; $p = 0.0178$, all subjects) from the prestudy and Step 1 concentrations ([Tables 1](#) and [5](#)). Compared with prestudy concentrations, triacylglycerol concentrations were 3% lower after the Step 1 diet. Triacylglycerol concentrations were 6%, 10%, and 16% lower after the low-, medium-, and high-soluble fiber diets than before the study and were significantly lower after the high-soluble fiber diet than before the study and after the Step 1 period in both the weight stable group and total subject group. Although triacylglycerol concentrations decreased with increasing soluble fiber, concentrations after the low-, medium- and high-soluble fiber diets were not significantly different.

Lipid fraction concentrations presented in [Table 6](#) includes all study subjects. The level of statistical significance, the differences between diet means and relative mean concentrations of lipid fractions with and without the two subjects who lost weight were equivalent. Concentrations of large, intermediate, and small fractions of VLDL or LDL cholesterol prestudy and after the Step 1 and three experimental diets were not significantly different. VLDL and LDL cholesterol lipid fractions after the low-, medium and high soluble fiber diets were not different. The concentration of small HDL cholesterol fractions after the experimental diets were significantly lower than the prestudy concentrations. Intermediate HDL cholesterol fractions after the experimental diets were significantly lower than concentrations after the Step 1 diet. However, these differences in HDL were not fiber specific. The large HDL particle concentrations did not significantly vary with diet.

Table 6. Fasting Lipid Fractions (mg/dl) of 18 Men Determined by Nuclear Magnetic Resonance Spectroscopy before and at the End of Each Controlled Dietary Period

	Prestudy	Step 1	Low	Medium	High	Diet effect
VLDL cholesterol						
Large	28.2 \pm 8.8 ^a	25.7 \pm 8.8	22.3 \pm 9.1	24.0 \pm 8.9	19.9 \pm 8.8	<i>p</i> = 0.661
Intermediate	52.5 \pm 7.7	55.8 \pm 7.7	53.1 \pm 8.0	58.7 \pm 7.8	50.2 \pm 7.7	<i>p</i> = 0.741
Small	22.1 \pm 2.7	22.7 \pm 2.7	25.6 \pm 2.9	17.9 \pm 2.8	20.8 \pm 2.7	<i>p</i> = 0.202
Mean size (nm)	45.16 \pm 1.85	45.41 \pm 1.83	44.62 \pm 1.88	46.41 \pm 1.85	44.07 \pm 1.83	<i>p</i> = 0.305
LDL cholesterol						
Large	71.9 \pm 9.6	57.2 \pm 9.7	46.2 \pm 10.1	55.1 \pm 9.8	59.2 \pm 9.7	<i>p</i> = 0.061
Intermediate	53.4 \pm 7.7	48.1 \pm 7.7	58.1 \pm 8.1	54.0 \pm 7.8	44.2 \pm 7.7	<i>p</i> = 0.451
Small	0.9 \pm 4.4	10.1 \pm 4.4	7.8 \pm 4.6	5.2 \pm 4.5	5.5 \pm 4.4	<i>p</i> = 0.257
Mean size (nm)	21.31 \pm 0.16 ^a	21.06 \pm 0.16 ^b	20.97 \pm 0.16 ^b	21.07 \pm 0.16 ^b	21.13 \pm 0.16 ^{ab}	<i>p</i> < 0.041
Number particles	1287 \pm 57 ^a	1229 \pm 57 ^{ab}	1212 \pm 58 ^b	1205 \pm 57 ^{bc}	1135 \pm 57 ^c	<i>p</i> < 0.007
HDL cholesterol						
Large	11.7 \pm 1.1	11.3 \pm 1.1	11.8 \pm 1.1	11.1 \pm 1.1	12.3 \pm 1.1	<i>p</i> = 0.588
Intermediate	0.8 \pm 0.3 ^a	1.5 \pm 0.3 ^b	0.6 \pm 0.3 ^a	0.4 \pm 0.3 ^a	0.2 \pm 0.3 ^a	<i>p</i> < 0.005
Small	23.9 \pm 0.6 ^a	21.9 \pm 0.6 ^b	21.7 \pm 0.6 ^b	21.9 \pm 0.6 ^b	21.0 \pm 0.6 ^b	<i>p</i> < 0.002
Mean size (nm)	8.62 \pm 0.05	8.58 \pm 0.05	8.62 \pm 0.05	8.59 \pm 0.05	8.64 \pm 0.05	<i>p</i> = 0.498

* Means within a row with different superscripts are significantly different.

Conversions of English to Metric units: cholesterol mg/dL * 0.0258 = mmol/L; triacylglycerols mg/dL * 0.01114 = mmol/L.

The mean number of LDL particles significantly decreased (*p* < 0.007) after all the experimental diets; the greatest decrease (12%) occurred after the high-soluble fiber diet. LDL mean size remained in the high-risk range after all diets for these subjects. LDL particle size did show a significant variation between the diets but no difference was observed due to the amount of soluble fiber in the diet. The size of VLDL and HDL particles did not significantly vary with diet.

► DISCUSSION

Most research studies using food as the soluble fiber source have fed oats or oat products [2,12,17,18,25–32]. Some controlled feeding studies compared a high-fiber diet with a

low-fiber diet. Many studies supplemented a self-selected diet with added fiber with and without controlling or monitoring other dietary factors. Significantly lower total cholesterol [2,25–27] and LDL cholesterol [2,25–27] concentrations have been reported after the consumption of oat bran compared to wheat bran or rice bran added to the self-selected diets of hypercholesterolemic subjects. Generally, no significant change was reported in triacylglycerols [2,25,28] or HDL cholesterol [2,26–28] concentrations in these subjects with the inclusion of oatmeal or oat bran in the diet. The lipids of normolipemic subjects usually do not decrease with the addition of soluble fibers to their diet [2,29,33,34].

Relatively few studies have reported the β -glucan content of the diet [2,8,16,30–32]. A reduction in total and LDL cholesterol from prestudy and maintenance diet concentrations was reported [8] after two levels of soluble oat extract (1.8 or 7.2 g β -glucan/day) were consumed by moderately hypercholesterolemic men and women for 5 weeks each. The greatest percentage decrease in total and LDL cholesterol occurred after the higher β -glucan intake. Davidson *et al.* [30] reported total cholesterol and LDL cholesterol were significantly lower after the 28–84 g (1–3 oz) of oatmeal or oat bran as the β -glucan source than after the farina. The greatest percentage change from baseline value occurred when 56 g oat bran (4.0 g β -glucan), 84 g oatmeal (3.6 g β -glucan), or 84 g oat bran (6.0 g β -glucan) were consumed, β -glucan intakes similar to that in our medium and high soluble fiber diets. However, Mackay and Ball [31] and Törrönen *et al.* [32] did not observe statistically significant decreases in total cholesterol [31,32], LDL cholesterol [31,32] after diets containing 1.9, 3.0, or 11.2 g β -glucan/day. These authors observed a significantly higher [31] and no difference [32] in HDL cholesterol concentrations. Jenkins *et al.* [16] supplemented diets of moderately hypercholesterolemic men and women consuming a self-selected Step II diet with high-fiber foods so that average soluble fiber was increased by 8 g/day compared to the diet with control foods. Compared to the control diet, total cholesterol and the ratios of total:HDL cholesterol and LDL:HDL cholesterol were significantly lower after the higher soluble fiber diet. Our results using a controlled Step 1 diet with 3 or 6 g soluble fiber/d were similar even though different soluble fiber sources were used on the two studies. Brown *et al.* [35] performed a meta-analysis of 67 controlled studies and calculated that for each gram of soluble fiber from oats, psyllium, or pectin, total and LDL cholesterol decreased by approximately 1.55 mg/dL (0.04 mmol/L). The meta-analysis showed no significant change in triacylglycerols and HDL cholesterol. The observed changes appeared to be independent of study design, treatment length, and dietary fat content.

Only one study has reported the effect of fiber on lipid subclass and particle numbers [36]. Overweight men consuming an oat (14 g dietary fiber/day, 5.5 g β -glucan) or wheat supplement (14 g dietary fiber/day) for 12 weeks had a significant reduction ($p < 0.05$) in LDL cholesterol concentration, LDL particle number, and the ratio of LDL to HDL cholesterol from prestudy concentrations after the oats whereas the lipids increased after the wheat. Total cholesterol, triacylglycerols, and VLDL cholesterol showed the same pattern as LDL cholesterol but the differences were not significant ($p < 0.08$, $p < 0.07$, $p < 0.08$, respectively). Concentrations of HDL cholesterol and HDL cholesterol subclasses and diameters of LDL, HDL, and VLDL particles were not significant by diet type. The

authors suggested that the decrease in small dense LDL cholesterol concentration and LDL particle number without changes in triacylglycerols or HDL cholesterol concentrations may contribute to the beneficial effect of oat fiber on cardiovascular disease. We observed similar pattern in the reduction of LDL particle number and concentration. However, we also observed some reduction in the intermediate and small HDL cholesterol subclasses. Freedman *et al.* [37] reported that men with relatively high concentrations of either small HDL or large VLDL particles were 3–4 times more likely to have extensive coronary artery disease than men with concentrations below average. Campos *et al.* [38] reported a significant association between large LDL size and coronary artery disease in normolipidemic men; large LDL particles were more prevalent in men with coronary artery disease (43%) than in control subjects (25%). The reduction in small HDL concentrations and LDL cholesterol particles we observed after subjects consumed the high-soluble fiber diet indicates an improvement in this risk factor.

Few studies have used barley as the source of β -glucan in the diet and most did not report the soluble fiber content of the diets. Similar to our findings the addition of barley to the diet resulted in lower total and LDL-cholesterol compared to concentrations prestudy or after a control grain. Newman *et al.* [34] reported men consuming 42 g dietary fiber for 4 weeks from wheat or barley had total and LDL cholesterol higher after the wheat products and lower after the barley products compared with prestudy concentrations. No decrease was observed in the men who had normal cholesterol concentrations before the addition of the barley. When oats or barley were consumed for 6 weeks by moderately hypercholesterolemic men and women [39], total and LDL cholesterol were lower after both grains. No significant difference between the two grains was observed, an indication that soluble fiber and not the source was critical in lipid reduction. A 50/50 mix of rice and barley (similar to the mixture in our medium diet containing 3.0 g beta glucan) consumed twice daily for 2–4 weeks resulted in significant decreases in total cholesterol, LDL cholesterol, LDL lipoproteins, and VLDL lipoproteins of hypercholesterolemic men and women, but not normolipemic subjects, from prestudy concentrations [33]. Lupton *et al.* [19] fed supplements of barley bran flour (30 g/day), barley oil (3 g/day), or cellulose (20 g/day) to hypercholesterolemic men and women following a Step 1 diet. Similar to our results, the addition of the barley bran flour and barley oil resulted in a significant decrease in total cholesterol ($p < 0.0001$ and $p < 0.002$, respectively) and LDL cholesterol ($p < 0.036$ and $p < 0.003$, respectively) whereas no change was observed after cellulose. Unlike our findings, HDL cholesterol decreased after the barley bran flour groups ($p < 0.006$). This may have been due, in part, to their subjects consuming approximately half the barley consumed by our subjects on the medium diet. McIntosh *et al.* [20] fed mildly hypercholesterolemic men barley or wheat products for 4 weeks. The diets averaged 25 g insoluble fiber and 13.4 g soluble fiber, which included 1.5 g and 8.0 g β -glucan, respectively, from the wheat and barley diets. Total cholesterol ($p < 0.05$) and LDL cholesterol ($p < 0.05$) were significantly lower after the barley diet than the wheat diet but triacylglycerols did not significantly change.

Several mechanisms have been suggested for the lowered cholesterol after increased soluble fiber consumption, including increased excretion of bile acids or neutral sterols, increased catabolism of LDL cholesterol, and reduced fat absorption [40–42]. Increased

viscosity of the gastric and intestinal contents can delay gastric emptying, decrease nutrient absorption, and interfere with micelle formation. Soluble fibers have been shown to be fermented in the colon [40–42], giving rise to short-chain fatty acids that can be absorbed and may inhibit hepatic cholesterol synthesis. Reduced cholesterol concentrations have also been associated with decreased postprandial insulin concentrations observed after soluble fiber ingestion [40]. In addition to the soluble fiber, barley contains a wide range of phytochemicals, some of which are being investigated for their effect on metabolism. A combination of factors and mechanisms appears to contribute to the reduction of lipids observed after consumption of barley.

► CONCLUSION

Overall, the subjects' cardiovascular risk factors improved with decreased total cholesterol, LDL cholesterol (especially large particle number), and ratio of total cholesterol to HDL cholesterol. The highest soluble fiber intake had the greatest effect on total and LDL cholesterol. These results indicate that the addition of barley to a healthy diet can reduce risk of cardiovascular disease.

► FOOTNOTES

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APPENDIX C

Barley β-glucan Health Claim

In the European Union

Article 14.1 Claim

COMMISSION REGULATION (EU) No 1048/2012

of 8 November 2012

on the authorization of a health claim made on foods and referring to the reduction of disease risk

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods⁽¹⁾, and in particular Article 17(3) thereof,

Whereas:

- (1) Pursuant to Regulation (EC) No 1924/2006 health claims made on foods are prohibited unless they are authorized by the Commission in accordance with that Regulation and included in a list of permitted claims.
- (2) Regulation (EC) No 1924/2006 also provides that applications for authorizations of health claims may be submitted by food business operators to the national competent authority of a Member State. The national competent authority is to forward valid applications to the European Food Safety Authority (EFSA), hereinafter referred to as 'the Authority'.
- (3) Following receipt of an application the Authority is to inform without delay the other Member States and the Commission thereof, and to deliver an opinion on the health claim concerned.
- (4) The Commission is to decide on the authorization of health claims taking into account the opinion delivered by the Authority.
- (5) Following an application from Cargill Incorporated, submitted pursuant to Article 14(1)(a) of Regulation (EC) No 1924/2006 and requesting the protection of proprietary data for one meta-analysis⁽²⁾ and for information pertaining to the production process of barley 'betafiber' (BarlivTM), the Authority was required to deliver an opinion on a health claim related to the effects of barley beta-glucans on lowering of blood cholesterol and reduced risk of (coronary) heart disease (Question No EFSA-Q-2011-00798)⁽³⁾. The claim

proposed by the applicant was worded as follows: 'Barley beta-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of (coronary) heart disease'.

- (6) On the basis of the data presented, the Authority concluded in its opinion received by the Commission and the Member States on 8 December 2011 that a cause and effect relationship had been established between the consumption of barley beta-glucans and lowering of blood LDL-cholesterol concentrations. Accordingly, a health claim reflecting this conclusion should be considered as complying with the requirements of Regulation (EC) No 1924/2006, and should be included in the Union list of permitted claims. The meta-analysis and information pertaining to the production process of barley 'betafiber' (BarlivTM), claimed by the applicant as proprietary, were not considered necessary by the Authority for reaching its conclusion. It is therefore considered that the requirement laid down in point (c) of Article 21(1) of Regulation (EC) No 1924/2006 is not fulfilled and accordingly, protection of proprietary data should not be granted.
- (7) Following an application from Valens Int. d.o.o., submitted pursuant to Article 14(1)(a) of Regulation (EC) No 1924/2006, the Authority was required to deliver an opinion on a health claim related to the effects of barley beta-glucans on lowering of blood cholesterol and reduced risk of (coronary) heart disease (Question No EFSA-Q-2011-00799)⁽⁴⁾. The claim proposed by the applicant was worded as follows: 'Barley beta-glucan has been shown to reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of heart disease'.
- (8) On the basis of the data presented, the Authority concluded in its opinion received by the Commission and the Member States on 8 December 2011 that a cause and effect relationship had been established between the consumption of barley beta-glucans and lowering of blood LDL-cholesterol concentrations. Accordingly, a health claim reflecting this conclusion should be considered as complying with the requirements of Regulation (EC) No 1924/2006, and should be included in the Union list of permitted claims.
- (9) Article 16(4) of Regulation (EC) No 1924/2006 provides that an opinion in favour of authorizing a health claim should include certain particulars. Accordingly, those particulars should be set out in the Annex to this Regulation as regards the authorized claim and include, as the case may be, the revised wording of the claim, specific

⁽¹⁾ OJ L 404, 30.12.2006, p. 9.⁽²⁾ Harland JL, 2011 (unpublished); Meta-analysis of the effects of barley beta-glucan on blood lipids.⁽³⁾ The EFSA Journal (2011); 9(12):2470.⁽⁴⁾ The EFSA Journal (2011); 9(12):2471.

- conditions of use of the claim, and, where applicable, conditions or restrictions of use of the food and/or an additional statement or warning, in accordance with the rules laid down in Regulation (EC) No 1924/2006 and in line with the opinions of the Authority.
- (10) One of the objectives of Regulation (EC) No 1924/2006 is to ensure that health claims are truthful, clear and reliable and useful to the consumer, and that wording and presentation are taken into account in that respect. Therefore where the wording of claims has the same meaning for consumers as that of an authorised health claim, because they demonstrate the same relationship that exists between a food category, a food or one of its constituents and health, they should be subject to the same conditions of use indicated in the Annex to this Regulation.
- (11) The comments from the applicants and the members of the public received by the Commission pursuant to Article 16(6) of Regulation (EC) No 1924/2006 have been considered when setting the measures provided for in this Regulation.
- (12) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health and neither the European Parliament nor the Council have opposed them,

HAS ADOPTED THIS REGULATION:

Article 1

1. The health claim listed in the Annex to this Regulation may be made on foods on the European Union market in compliance with the conditions laid down in that Annex.

2. The health claim referred to in paragraph 1 shall be included in the Union list of permitted claims as provided for in Article 14(1) of Regulation (EC) No 1924/2006.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the Official Journal of the European Union.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 8 November 2012.

For the Commission
The President
José Manuel BARROSO

ANNEX

Permitted health claim

Application — Relevant provisions of Regulation (EC) No 1924/2006	Applicant — Address	Nutrient, substance, food or food category	Claim	Conditions of use of the claim	Conditions and/or restrictions of use of the food additive, additional statement or warning	EUCA opinion reference
Article 14(1)(a) health claim referring to a reduction of a disease risk	Cargill Incorporated, acting through Cargill Health and Nutrition, c/o Cargill R & D Centre Europe, Havenstraat 84, 1800 Vilvoorde, Belgium	Barley beta-glucan	Barley beta-glucan has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of barley beta-glucan.	The claim can be used for foods which provide at least 1 g of barley beta-glucan per quantified portion.	Q-2011-00798
	Valenz Int. d.o.o., Kldričeva ulica 24b, SI-1000 Celje, Slovenia					Q-2011-00799

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Valens Int. d.o.o. submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Slovenia, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease, referring to disease risk reduction and including a request for the protection of proprietary data. The food constituent that is the subject of the health claim, barley beta-glucans, is sufficiently characterised. Lowering blood LDL-cholesterol concentration is a beneficial physiological effect by decreasing the risk of coronary heart disease. The applicant identified a total of eight references as being pertinent to the health claim. These references included two meta-analyses and six human studies. In weighing the evidence, the Panel took into account that one meta-analysis including 11 RCTs, and one additional RCT, which investigated the effects of barley beta-glucans at doses of at least 3 g/day showed a decrease in total and LDL-cholesterol concentrations in both normo- and hypercholesterolaemic subjects, and that there is evidence supporting the biological plausibility of the mechanism of the effect. The Panel concludes that a cause and effect relationship has been established between the consumption of barley beta-glucans and lowering of blood LDL-cholesterol concentrations. The following wording reflects the scientific evidence: "Barley beta-glucans have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease". At least 3 g of barley beta-glucans should be consumed per day in order to obtain the claimed effect. The target population is adults who want to lower their blood cholesterol concentrations. © European Food Safety Authority, 2011

KEY WORDS

Barley beta-glucans, fibre, blood cholesterol, LDL-cholesterol, health claims.

¹ On request from the Competent Authority of Slovenia following an application by Valens Int. d.o.o., Question No EFSA-Q-2011-00799, adopted on 23 November 2011.

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SUMMARY

Following an application from Valens Int. d.o.o. submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Slovenia, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease.

The scope of the application was proposed to fall under a health claim referring to disease risk reduction and including a request for the protection of proprietary data.

The food constituent that is the subject of the health claim is barley beta-glucans. Beta-glucans are non-starch polysaccharides composed of glucose molecules in long linear polymers. Beta-glucans occur naturally in barley and are measurable in foods by established methods. This opinion applies to barley beta-glucans naturally present in foods, and to those forms added to foods. The Panel considers that the food constituent, barley beta-glucans, which is the subject of the health claim, is sufficiently characterised.

The claimed effect is “reduction of blood cholesterol, which may reduce the risk of heart disease”. The target population proposed by the applicant is adults who want to lower their blood cholesterol concentrations. The Panel considers that lowering blood LDL-cholesterol concentrations is a beneficial physiological effect by decreasing the risk of coronary heart disease.

The Panel has already issued an opinion on beta-glucans from various sources, including barley, and maintenance of normal blood cholesterol concentrations, pursuant to Article 13(1) of Regulation (EC) No 1924/2006, with a favourable outcome. The Panel has also issued an opinion on barley beta-glucans and reduction of blood cholesterol concentrations, which may reduce the risk of (coronary) heart disease, pursuant to Article 14 of Regulation (EC) No 1924/2006, with a favourable outcome.

The applicant identified a total of eight references as being pertinent to the health claim. These references included two meta-analyses and six human studies.

Three of the six human studies were uncontrolled. One trial was an acute study which measured postprandial plasma lipids after the intake of test meals. One study was carried out in ileostomy patients who are not representative of the target population with respect to digestion and absorption of dietary fats. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

The first meta-analysis comprised 11 studies (17 treatment arms) which included a total of 591 subjects. Seven studies had a crossover design and four had a parallel design. The duration of the intervention lasted from 4 to 12 weeks (mean 5.2 weeks) and 10–62 subjects were enrolled in the studies. Study populations were both normo- and hypercholesterolaemic, with mean blood cholesterol ranges from 3.6 to 8.6 mmol/L. The mean age ranged from 20 to 63 years. Mean baseline body mass index ranged from 19 to 35 kg/m². The estimated daily consumption of barley beta-glucans ranged from 3 to 12 g, with a median intervention dose of 5 g/day. The sources of barley beta-glucans included barley flour, barley flakes, pearled barley and barley bran. Most control groups received comparable products based on wheat or rice. Overall, barley beta-glucans lowered total and LDL-cholesterol concentrations by 0.30 mmol/L (95 % CI: 0.39 to 0.21, p<0.00001) and by 0.27 mmol/L (95 % CI: 0.34 to 0.20, p<0.00001), respectively. HDL-cholesterol concentrations were not affected.

The second meta-analysis included eight studies, seven of which were already included in the meta-analysis above. The additional study was a randomised, parallel, 30-day intervention trial in 79 hypercholesterolaemic subjects. The results from this meta-analysis were similar to those obtained in the first meta-analysis. Barley beta-glucans lowered total and LDL-cholesterol concentrations by

0.35 mmol/L (95 % CI: 0.48 to 0.21, p<0.05) and by 0.26 mmol/L (95 % CI: 0.36 to 0.16, p<0.05), respectively. Other blood lipid parameters were not affected. To address publication bias, visual inspection of funnel plots, Egger's weighted regression statistics, and the trim and fill method were applied. When potentially missing studies (i.e. unpublished negative studies) were taken into account, barley beta-glucans still had a significant, albeit reduced, effect on total and LDL-cholesterol concentrations.

In a randomised controlled crossover trial, 24 mildly hypercholesterolaemic men consumed a barley beta-glucan enriched diet (6 g barley beta-glucans per day) or a rice bran-enriched control diet for 4 weeks each (with a 3-week washout in between) after a 3-week run-in period. Compared to the rice-bran diet, the barley beta-glucan diet induced a significant decrease in total and LDL-cholesterol concentrations of 0.34 mmol/L (95 % CI: 0.47 to 0.20, p<0.001) and 0.21 mmol/L (95 % CI: 0.40 to 0.02, p=0.033), respectively. No effects were observed on other blood lipid parameters.

The cholesterol-lowering effect of barley beta-glucans is considered to depend on increased viscosity that reduces the reabsorption of bile acids and increases both the synthesis of bile acids from cholesterol as well as the faecal excretion of neutral sterols. Viscosity in the small intestine is determined by the concentration, molecular weight and solubility of the barley beta-glucans.

In weighing the evidence, the Panel took into account that one meta-analysis including 11 randomised controlled trials (RCTs), and one additional RCT, which investigated the effects of barley beta-glucans at doses of at least 3 g/day showed a decrease in total and LDL-cholesterol concentrations in both normo- and hypercholesterolaemic subjects, and that there is evidence supporting the biological plausibility of the mechanism of the effect.

The Panel concludes that a cause and effect relationship has been established between the consumption of barley beta-glucans and the lowering of blood LDL-cholesterol concentrations.

The Panel considers that the following wording reflects the scientific evidence: "Barley beta-glucans have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease".

The Panel considers that at least 3 g of barley beta-glucans should be consumed per day in order to obtain the claimed effect. This amount can reasonably be consumed as part of a balanced diet. The target population is adults who want to lower their blood cholesterol concentrations.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of this Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 10/06/2011.
- The scope of the application was proposed to fall under a health claim referring to disease risk reduction and including a request for the protection of proprietary data.
- On 07/07/2011, during the validation process of the application, EFSA sent a request to the applicant to provide additional information.
- The applicant provided the requested information on 29/07/2011.
- The scientific evaluation procedure started on 10/08/2011.
- During its meeting on 23/11/2011, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to barley beta-glucans and the lowering of blood LDL-cholesterol concentrations.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of barley beta-glucans, a positive assessment of its safety, nor a decision on whether barley beta-glucans are, or are not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Valens Int. d.o.o., Kidričeva ulica 24b, SI-3000 Celje, Slovenia.

The applicant claimed proprietary rights for the information pertaining to the manufacturing process of the applicant's barley flour enriched in beta-glucan.

Food/constituent as stated by the applicant

According to the applicant, barley beta-glucans, which are soluble cereal fibres naturally occurring in barley.

Health relationship as claimed by the applicant

According to the applicant, the consumption of barley beta-glucans has been shown to reduce blood cholesterol. Blood cholesterol has been shown as a risk factor for coronary heart disease.

Wording of the health claim as proposed by the applicant

The applicant proposed the following wording for the health claim: "Barley beta-glucan has been shown to reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of heart disease".

Specific conditions of use as proposed by the applicant

According to the applicant, to bear the claim:

- Foods should provide at least 3 g/day of barley beta-glucans. The total 3 g of barley beta-glucans can be consumed in one or more servings throughout the day. The claim should be permitted on foods containing at least 0.75 g barley beta-glucans per serving and such products should be equipped with information about the necessary daily consumption of beta-glucans required to obtain the claimed beneficial effect.
- The source of barley beta-glucans in foods should be barley or barley fractions with improved beta-glucan content produced by use of physical methods. Enzymatically treated beta-glucans are not the subject of this claim.

The target population proposed by the applicant is adults who want to lower their blood cholesterol concentrations.

Similar claims as proposed/authorised by other entities

The US Food and Drug Administration (FDA, 2005, 2008) has already approved a health claim for barley beta-glucans which is similar to the one proposed in this application.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is barley beta-glucans, which are soluble fibres present in barley (*Hordeum vulgare* L.). Beta-glucans are non-starch polysaccharides composed of glucose molecules in long linear polymers consisting in blocks of 2-4 glucose units linked by β - $(1\rightarrow 4)$ bonds, separated generally by a single glucose molecule with a β - $(1\rightarrow 3)$ link, leading to an approximate distribution of 70 % to 30 % for the two types of linkages. The molecular weight (MW)

varies between 50 and 2000 kDa. The mixed linkages are important for the physical properties, such as solubility and viscosity. Viscosity is a function of the concentration of dissolved beta-glucans and of its molecular weight (Wood et al., 2000), and further depends on differences in raw materials, processing and methods of determination. Beta-glucans occur naturally in barley (4-7 %) and are measurable in foods by established methods. This opinion applies to barley beta-glucans naturally present in foods, and to those forms added to foods.

The Panel considers that the food constituent, barley beta-glucans, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is “reduction of blood cholesterol, which may reduce the risk of heart disease”. The target population proposed by the applicant is adults who want to lower their blood cholesterol concentrations.

Coronary heart disease (CHD) is a leading cause of mortality and morbidity in European populations, with over 1.9 million deaths in the European Union and over 4.35 million deaths in Europe each year (Pedersen et al., 2005). Elevated blood cholesterol is an important modifiable risk factor in the development of CHD (WHO, 2002a, b).

It has been shown that blood cholesterol concentrations can be decreased by drugs, and by dietary and lifestyle changes (Denke, 2005; Gordon, 2000; Ornish et al., 1998; van Horn et al., 2008).

The Panel considers that lowering blood LDL-cholesterol concentrations is a beneficial physiological effect by decreasing the risk of coronary heart disease.

3. Scientific substantiation of the claimed effect

The Panel has already issued an opinion on beta-glucans from various sources, including barley, and maintenance of normal blood cholesterol concentrations, pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2009). On the basis of the studies provided for oat beta-glucans, and of eight studies provided for barley beta-glucans (Behall et al., 2004a, b; Biörklund et al., 2005; Keenan et al., 2007; Keogh et al., 2003; McIntosh et al., 1991; Newman et al., 1989; Shimizu et al., 2008), the Panel concluded that a cause and effect relationship had been established between the consumption of beta-glucans and the maintenance of normal blood cholesterol concentrations. Six of the studies conducted with barley beta-glucans (Behall et al., 2004a, b; Keenan et al., 2007; McIntosh et al., 1991; Newman et al., 1989; Shimizu et al., 2008) showed significant lowering of LDL-cholesterol concentrations, whereas two studies did not (Biörklund et al., 2005; Keogh et al., 2003).

The Panel has also issued an opinion on barley beta-glucans and reduction of blood cholesterol concentrations, which may reduce the risk of (coronary) heart disease, pursuant to Article 14 of Regulation (EC) No 1924/2006, with a favourable outcome (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011).

The applicant identified a total of eight references as being pertinent to the health claim. These references included two meta-analyses and six human studies. No information was provided on the literature search.

Three of the six human studies were uncontrolled, i.e. single arm interventions without a control (placebo) group (Dongowski et al., 2006; Ikegami et al., 1996; Smith et al., 2008). One acute trial (Bourdon et al., 1999) measured postprandial plasma lipids after the intake of test meals. One study was carried out in ileostomy patients (Zhang et al., 1991) who are not representative of the target

population with respect to digestion and absorption of dietary fats. The Panel considers that no conclusions can be drawn from these studies for the scientific substantiation of the claim.

The first meta-analysis (AbuMweis et al., 2010) comprised 11 studies (17 treatment arms) published between 1989 and 2008, and which included a total of 591 subjects (Behall et al., 2004a, b; Biörklund et al., 2005; Keenan et al., 2007; Keogh et al., 2003; Li et al., 2003; McIntosh et al., 1991; Newman et al., 1989; No authors listed, 2005, unpublished clinical study report; Shimizu et al., 2008; Sundberg, 2008). Seven studies had a cross-over design and four had a parallel design. The duration of the intervention lasted from 4 to 12 weeks (mean 5.2 weeks) and study size ranged from 10 to 62 subjects. Study populations were both normo- and hypercholesterolaemic (mean blood cholesterol concentrations 3.6 to 8.6 mmol/L), had a mean age of 20-63 years and a mean body mass index (BMI) of 19-35 kg/m². Five studies recruited only males and one study recruited only females. The estimated daily consumption of barley beta-glucans ranged from 3 to 12 g, with a median intervention dose of 5 g/day. The sources of barley beta-glucans included barley flour, barley flakes, pearl barley and barley bran which were consumed as breakfast cereals, biscuits, bread, pancakes, muffins, tabbouleh, steamed grains, powders, juice/drinks and a low MW gelled form. Most control groups received comparable products based on wheat or rice. In five studies, subjects consumed their habitual diet, and in two studies, an American Heart Association Step I diet. Two other studies were controlled feeding trials with typical Japanese and Western diets. In one study subjects were given instructions to follow a low saturated and low *trans* fat diet. The primary outcome of this meta-analysis was to quantify the effect of beta-glucans from barley on total and LDL-cholesterol concentrations. Estimates of the pooled treatment effect size and 95 % confidence intervals (CI) were calculated using both fixed effect and random effects models. Overall, barley beta-glucans lowered total and LDL-cholesterol concentrations by 0.30 mmol/L (95 % CI: 0.39 to 0.21, p<0.00001) and by 0.27 mmol/L (95 % CI: 0.34 to 0.20, p<0.00001), respectively. The ingestion of barley beta-glucans did not affect HDL-cholesterol concentrations. The cholesterol-lowering effect of beta-glucans from barley was apparently independent of baseline cholesterol concentrations, type of intervention and food matrix. High doses of barley beta-glucans (>7 g/day) did not appear to have a greater effect on blood cholesterol than modest consumption (3-5 g/day). In one study, consumption of either high or low MW barley beta-glucans at doses of 3 and 5 g/day for six weeks significantly decreased LDL-cholesterol concentrations relative to control (Keenan et al., 2007). However, the MW of beta-glucans was not reported in the majority of studies and therefore it was not possible to assess the impact of MW on the cholesterol-lowering effect of barley beta-glucans in these studies. Funnel plots were used to assess publication bias. Their slightly asymmetrical appearance indicated that small negative studies might have remained unpublished. The Panel notes that nine out of the 11 studies considered in the meta-analysis reported a significant effect of barley-beta glucans on total and LDL-cholesterol concentrations.

The second meta-analysis (Talati et al., 2009) included eight studies (13 treatment arms, 391 subjects), seven of which (Biörklund et al., 2005; Keenan et al., 2007; Keogh et al., 2003; Li et al., 2003; McIntosh et al., 1991; Newman et al., 1989; Shimizu et al., 2008) were already included in the meta-analysis by AbuMweis et al. (2010). The additional study (Lupton et al., 1994) was a randomised, parallel, 30-day intervention trial in 79 hypercholesterolaemic subjects. The results from this meta-analysis were similar to those obtained by AbuMweis et al. (2010). Barley beta-glucans lowered total and LDL-cholesterol concentrations by 0.35 mmol/L (95 % CI: 0.48 to 0.21, p<0.05) and by 0.26 mmol/L (95 % CI: 0.36 to 0.16, p<0.05), respectively. Other blood lipid parameters (HDL-cholesterol, triglycerides) were not affected. To address publication bias, visual inspection of funnel plots, Egger's weighted regression statistics, and the trim and fill method were applied. When potentially missing studies (i.e. unpublished negative studies) were taken into account, barley beta-glucans still had a significant, albeit reduced, effect on total and LDL-cholesterol concentrations.

One human intervention study (Rondanelli et al., 2011), which was not available at the time the two meta-analyses above were conducted, was also provided by the applicant. In this randomised controlled cross-over trial, 24 mildly hypercholesterolaemic men (mean age 50.3±5.3 years)

consumed a barley beta-glucan-enriched diet or a rice bran-enriched control diet for four weeks each (with a three-week washout in between) after a three-week run-in period on a Step 1 American Heart Association diet containing rice bran-enriched foods (providing 19.7 g/day total dietary fibre, including 7 g/day soluble fibre). The barley beta-glucan diet provided 39.3 g/day total dietary fibre, of which 13.7 g/day was soluble fibre and 6 g/day of this was barley beta-glucans from a dry, processed, ‘high’ (but undeclared) molecular weight concentrate included in various foods. The rice bran-enriched control diet provided 45.7 g/day total fibre, of which 10.4 g/day was soluble fibre. A significant decrease in total cholesterol (-0.54 mmol/L, 95 % CI: -0.75 to -0.33, p<0.001) and LDL-cholesterol (-0.53 mmol/L, 95 % CI: -0.79 to -0.26, p<0.001) was observed during the run-in period. The treatment effect of the barley beta-glucan and the rice diets was presented as the mean difference between final values (95 % CI) adjusted for baseline values of each period and for period effect. Compared to the rice-bran diet, the barley beta-glucan diet induced a significant decrease in total and LDL-cholesterol concentrations of 0.34 mmol/L (95 % CI: 0.47 to 0.20, p<0.001) and 0.21 mmol/L (95 % CI: 0.40 to 0.02, p=0.033), respectively. No effects were observed on other blood lipid parameters (HDL-cholesterol, apolipoprotein A-I and B, triglycerides, or various calculated ratios).

The cholesterol-lowering effect of barley beta-glucans is considered to depend on increased viscosity that reduces the reabsorption of bile acids and increases both the synthesis of bile acids from cholesterol as well as the faecal excretion of neutral sterols (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2011). Viscosity in the small intestine is determined by the concentration, molecular weight and solubility of the barley beta-glucans. Barley beta-glucans may be degraded during the purification and manufacturing of foods by factors such as shear, heat and the action of enzymes, and its cholesterol-lowering effect may be weakened or even disappear. Differences in viscosity are thought to explain, at least in part, the large variation between the LDL-cholesterol lowering effects found in individual studies (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2009, 2010).

In weighing the evidence, the Panel took into account that one meta-analysis including 11 randomised controlled trials (RCTs), and one additional RCT, which investigated the effects of barley beta-glucans at doses of at least 3 g/day showed a decrease in total and LDL-cholesterol concentrations in both normo- and hypercholesterolaemic subjects, and that there is evidence supporting the biological plausibility of the mechanism of the effect.

The Panel concludes that a cause and effect relationship has been established between the consumption of barley beta-glucans and the lowering of blood LDL-cholesterol concentrations.

4. Panel's comments on the proposed wording

The Panel considers that the following wording reflects the scientific evidence: “Barley beta-glucans have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease”.

5. Conditions and restrictions of use

The Panel considers that at least 3 g of barley beta-glucans should be consumed per day in order to obtain the claimed effect. This amount can reasonably be consumed as part of a balanced diet. The target population is adults who want to lower their blood cholesterol concentrations.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, barley beta-glucans, which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is “reduction of blood cholesterol, which may reduce the risk of heart disease”. The target population proposed by the applicant is adults who want to lower their blood cholesterol concentrations. Lowering blood LDL-cholesterol concentration is a beneficial physiological effect by decreasing the risk of coronary heart disease.
- A cause and effect relationship has been established between the consumption of barley beta-glucans and the lowering of blood LDL-cholesterol concentrations.
- The following wording reflects the scientific evidence: “Barley beta-glucans have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease”.
- At least 3 g of barley beta-glucans should be consumed per day in order to obtain the claimed effect. This amount can reasonably be consumed as part of a balanced diet. The target population is adults who want to lower their blood cholesterol concentrations.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on barley beta-glucans and lowering of blood cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0306_SI). June 2011. Submitted by Valens Int. d.o.o.

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