NATIONAL
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FOODS COUNCIL

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August 2, 2004

Dr. James Hoadley
Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Nutritional Products, Labeling and Dietary Supplements
Division of Nutrition Programs and Labeling (HFS-832)
5100 Paint Branch Pkwy.
College Park, MD 20740

Subject: Petition for Health Claim – Barley β-glucan Soluble Fiber and Barley Foods Containing β-glucan Soluble Fiber and Coronary Heart Disease

Dear Dr. Hoadley,

On behalf of The National Barley Foods Council, I am writing to respectfully request that the FDA accept the enclosed amendment to the above mentioned petition which was originally submitted to the FDA on September 25, 2003 and withdrawn (pending submission of this amendment) on December 29, 2003. Enclosed are two copies of the amendment and two copies of all research papers cited which were not submitted with the original petition. Appendices to the amendment include a letter from Dr. Kay Behall, Research Nutritionist, USDA, and a letter from B. E. Knuckles, Chemist, USDA.

We very much appreciate the FDA's patience in this matter.

Sincerely,

Christine E Fastnaught

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2004P-0512 CP2

Date: August 2, 2004

TO:

Office of Nutritional Products, Labeling
and Dietary Supplements
Food and Drug Administration
5100 Paint Branch Pkwy.
College Park, MD 20740

FROM PETITIONERS:

NATIONAL BARLEY FOODS COUNCIL
905 W. Riverside, Suite 501
Spokane, WA 99201

SUBJECT:

AMENDMENT TO THE PETITION FOR AN UNQUALIFIED HEALTH CLAIM FOR BARLEY β-GLUCAN SOLUBLE FIBER AND BARLEY PRODUCTS CONTAINING β-GLUCAN SOLUBLE FIBER AND CORONARY HEART DISEASE

AMENDMENT TO THE BARLEY HEALTH CLAIM PETITION

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INTRODUCTION

A petition for an unqualified health claim titled "Barley β-glucan Soluble Fiber and Barley Products Containing β-glucan Soluble Fiber and Coronary heart Disease" was submitted to the FDA on September 25, 2003 by the National Barley Foods Council. The petition requested that 21 CFR 101.81 "Health claims: Soluble fiber from certain foods and risk of coronary heart disease" be amended to include soluble fiber from barley and barley products. This document is being submitted to amend that petition, herein referred to as the "NBFC petition", which was withdrawn on December 29, 2003 and incorporates that petition and all of its parts by reference (National Barley Foods Council 2003).

The FDA requested clarification of three terms used in the NBFC petition: barley flour, barley meal and β-glucan enriched flour fractions. This document provides these definitions based on processes known in the industry and documented in the scientific literature. Further, definitions for all of the barley products have been revised to include a minimum level of β-glucan.

The FDA also requested clarification of the relationship between total β-glucan and β-glucan soluble fiber in barley and the content of β-glucan soluble fiber in diets consumed in three studies, Newman et al 1989, McIntosh et al 1991 and Behall et al 2004a. These studies reported total fiber or total β-glucan or soluble fiber content rather than β-glucan soluble fiber. These have been reviewed and data is submitted for the barley foods consumed in Behall et al. 2004a. Additionally, the conclusions from an expanded review of barley and oat β-glucan solubility is presented here with the overall conclusion that under most of the conditions in which β-glucan solubility is measured, including as a component of the soluble fiber, oat and barley are comparable. It was further concluded that the AOAC Method 992.28 (AOAC 2000) for total β-glucan content is an adequate marker for soluble β-glucan in barley and oats.

In the months since submission of the NBFC petition, additional data and clinical trials pertinent to the NBFC petition have become available. A manuscript with final results from a second USDA clinical trial (submitted as an abstract by Behall et al 2003 in the NBFC petition) has been accepted for publication (Behall et al 2004b) and the results are provided for inclusion in this amendment. Two additional human clinical studies that included barley (Li et al 2003) or barley β-glucan (Keogh et al 2003) in intervention diets have also been recently published and are presented here. Finally, two studies previously submitted as abstracts were recently published and are submitted with additional discussion (Hallfrisch et al 2003; Yang et al 2003).

AMENDED SUMMARY OF SCIENTIFIC EVIDENCE

Definitions

Clarification of three of the barley products discussed in the NBFC petition was requested: barley flour, barley meal, and β-glucan enriched flour fractions. Definitions and clarification for all of the barley products submitted for eligibility for the health claim are given here along with specification of the β-glucan and total fiber content (Table 1). These definitions are based on the definitions found in Appendix 1 of the NBFC petition (AACC Method 55-99: Barley Glossary, AACC 2000) or on processes known in the industry and documented in the scientific literature.

The production of barley flour, regardless of type of milling equipment, incorporates a size separation process with the flour having the smaller particle size. The size separation typically results in all or a portion of the bran and germ being separated from the starchy endosperm. The following definition is based on the AACC Barley Glossary (2000):

> "**Barley Flour** is produced by any roller/separation dry milling of barley such that it consists principally of endosperm tissue and provides at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 8%."

For clarification, the term flour is frequently incorrectly used to refer to any ground grain.

The Dictionary of Milling Terms and Equipment (Wingfield 1989) has two similar definitions for a meal product:

1. The edible seed or other edible part of any grain, coarsely ground. 2. An ingredient which has been ground or otherwise reduced in size.

Based on these definitions and knowledge of barley processing, the following definition is incorporated into the NBFC petition:

> "**Barley meal** is an unsifted, ground product made from dehulled, pearled or hulless barley grain that may contain all portions of the grain including bran, germ and endosperm and provides at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 8%."

Depending on the type of grinding equipment, barley meal may be coarse or fine. Finely ground barley meal made from whole grain barley (dehulled, hulless, lightly pearled) may be called whole grain barley flour in consumer markets even though it does not undergo a separation process.

β-glucan enriched flour fractions can be made during the production of flour. The β-glucan in the barley endosperm cell walls increase the softness of the endosperm so that during the milling process the cell walls break up into larger fragments than the starch and protein. These fragments can be efficiently separated from the flour and bran during the sieving process of roller milling or by air classification typically used in pin milling. Depending on the type of mill, they may be coarse or fine, but are generally not as fine as the resulting flour. Based on this information the following definition is incorporated into the NBFC petition:

> "**β-glucan enriched barley fractions** are the portion of ground barley separated from flour by milling that contains endosperm cell walls and may contain a portion of the bran and provide at least 5.5% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 15%".

The remaining definitions are based on the AACC Barley Glossary (2000):

"**Barley Bran** is a product of milling *dehulled* or *hulless barley*, that contains the outer covering such as pericarp, testa (seed coat), aleurone and subaleurone layers and provides at least 5.5% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 15%".

"**Barley Flakes** are made from d*ehulled, pearl* or *hulless barley* that may be enzyme deactivated and/or tempered followed by the process of being rolled, dried and cooled and provide at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 8%".

"**Barley Grits** are d*ehulled, pearl* or *hulless barley* that has been cut into small pieces and provide at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 8%".

"**Dehulled Barley** is barley from which the hulls have been removed by a physical process and contains at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 10%".

"**Hulless Barley** is barley having the homozygous recessive gene *nud*, which prevents the hulls from adhering to the seed and contains at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 10%".

"**Pearl Barley** is produced by an abrasive scouring process in which the hulls and portions of the pericarp, testa, germ and outer endosperm are removed from barley and contains at least 4% (dwb) of β-glucan soluble fiber and a total dietary fiber content of at least 8%".

Table 1. Recommended Minimum β-glucan and Total Dietary Fiber Content of Typical Milled Barley Products to be Eligible for a Health Claim.

Barley Product	**β-glucan Soluble Fiber Content (% dwb)**	**Total Dietary Fiber Content (% dwb)**
Barley Flour	4.0	8.0
Barley Grits	4.0	8.0
Barley Flakes	4.0	8.0
Pearl Barley	4.0	8.0
Barley Bran	5.5	15.0
Dehulled Barley	4.0	10.0
Hulless Barley	4.0	10.0
Barley Meal	4.0	8.0
β-glucan Enriched Barley Fractions	5.5	15.0

Barley and Oat β-glucan Solubility

The functional characteristics of barley β-glucan were reviewed in the NBFC petition, Section III.C. Barley β-glucan Chemistry – 5. Solubility and Viscosity (NBFC 2003). Our conclusion was even though β-glucan in barley grain had lower solubility than β-glucan in oat groats when measured with aqueous extraction at 38°C for 2 hours (Aman and Graham 1987), this did not appear to be related to *in vivo* solubility. In addition, since the animal trials directly comparing barley and oats reported no significant difference between the two with respect to cholesterol reduction, we concluded that they were functionally equivalent.

Additional Background

21CFR § 101.81(c)(2)(ii)(A) lists β-glucan soluble fiber from whole oat sources as an eligible source of soluble fiber for a health claim. It also specifies the Association of Official Analytical

Chemists International (AOAC 2000) Method 992.28 to determine the β-glucan soluble fiber content of the food. It appears this decision was based on a statement in the original oat petition (Quaker Oats 1995) that the soluble fiber content of whole oats is predominantly (approximately 87 percent or more) β-glucan. This value was based on analyses reported by Marlett (1992) for old-fashioned oatmeal that had been cooked. Indeed, Marlett reported that the cooked oatmeal had 0.7% β-glucan and 0.7% soluble fiber. The soluble fiber contained 0.6% β-glucan, thus, the β-glucan solubility was 86% and coincidentally the β-glucan represented 86% of the soluble fiber. So, in this case, total β-glucan, as measured by AOAC Method 992.28 provided a good estimate of β-glucan soluble fiber content. However, in the same paper, oat bran was reported to contain 4.9% β-glucan in the soluble fiber which totaled 6.5%. In this case, β-glucan represents 75% of the soluble fiber. Since total β-glucan in the oat bran was 6.9%, β-glucan solubility was only 71%. In this case, the soluble fiber and β-glucan contents are close, but neither provide a good estimate of the β-glucan soluble fiber.

Since β-glucan soluble fiber is eligible for the health claim, it becomes necessary to establish the β-glucan soluble fiber content of barley. Unfortunately, this is not a determination ordinarily made. In fact, to our knowledge, none of the clinical trials using foods containing β-glucan have made this determination. Typically either soluble fiber is analyzed and/or total β-glucan. The real problem is that there is not a single definition for soluble β-glucan nor a single standard method. Soluble β-glucan has been reported in many studies, but the methods vary and do not necessarily reflect the β-glucan content of the soluble fiber (as measured in either an enzymatic-gravimetric or enzymatic-chemical fiber analysis) or its functionality.

Comparison of Barley and Oat Products

As mentioned in the NBFC petition, the test frequently used to report β-glucan solubility begins with aqueous extraction at 38°C (Aman and Graham 1987) followed by β-glucan analysis of either extract or insoluble materials using the standard method for total β-glucan AOAC 992.28. This method was slightly modified to examine the β-glucan solubility characteristics of the barley raw materials used by Dr. Kay Behall in recent clinical trials (Behall et al 2004a, b). Samples of the barley used in these trials were obtained from the USDA Beltsville Human Nutrition Research Center, and sent to B.E. Knuckles, a Research Chemist and expert in β-glucan chemistry at the USDA Western Regional Research Center, Albany, CA. Total β-glucan and solubility were measured for pearl barley and flakes from both clinical trials and compared with a sample of quick oatmeal that was purchased in Dec. 2000 and quick oatmeal and old-fashioned oatmeal purchased in Dec. 2004. The methods and results are submitted via a letter from B.E. Knuckles (Appendix A) and are summarized in Table 2 of this amendment. Solubility was determined using aqueous extraction at 40°C for 2 hours (similar to Aman and Graham 1987) and separately at 100°C for 1 hour (to eliminate the effect of β-glucanase).

The total β-glucan content of the pearl barley and flakes (Table 2) was almost identical to the results reported in Appendix 3 of the NBFC petition (NBFC 2003). The β-glucan solubility of the barley products ranged from 49.9 to 57.4% at 38°C for 2 hours and 50.0 to 59.9% at 100°C for 1 hour. In comparison, the oatmeal had 41.5 to 47.6% soluble β-glucan at 38°C and 38.1 to 55.9% at 100°C. These values are comparable to those reported by Carr et al (1990) using a similar method. Using this methodology, the barley and oat products are similar for β-glucan solubility. This is in contrast to the difference frequently reported for barley grain and oat groats.

Table 2. β-glucan Solubility[1] of Barley Products from the USDA 2001 and 2002 Clinical Trials[2] and Standard Oat Samples.[3]

Sample	Total β-glucan (%)	Soluble β-glucan (% of total) 40°C	Soluble β-glucan (% of total) 100°C
Oatmeal - Quick purchased in 2004	4.34	47.6	55.9
Oatmeal - Quick purchased in Dec. 2000	4.22	44.4	38.1
Oatmeal - Old Fashioned purchased in 2004	4.64	41.5	43.4
Barley pearled, 2001	4.98	49.9	59.6
Barley flakes, 2001	4.94	51.0	50.0
Barley pearled, 2002	5.14	57.4	59.9
Barley flaked, 2002	5.35	50.9	50.1

[1]Aqueous extraction at either 40°C for 2 hrs or 100°C for 1 hr without pretreatment.
[2]Barley from 2001 was used in Behall et al 2004a. Barley from 2002 was used in Behall et al 2004b.
[3]Data taken from a letter from B.E. Knuckles submitted in Appendix A.

Solubility Methods and β-glucan

After reviewing the solubility methods and available data some general points and conclusions can be made about methodology and the relationship between total and soluble β-glucan in barley and oats.

1. There are four general procedures that have been used to measure β-glucan solubility.
    a. Aqueous extract at 38°C for 2 hours – temperature, time can be modified (Aman and Graham, 1987).
    b. Aqueous extract with a hot ethanol pretreatment – temperature, time vary (Anderson et al 1978; Henry 1985).

   c. Aqueous extract with hot ethanol pretreatment followed by thermostable α-amylase – temperature 80-100°C, times vary (Beer et al 1997).
   d. Soluble dietary fiber analysis with buffered extractions and enzymes, β-glucan or glucose measured in the soluble portion of fiber (Englyst and Cummings 1985b; Gaosong and Vasanthan 2000; Shinnick et al 1988).

2. Aqueous extraction at 38°C is confounded by β-glucanase activity that can be present. Limited digestion of the β-glucan molecule increases its solubility. Oat varieties have higher soluble β-glucan than barley varieties under these conditions, but processed oatmeal and oat bran do not as β-glucanase is destroyed by processing (Aman and Graham 1987; Aman et al 1989; Knuckles et al 1992; Lee et al 1997).
3. β-glucanase can be inactivated by pretreatment with hot ethanol, by heat processing of materials, by low or high pH during extraction, or by high temperature during extraction (Anderson et al 1978; Beer et al 1997; Henry 1985; Hogberg 2003).
4. β-glucan solubility of enzyme inactivated barley and oats increases with increasing temperature and the addition of amylase and/or protease enzymes (Rooney Duke 1996; Temelli 1997; Wood et al 1991).
5. Soluble β-glucan of barley and oats measured as the β-glucan content of extracted soluble fiber (using any of the enzymatic dietary fiber methods) represents from 68 to 96% of total β-glucan. Oatmeal has the highest β-glucan solubility reported (80-96%) with oat bran (71-77%), oat groats (68-75%) and barley products (69-83%) being similar (Englyst and Cummings 1985a, 1985b, 1988; Gaosong and Vasanthan 2000; Graham et al 1988; Manthey et al 1999; Marlett 1992; Shinnick et al 1988).

6. Limited research has examined the relationship between *in vitro* and *in vivo* β-glucan solubility (Englyst and Cummings 1985a; Robertson et al 1997a, 1997b; Johansen et al 1997). In general, the amount of β-glucan soluble in ileal effluent following consumption of either a barley or oat product is similar or slightly lower than the amount of soluble β-glucan as measured in extracted soluble fiber from the product.
7. Sundberg et al (1996) made a direct comparison between barley and oat β-glucan solubility in human ileal effluent finding that it decreased from 46% (in prepared barley and oat breads) to 34% for barley and 39% for oat.
8. β-glucan solubility measured in extracted soluble fiber was related to lipid response in rats by Shinnick et al (1988). All of the oat products, having from 51% to 88% soluble β-glucan, were identical in lowering cholesterol in rats compared to a cellulose control.

Conclusions

Total β-glucan content has successfully been used as a marker for β-glucan soluble fiber in all oat products even though the data shows less than 70% solubility for some oat products. Under most of the methods in which β-glucan solubility has been measured, oats and barley are comparable. Thus, at this time, the data supports using similar guidelines for barley. While solubility of β-glucan continues to be of interest to researchers, the data has not established a clear relationship between an *in vitro* level of solubility and the cholesterol-lowering abilities of barley or oats. But, the comparable β-glucan solubility of oats and barley does support the conclusion that these two grain sources of β-glucan are bioequivalent.

Human Clinical Trials

Supplemental Data

The level of β-glucan soluble fiber (measured as soluble β-glucan or total β-glucan) becomes especially important in human clinical trials in which the level of a perceived functional component is correlated to an endpoint such as cholesterol. The FDA requested clarification as to the level of β-glucan soluble fiber in the barley diets reported in the clinical trials of Newman et al (1989), McIntosh et al (1991) and Behall et al (2004a).

In the Newman et al (1989) trial, a diet containing barley products supplied 13g more total fiber than a diet containing an equivalent amount of oat products. The authors were unable to provide additional data documenting actual β-glucan content of the diets.

McIntosh et al (1991) fed barley and wheat foods in an 8 week crossover trial in which barley diet supplied 8g of β-glucan and the wheat diet provided 1.5 g β-glucan. Thus, there was a 6.5 g/day difference in β-glucan between the two diets. While the data from this study was reported correctly in the NBFC petition, this difference was previously overlooked. Thus, the significant reduction in total cholesterol observed while participants consumed the barley diet may be associated with consumption of 6.5g β-glucan rather than 8 g of β-glucan.

The two clinical studies reported by Behall et al (2004a, 2004b) in the NBFC petition were originally submitted as a manuscript pending publication and an abstract containing preliminary data. Since that time, the first study has been published and is submitted with this amendment

(Behall et al 2004a) and the second study data analysis has been completed and the manuscript accepted for publication (Behall et al 2004b).

In the first study, Behall et al (2004a) reported soluble fiber content of the barley was 5%. The three intervention diets contained either low levels of soluble fiber, or 3 or 6g of soluble fiber from barley. The β-glucan content was not reported in this publication. However, a report submitted to the National Barley Foods Council and included as Appendix 3 of the NBFC petition reported that the barley donated for this study contained 4.9-5% β-glucan, almost identical to the soluble fiber content. Thus, the levels of soluble fiber in the experimental diets reflect the β-glucan content. Dr. Behall further explains in a letter submitted July 19, 2004 (Appendix B) that the terms "added soluble fiber" and "added β-glucan" were used interchangeably when discussing the amount of β-glucan in the diets. Dr. Behall also provided new data from analyses of the β-glucan content of the barley containing diets which were recently completed. The analyzed levels of β-glucan in the barley diets are slightly higher than the reported calculated levels, 3.3 g vs. 3.0 g in the mid barley soluble fiber diet and 6.7g vs. 6.0 g in the high barley soluble fiber diet.

The second study had an identical design as the first study by this group, i.e., a 2 week Step 1 diet adaptation period followed by a 15 week crossover study of 3 experimental diets, low, mid and high soluble fiber from barley. Test diets included foods made with brown rice, whole wheat flakes and flour in the low soluble fiber diet (control) and with pearl barley, barley flakes and flour in the mid and high soluble fiber diets. The barley products were made with a new lot of barley selected with a similar β-glucan content as the first study. The barley mid diet contained 5.6g/day soluble fiber and 3g/day β-glucan (calculated). The barley high diet contained 8.8g/day of soluble fiber and 6g/day of β-glucan (calculated). Again, Dr. Behall reports (Appendix B) that the calculated β-glucan content of the diets is slightly lower than the analyzed content. In this study the subjects included mildly hypercholesterolemic men (n=7), pre-menopausal women (n=9) and post-menopausal women (n=9). The average baseline cholesterol was slightly lower than in the first study (224 vs. 235 mg/dl) in which the subjects were all men.

As with the first study, the addition of barley products to the control diet had a significant effect upon total, LDL-, and HDL-cholesterol. When subjects consumed either the mid or high barley β-glucan soluble fiber diets, they had significantly lower total (-4.9% and –5.8%) and LDL-cholesterol (-6.5% and –8.4%) than when consuming the control diet. A significant difference was not detected between the mid and high barley β-glucan soluble fiber diets. This data (Table 3) is submitted as changes to Table 3 of the NBFC petition.

These significant changes are in contrast to the first study in which only the high barley soluble fiber diet lowered cholesterol significantly compared to the control diet. In both studies, the control diet reduced cholesterol compared to the Step 1 diet period. In this study that reduction was only 3.7% while in the first study it was 9.3%. It was suggested that the high consumption of whole grains in the control diet compared to the Step 1 diet in the first study may have played a role in this significant decrease. In fact, subjects consumed approximately 1 serving/day of whole grains while on the Step 1 diet and this increased to 3-4 servings/day on all of the test diets. This did not appear to have as great an impact on subjects in the second study.

Table 3. Changes to Table 3 of the NBFC Petition: Summary of Human Clinical Trials Utilizing Barley Foods Containing β-glucan Soluble Fiber as a Dietary Intervention to Reduce Risk of CHD[a].

Study	Subjects/ Initial TC	Diet Intervention	Methods	Results[b]
Behall et al 2004a	18 male 235 mg/dl	Barley Pearl, Flakes, Sieved Flour Average – 5.0% BG Brown rice / whole wheat (BR/WW)	2 weeks NCEP Step 1 diet; then 15 week crossover with Latin Square design of three 5 week diet periods; 3 treatments: 0, 3g, 6g barley soluble fiber; all with NCEP Step 1 diet *Barley BG g/day:* Low = < 0.5 (BR/WW) Mid = 3.3g (BR/WW + barley foods) High = 6.7g (barley foods) Dietary nutrients equivalent.	Barley Mid vs. Low diet – TC: - 1.0 mg/dl (- 1.0% ns) LDL: - 0.4 mg/dl (- 0.3% ns) HDL: - 1.8 mg/dl (- 4.3% ns) Barley High vs. Low diet – TC: - 17.9 mg/dl (- 8.8%) LDL: - 14.3 mg/dl (- 11.0%) HDL: - 0.5 mg/dl (-1.5% ns)
Behall et al 2004b	7 male 216 mg/dl 9 female/ pre-M 218 mg/dl 9 female/ post-M 237 mg/dl	Barley Pearl, Flakes, Sieved Flour Average – 5.0% BG Brown rice / whole wheat (BR/WW)	2 weeks NCEP Step 1 diet; then 15 week crossover with Latin Square design of three 5 week diet periods; 3 treatments: 0, 3g, 6g barley soluble fiber ; all with NCEP Step 1 diet *Barley BG g/day:* Low = < 0.5 (BR/WW) Mid = 3.3g (BR/WW + barley foods) High = 6.6g (barley foods) Dietary nutrients equivalent.	Barley Mid vs. Low diet – All TC: - 10.3 mg/dl (- 4.9%) LDL: - 9.7 mg/dl (-6.5%) HDL: no change Barley High vs. Low diet – All TC: - 12.3 mg/dl (- 5.8%) LDL: - 12.4 mg/dl (- 8.4%) HDL: no change

[a] Abbreviations: CHD = coronary heart disease; TC = total cholesterol; LDL = low density lipoprotein (cholesterol); HDL = high density lipoprotein; M=menopause; TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber; NCEP = National Cholesterol Education Program; BG = β-glucan; dwb = dry wt basis ; %E = % energy; Sat. Fat % E = % energy from saturated fat.
[b] All changes reported are significant ($p < 0.05$) unless followed by ns (nonsignificant);

In the NBFC petition, we concluded that the cholesterol reduction achieved with 6g of soluble fiber (β-glucan) from barley (Behall et al 2004a) was consistent with the dose response curve for oat β-glucan that was plotted using the data from the Davidson et al (1991) study. The regression equation based on Davidson et al (1991) data, predicted 6g of oat β-glucan would lower total cholesterol 8.6%. The 6.7g of barley soluble fiber (β-glucan) lowered cholesterol 8.8% compared to the control. Figure 1 is a plot of the barley data from Behall et al (2004a, 2004b) and McIntosh et al (1991) with the oat data from Davidson et al 1991. The cholesterol reduction reported for the 3.3g and 6.6g level of β-glucan in the second Behall et al (2004b)

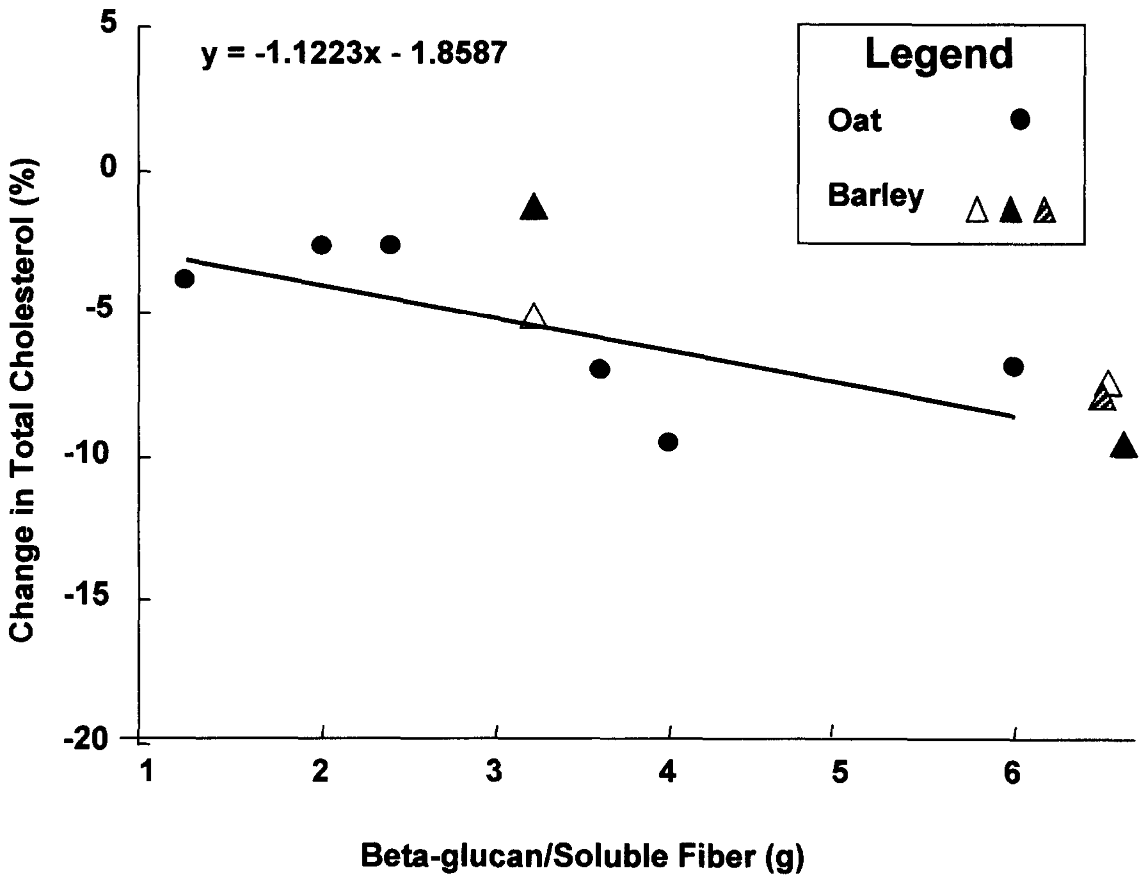

Figure 1. Change in total cholesterol in subjects consuming 6 levels of oat β-glucan soluble fiber (Davidson et al 1991) and 2 levels of barley β-glucan soluble fiber (Behall et al 2004a △ , 2004b ▲ ; McIntosh et al 1991 ▲).

study are also consistent with the oat data. Additionally, the data from McIntosh et al (1991), with a 6.5 g β-glucan difference between the wheat diet and the barley diet, are consistent with the oat data [we could not include the Newman et al (1989) since we did not have reliable β-glucan data]. This supports our conclusion that: 1) barley and oat β-glucan are very similar; 2) 3g of barley β-glucan will reduce LDL-cholesterol on the average about 5% when consumed with a low fat, low cholesterol diet, and 3) the dose response for barley β-glucan appears to be similar to the dose response for oat β-glucan.

In the NBFC petition, reference was made to a study by Hallfrisch et al (2002) in which blood pressure reductions were associated with whole grain diets containing barley or whole wheat and brown rice. This data was collected from the same clinical trial reported by Behall et al (2004a) and was initially presented as an abstract. This work has been recently published (Hallfrisch et al 2003) and is submitted for FDA review.

New Human Studies

Two additional clinical trials that included barley as a dietary intervention have been published since the NBFC petition was submitted in September 2003. The data is summarized in Table 4.

Keogh et al (2003) incorporated an extracted barley β-glucan supplement (Glucagel) providing 9.9g β-glucan/day into snacks and meals that were consumed by 18 mildly hyperlipidemic men for 4 weeks. At the end of this period these individuals displayed no difference in total or LDL cholesterol in comparison to a control diet. The authors suggested that the lack of efficacy of this product might be due to the decreased molecular weight and viscosity of the product

Table 4. Additions to Table 3 of the NBFC Petition: Summary of Human Clinical Trials Utilizing Barley Foods Containing β-glucan Soluble Fiber as a Dietary Intervention to Reduce Risk of CHD[a].

Study	Subjects/ Initial TC	Diet Intervention	Methods	Results[b]
Keogh et al 2003	18 male 228 mg/dl	Glucagel – Barley extract – 75% β-glucan Control – glucose	12 week crossover; 4 week washout between two 4 week diet periods; 2 treatments: 0 and 9.9g barley β-glucan both with a controlled Westernized diet as background (fat=38%E)	Barley vs. Control – TC: - 1.3%, ns LDL: - 3.8%, ns
Li et al 2003	10 female pre-M 140 mg/dl	Barley- whole grain Control - rice	12 week crossover; 4 week washout between two 4 week diet periods; 2 treatments: 0 or 89g barley/day both with a controlled Japanese standard diet as background (fat=25% E) *SDF g/day:* Barley = 8.9g/day Control = 3.9/day	Barley vs. Control – TC: - 20.0 mg/dl (- 14.5%) LDL: - 11.1 mg/dl (- 21.0%) HDL: - 2.6 mg/dl (- 4.1%, ns) Body weight and BMI did not change

[a] Abbreviations: CHD = coronary heart disease; TC = total cholesterol; LDL = low density lipoprotein (cholesterol); HDL = high density lipoprotein; M=menopause; SDF = soluble dietary fiber; %E = % energy.
[b] All changes reported are significant ($p < 0.05$) unless followed by ns (nonsignificant).

resulting from β-glucanase activity present during extraction at 50-60°C. This study provides an excellent example of β-glucan that has probably been depolymerized to the point of being nonfunctional.

Li et al (2003) replaced 30% of the carbohydrates of a standard Japanese diet with a whole grain barley in a 12 week crossover study that had a 4 week washout period. The barley replaced rice in the test diet and provided, on the average, 5g of soluble dietary fiber from barley/ day. The authors reported that the 10 female participants averaged a significant reduction in total and LDL cholesterol of 14.5 and 21%, respectively. These results are somewhat surprising since the participants were very atypical for this type of study, young women (avg. age 20) with an initial total cholesterol of 140 mg/dl. The authors also reported a significant increase in stool volume but no change in fasting plasma glucose or glucose tolerance. While the amount of β-glucan consumption was not reported, the barley provided 5g of soluble fiber/day. This would represent anywhere from about 3-4.5g of β-glucan soluble fiber/day.

Animal Studies

<u>Supplemental Data</u>

Yang and Moon (2002) presented data in an abstract concerning significant reductions in serum cholesterol of rats fed milled waxy barley and a barley β-glucan isolate. More complete data is available now that the research has been published (Yang et al 2003). In addition to cholesterol reduction (Table 5), the barley and barley β-glucan increased bile acid excretion and hepatic cholesterol 7α-hydroxylase activity.

Table 5. Changes to Table 7 of the NBFC Petition: Lipid Response in Animals Consuming Barley Products Compared to a Control.[a]

Reference	Species	N/trt[a]	No. Days	Treatments	Diet % Fiber or BG	Response %[b] TC	LDL	HDL	% Chol[c]	Control
Yang et al 2003 (replace Yang and Moon 2002)	Rat	7	14	Barley BG extract	2.5% BG	-13.5	ns	ns	0.5%	No fiber
		7	14	Waxy Barley	2.5% BG	-18.9	-24.3	ns		

[a]Abbreviations: N/trt=number/treatment; BG=β-glucan; TC=total cholesterol; LDL=low density lipoprotein cholesterol; HDL=high density lipoprotein cholesterol; ns=nonsignificant.

[b] All changes are statistically significant at P<0.05 unless noted or nonsignificant.

[c]% Chol.=% cholesterol or cholesterol source in diet.

OVERALL CONCLUSIONS

At this time, the data on β-glucan solubility indicates that barley and oat β-glucan are similar. Presently, the test for total β-glucan is an adequate marker for the functionality of oat and barley foods.

The new barley clinical data adds to the evidence that 3g of barley β-glucan lowers total cholesterol about 5% confirming the bioequivalence of barley and oat β-glucan soluble fiber. The data from McIntosh et al (1991) and Behall et al (2004a, 2004b) indicate that barley has a very similar dose response as oats in lowering elevated cholesterol levels. The NBFC requests that the FDA append the NBFC petition to include all of this data and continue its review of the petition.

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[1]*Copies of article were included in the original NBFC petition.*