CHAPTER 6

Introduction of Oats in the Diet of Individuals with Celiac Disease: A Systematic Review

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Abstract

Celiac disease is an immune-mediated disease, triggered in genetically susceptible individuals by ingested gluten from wheat, rye, barley, and other closely related cereal grains. The only treatment for celiac disease is a strict gluten-free diet for life. This paper presents a systematic review of the scientific literature on the safety of pure oats for individuals with celiac disease, which historically has been subject to debate.

Limitations identified within the scientific database include: limited data on long-term consumption, limited numbers of participants in challenge studies, and limited reporting about the reasons for withdrawals from study protocols. Furthermore, some evidence suggests that a small number of individuals with celiac disease may be intolerant to pure oats and some evidence from *in vitro* studies suggests that an immunological response to oat avenins can occur in the absence of clinical manifestations of celiac disease as well as suggesting that oat cultivars vary in toxicity.

Based on the majority of the evidence provided in the scientific database, and despite the limitations, Health Canada and the Canadian Celiac Association (CCA) concluded that the majority of people with celiac disease can tolerate moderate amounts of pure oats. The incorporation of oats into a gluten-free diet provides high fiber and vitamin B content, increased palatability, and beneficial effects on cardiovascular health. However, it is recommended that individuals with celiac disease should have both initial and long-term assessments by a health professional when introducing pure oats into a gluten-free diet.

I. INTRODUCTION

Celiac disease is an autoimmune disorder in which ingestion of gluten causes damage to the small-intestinal mucosa in genetically susceptible individuals (Fasano *et al*., 2008; Green and Cellier, 2007; Losowsky, 2008; Presutti *et al*., 2007). Celiac disease is also known as celiac sprue or gluten-sensitive enteropathy. The clinical presentation of celiac disease is highly variable. In addition to the intestinal symptoms, celiac disease is associated with various extraintestinal complications. It is, therefore, considered as a multisystem disorder (Briani *et al*., 2008). Patients with celiac disease also have an increased risk of developing other autoimmune disorders, such as type I diabetes mellitus (Barton and Murray, 2008; Gianfrani *et al*., 2008). In children, celiac disease can be associated with growth failure and delayed puberty (Tully, 2008). Furthermore, the symptoms and associated conditions of celiac disease can vary greatly in number and severity resulting in frequent delays in diagnosis, and/or misdiagnosis. Common examples of misdiagnoses include: irritable bowel syndrome, chronic fatigue syndrome, and fibromyalgia (Catassi and Fasano, 2008; Cranney *et al*., 2007; Rashid *et al*., 2005).

Dermatitis herpetiformis[1] is a condition of the skin that is also triggered by the ingestion of gluten in genetically susceptible individuals and is considered a dermatological form of celiac disease (Abenavoli *et al*., 2006; Alaedini and Green, 2005; Briani *et al*., 2008; Losowsky, 2008).

Gluten is a generic name given to storage proteins in wheat, barley, rye, and other closely related cereal grains. It is the gluten in wheat flour that binds and gives structure to bread, baked goods, and other foods, making it widely used in the production of many processed and packaged foods. For individuals with celiac disease, these proteins trigger an inflammatory injury in the absorptive surface of the small intestine resulting in malabsorption of protein, fat, carbohydrate, fat-soluble vitamins, folate, and minerals, especially iron and calcium (Koning, 2008; Tye-Din and Anderson, 2008; Wieser and Koehler, 2008).

Celiac disease is a lifelong condition. If celiac disease is not diagnosed early and treated with a strict gluten-free diet, it can be associated with serious complications, including: osteoporosis, lymphoma, and infertility in both men and women (Bianchi and Bardella, 2008; Fasano *et al*., 2008; Freeman, 2008; Green and Cellier, 2007; Malandrino *et al*., 2008; Mangione, 2008; Pastore *et al*., 2008; Pellicano *et al*., 2007; Pope and Sheiner, 2009).

A small-intestinal biopsy is necessary to confirm the diagnosis of celiac disease (Dickson *et al*., 2006; Haines *et al*., 2008). However, the advent of

[1] Throughout this publication, when not otherwise specified, dermatitis herpetiformis is included under the general term celiac disease.

new diagnostic serological tests, particularly for anti-endomysial and anti-tissue transglutaminase antibodies (Alaedini and Green, 2008; Fasano *et al.*, 2008; Gianfrani *et al.*, 2008; Hill and Holmes, 2008; Hopper *et al.*, 2008), has now estimated the worldwide prevalence of celiac disease to be between 1 in 100 and 200 individuals (Catassi, 2005; Catassi *et al.*, 2007b; Fasano *et al.*, 2003; Harrison *et al.*, 2007; National Institute of Health, 2004). Certain groups of people have markedly elevated risks of developing celiac disease. First-degree relatives of individuals diagnosed with celiac disease have a 10–20% increased risk of developing celiac disease (Jolobe, 2008). A high prevalence of celiac disease is also found in individuals with Down syndrome and IgA deficiency (Presutti *et al.*, 2007).

Presently, the only treatment of celiac disease is a strict lifelong exclusion of wheat, rye, barley, and other related cereal grains[2] from the diet (Akobeng and Thomas, 2008; Buchanan *et al.*, 2008; Catassi *et al.*, 2007a; Ciclitira *et al.*, 2005; Collin *et al.*, 2007; Guandalini, 2007; Hopman *et al.*, 2008; Kupper, 2005; Zarkadas and Case, 2005; Zarkadas *et al.*, 2006). The amount of gluten that can be tolerated varies amongst people with celiac disease. Some patients tolerate an average of 34–36 mg gluten/day without any clinical manifestations of celiac disease, while others who consume approximately 10 mg gluten/day developed mucosal abnormalities (Akobeng and Thomas, 2008; Catassi *et al.*, 2007a). Although there is no evidence to suggest a single definitive threshold for a tolerable gluten intake, there is evidence that a daily gluten intake of <10 mg is unlikely to cause significant histological abnormalities (Akobeng and Thomas, 2008; Buchanan *et al.*, 2008; Catassi *et al.*, 2007a; Collin *et al.*, 2007; FDA, 2006; Hopman *et al.*, 2008).

Whether or not individuals with celiac disease can safely consume oats has been an issue of interest and investigation in recent scientific literature. Using a systematic approach, we conducted a comprehensive review of the available scientific literature from 1995 to 2008 that was relevant to the safety of oats in the diet of individuals with celiac disease, including those with dermatitis herpetiformis. It is agreed in the literature that the first large controlled trial on the safety of oats in celiac disease was conducted in 1995 (Janatuinen *et al.*, 1995), marking the starting point of investigations in the field. Earlier publications were uncontrolled and their limitations were previously reviewed (Garsed and Scott, 2007). Hence, 1995 was selected as starting point.

[2] Cereal grains that are known to trigger celiac disease/dermatitis herpetiformis reactions include the following: wheat (including durum wheat or ''durum,'' spelt wheat or ''spelt'', kamut), barley, rye, triticale, atta, bulgur, einkorn, emmer, and farro. Also of concern are: wheat bran, wheat farina, wheat flour, wheat germ, wheat-based semolina, wheat starch (in some countries ''gluten-free'' can be made with wheat starch), and graham flour. Commercial oats that may be contaminated with the foregoing grains are also of concern (Case, 2008; Zarkadas and Case, 2005).

II. METHODS

A computer-assisted search of available English literature databases was conducted covering January 1995 to November 2008, using Ovid, Medline, and Pub Med. The search was performed in Food Science and Technology Abstracts, Medline, Embase, CINAHL, Global Health, and Current Contents. The following search terms were used: ''Celiac disease'' and ''oats,'' oat challenge or rechallenge, oats and the immune system, oats clinical trials, and oats dietary consumption. The following keywords were used with adaptations for each database platform: gluten tolerance or gluten intolerance or coeliac or celiac, oat or oats or avena or avenin. The reference list of publications generated by the database search terms were reviewed and compared for further identification of potentially relevant papers. Search results were managed using ''Reference Manager® Soft Ware.'' Other publications, which were not directly relevant to celiac disease and the consumption of oats such as oats atopic sensitization and allergy, were excluded.

Two independent investigators evaluated the publications and the results were tabulated (Tables 6.1 and 6.2). The safety assessment included all publications identified in the database search as: (A) pivotal *in vivo* clinical studies conducted in patients, adults, or children, with celiac disease or dermatitis herpetiformis, who were challenged with oats and underwent a small bowel or skin biopsy, with or without additional diagnostic serological tests (anti-endomysial; anti-tissue transglutaminase) in order to assess the biological response to the introduction of oats to an otherwise gluten-free diet (Table 6.1); (B) nonpivotal studies conducted in patients, adults, or children, with celiac disease or dermatitis herpetiformis using *in vitro* methods or serology without a biopsy to test the biological response to oats (Table 6.2); (C) other relevant studies not fulfilling either of the above selection criteria (not tabulated).

The following documents were also considered in the safety/benefit evaluation: Threshold Working Group on ''Approaches to Establish Thresholds for Major Food Allergens and for Gluten'' (Food And Drug Administration, 2006); Gluten-free Labelling of Foods (Food and Drug Administration, 2007); Health Claims from Certain Foods and Risk of Coronary Heart Disease (Food And Drug Administration, 2008); publications assessing the benefits of oats for celiac individuals (Peräaho *et al.*, 2004a,b; Sadiq Butt *et al.*, 2008); and for the general population (Andon and Anderson, 2008; Makelainen *et al.*, 2007; Maki *et al.*, 2007a,b; Reyna-Villasmil *et al.*, 2007).

The available information is summarized in Appendix 1 and in Tables 6.1 and 6.2, and is organized accordingly.

TABLE 6.1 Pivotal *in vivo* clinical studies of the effects of oats in patients with celiac disease and dermatitis herpetiformis

Reference authors/ year	Study design	Number of subjects tested	Study duration	Amount of oats added to a GFD	Purity verified	Withdrawal	Clinical and lab tests	Intestinal biopsy findings after challenge	Summary comments
Adults									
Janatuinen *et al.* (1995)	Randomized controlled	92 CD subjects			Not reported	11 subjects dropped out	No serology (refer to Janatuinen *et al.*, 2000)	Normal villous architecture at 6 and 12 months	Most subjects tolerated oats in the amounts of 50–70 g/day in an otherwise GFD
		52 in remission	6 months	Mean: 49.9 ± 14.7 g/day		6 from remission group (3 control (1 +, 2 −), 3 oats (2+, 1 −))		One out of 21 new Dx controls did not enter remission after 12 months	
		26 oats/26 control							
		40 new Dx of CD	12 months	Mean: 46.6 ± 13.3 g/day		5 from new Dx group (2 control (1+, 1−), 3 oats (1+, 2−)) (+) Reported symptoms of itching and/or abdominal bloating			
		19 oats/21 control				(−) Refused to continue, no reason provided			
Srinivasan *et al.* (1996)	Baseline controlled	10 CD subjects in remission	12 weeks	50 g/day	Yes	None	No change in gliadin and EM Abs	No morphological damage, no change in IEL counts or enterocyte height	Oats are not toxic to celiac patients
Hardman *et al.* (1997)	Baseline controlled	10 DH subjects in remission	12 weeks	Mean: 62.5 ± 10.8 g/day	Yes	None	Gliadin, Ret, and EM Abs	Normal histology after Oats challenge, no change in IEL and enterocyte height	Adults with DH tolerate moderate amounts of oats
							Not detected after oats challenge	No change in dermal IgA	

Reuanala *et al.* (1998)	Controlled	22 DH subjects in remission 11 oats/11 control	6 months	Median: 53.2 g/day (range 30–66 g/day)	Yes	2 subjects dropped out: both from oats group (1 due to persistent mild rash, 1 no reason provided)	8 no symptoms with oats 2 oats and 3 control developed transient rash EM Abs negative in all subjects	Unaltered villous architecture, IELs, and IH markers Skin biopsy not different between groups	Absence of oats toxicity in small bowel and suggest that the rash observed in DH subjects is not activated by eating oats
Janatuinen *et al.* (2000) (*continuation of Janatuinen *et al.*, 1995)	Randomized controlled	*Same study population: Serum from 92 CD subjects 52 in remission 26 oats/26 control 40 new Dx of CD 19 oats/21 control	6 months 12 months	Mean: 49.9 ± 14.7 g/day Mean: 46.6 ± 13.3 g/day	Yes	*N/A	Gliadin and Ret Abs not significantly different between oats and controls	No increase in IELs among subjects in remission with and without oats IELs decreased among new Dx subjects with and without oats	Results strengthen the view that most subjects tolerated oats in the amounts of 50–70 g/day in an otherwise GFD
Janatuinen *et al.* (2002) (*continuation of Janatuinen *et al.*, 1995)	Follow-up; controlled	*Follow-up with CD subjects from previous study 35 oats/28 control	5 years after original trial subjects reexamined	Median: 34 g/day (range 10–70 g/day)	Yes at 6–12 months, but not thereafter	*12 subjects no longer eating oats after 5 years citing fears about safety	Gliadin, EM, and Ret Abs not significantly different between oats and controls	Villous architecture and inflammatory cell infiltration not significantly different between oats and control group	Supports the view that long-term (up to 5 years) consumption of oats in moderate amounts are tolerated by CD subjects Limitations: higher number of drop outs; purity of oats not tested after long term
Lundin *et al.* (2003)	Baseline controlled	19 CD subjects	12 weeks	50 g/day	Yes	1 subject dropped out after 2 weeks due to GI symptoms	Normal gliadin, EM, and tTG Abs	18 patients with normal histology 1 villous atrophy 5 with IFN-γ mRNA	Some concerns remain with respect to the safety of oats
	Follow-up; baseline controlled	Follow-up with 12 subjects who continued with oats regularly	1.5 years	Less than 50 g/day	No		Normal serology	Normal histology 2 with IFN-γ mRNA	Most CD patients tolerate uncontaminated oats in the diet

(*continued*)

TABLE 6.1 (*continued*)

Reference authors/ year	Study design	Number of subjects tested	Study duration	Amount of oats added to a GFD	Purity verified	Withdrawal	Clinical and lab tests	Intestinal biopsy findings after challenge	Summary comments
Størsrud *et al.* (2003a,b)	Baseline controlled	20 CD subjects in remission	2 years	Median: 93 g/ day (range 70–100 g/ day)	Yes	5 subjects dropped -out: 2 because of abdominal distension/ flatulence (histology and serology normal) 3 nonmedical	Normal gliadin and EM Abs	13 subjects no change from baseline after oats 3 partial atrophy at baseline had histological improvement and inflammation remained unchanged or improved after oats 1 slight inflammation increase	Adult patients in remission can tolerate 70–100 g/day of oats with nutritional and compliance benefits
Peräaho *et al.* (2004a)	Randomized controlled	39 CD subjects with mucosal recovery 23 oats/16 control	1 year	Median: 31.5 g/ day (range 0–70 g/day)	Not reported	3 subjects dropped out from oats group due to GI symptoms (serology normal, biopsy incomplete recovery)	EM and tTG Abs not significantly different between oats and controls	Biopsy available from 18 treated and 13 control (Others refused) No morphology differences between groups IEL higher in oats versus control	Inclusion of oats did not disturb intestinal integrity but did cause more GI symptoms Limitations: it is not clear if oats used were uncontaminated
Kemppainen *et al.* (2007) (*continuation of Janatuinen *et al.*, 2002)	Follow-up; controlled	*Follow-up with 22 CD subjects from oat group in previous study 20 control subjects with strict GFD and no history with oats	5 years	Median: 30 g/ day (range 10–70 g/ day)	Yes at 6–12 months, but not thereafter	*10 subjects no longer eating oats after 5 years citing fears about safety	No serology	Intestinal biopsy indicated no differences in IELs biomarkers	Long-term use of oats does not stimulate local immunologic response in the small bowel

Kemppainen *et al.* (2008)	Randomized controlled trial	32 CD subjects in remission who followed GFD + oats 16 kilned oats/16 unkilned oats Self controlled groups switched treatments after 6 months	1 year	Increased from 20 (range 0–89 g/day) to 93 g/day (range 35–158 g/day) at 6 months	Yes	2 drop outs; 1 due to reported GI symptoms after 1 week and 1 due to unrelated circumstances	Normal levels of EM Abs throughout the study	No marked differences in the histopathology of small-intestinal biopsies Duodenal villous architecture and mucosal inflammation improved throughout the study	The lack of reactivity of patients' sera against avenin, suggests that oats is not harmfully antigenic in CD, even in an unkilned form
Children									
Hoffenberg *et al.* (2000)	Baseline controlled	10 CD subjects with New Dx	6 months	24 g/day	Yes	None	Decrease in tTG Abs	Villous architecture and IEL count improved during study	Commercially available oats, tested for purity was tolerated by newly Dx celiac children
Högberg *et al.* (2004)	Double-blind randomized controlled	116 CD subjects with new Dx 57 oats/59 control	1 year	Median: 15 g/day (range 5–40 g/day)	Yes	22 subjects dropped out 15 from oats (5 because of GI symptoms, 1 due to poor growth, 7 no symptoms) 7 from control (2 with GI symptoms, 5 no symptoms)	Gliadin, EM, and tTG Abs not significantly different between oats and controls	All with normal mucosal architecture except 2 control subjects after 1 year No significant difference in IELs count between groups	Addition of oats to GFD is tolerated by newly Dx CD children
Hollén *et al.* (2006a) (*continuation of Högberg *et al.*, 2004)	Controlled	*Same study population 86 subjects from original study sampled Serum from 38 CD subjects on GFD + oats Control: serum from 48 CD subjects on GFD	1 year	*Subjects consuming at least 10 g/day were selected from original study population	Yes	N/A	IgA antiavenin Abs decline after 3 months, IgG Abs significantly decreased but remained high in majority of patients both groups; nitric oxide levels high in 4 urine samples	No biopsy	Oats was not producing a humoral immune reaction; however, findings do not exclude the possibility that some CD patients are susceptible to oats

(*continued*)

TABLE 6.1 (*continued*)

Reference authors/ year	Study design	Number of subjects tested	Study duration	Amount of oats added to a GFD	Purity verified	Withdrawal	Clinical and lab tests	Intestinal biopsy findings after challenge	Summary comments
Hollén *et al.* (2006b) (*continuation of Högberg *et al.*, 2004)	Controlled	*Same study-population 87 subjects who completed original study sampled Urine from 39 CD subjects on GFD + oats Control: Urine from 48 CD subjects on GFD	1 year	Median: 15 g/day (range 5–40 g/day)	Yes	N/A	No significant differences observed between groups Nitrite/nitrate values of 9 GFD + oats and 8 GFD remained high after 1 year	No biopsy	Children with CD consuming oats have similar reduction in urinary nitrite/nitrate excretion as GFD children; yet for some, nitrite/nitrate levels remain high, long-term follow-up needed
Holm *et al.* (2006)	Baseline controlled	32 CD subjects 9 new Dx on GFD + oats 23 in remission 13/23 challenged with oats and 10/23 challenged with gluten, after relapse with gluten placed on GFD + oats 22 long-term clinical follow-up	2 years 7 years	Median for new Dx subjects 43 g/day (range 19–64 g/day) Median for subjects in remission 45 g/day (range 13–81 g/day)	Yes	3 remission subjects dropped out: 2 from oats challenge due to GI symptoms (serology negative, villous morphology normal, and IELs decreased) and 1 from gluten challenge due to laborious study protocol (asymptomatic)	EM, gliadin, and tTG Abs were negative except during gluten challenge	Relapse after gluten but not oats challenge Recovery of all groups on GFD + oats as per histology and IELs biomarkers	Pure oats can be safely added to the GFD of most children with CD Consumption of oats does not result in mucosal deterioration or immune activation

Abs, Antibodies; CD, celiac disease; DH, dermatitis herpetiformis; Dx, Diagnosis; EM, endomysial antibodies; GI, gastrointestinal; GFD, gluten-free diet; IELs, intraepithelial lymphocytes; IFN, interferon; Lab, laboratory; tTG, tissue transglutaminase antibodies; Ret, reticulin; IH, immunohistochemistry.

TABLE 6.2 Nonpivotal studies testing the effects of oats in patients with celiac disease by *in vitro* methods or serology

Reference authors/year	Number of subjects tested	Method of assessment	Results	Summary comments
***In vitro*—duodenal mucosal cultures**				
Srinivasan *et al.* (1999) (*continuation of Srinivasan *et al.*, 1996)	9 CD and 11 non-CD controls *Additionally same study population: 10 CD subjects in remission who supplemented GFD with 50 g oats/day for 3 months	Lactase expression of *in vitro* duodenal mucosal cultures	Lactase was expressed in 11 control samples from subjects with normal histology Lactase was not expressed in samples from 9 subjects with active CD Lactase was expressed in samples from 10 CD subjects in remission who were challenged with oats	Results corroborate the lack of oats toxicity in adult CD subjects
Picarelli *et al.* (2001)	13 treated CD subjects	Immune responses of *in vitro* duodenal mucosal culture to gliadin and avenin	EM Abs produced in response to gliadin but not avenin by celiac mucosa. No EM Abs from controls	Oats appear to have no harmful effect on CD

(*continued*)

TABLE 6.2 (*continued*)

Reference authors/year	Number of subjects tested	Method of assessment	Results	Summary comments
Kilmartin *et al.* (2003)	17 CD and 16 non-CD controls	Immune responses of *in vitro* duodenal mucosal culture to gliadin and avenin	Gliadin caused increase in IFN-γ mRNA in all CD subject samples Increased IFN-γ protein in 4 CD subject samples Smaller increases in IL-2 cytokine mRNA detected in 6 CD subject samples and increased IL-2 cytokine protein in 2 CD subject samples No significant response with avenin No response to gliadin or avenin among controls	Immunogenic sequences in gliadin are not present in avenin. These results support that oats are safe for consumption by CD subjects
Arentz-Hansen *et al.* (2004) (*continuation of Lundin *et al.*, 2003)	9 CD subjects who had history of oats exposure (*5/9 from same study population)	Derivation of polyclonal T cell lines *In vitro* duodenal mucosal cultures were challenged with either pepsin or	Avenin-reactive T cell lines recognized avenin peptides in the context of HLA-DQ2 A substantial proportion of the avenin-reactive T cell appears to be specific to avenin	Some CD patients have avenin-reactive mucosal T cells that can cause mucosal inflammation

		trypsin digest or a chymotrypsin digest of avenin	T cell lines challenged with avenin responded more strongly to avenin than to gluten in 4 of 5 samples	
Srinivasan *et al.* (2006) (*continuation of Srinivasan *et al.*, 1996)	*Same study population: 10 CD subjects who supplemented GFD with 50 g oats/day for 3 months	Immune responses of *in vitro* duodenal mucosal culture treated with Abs against: HLA D-related, Ki-67, CD25, CD54, ICAM-1, and mast cell tryptase	None of the patients developed clinical or lab evidence of adverse effects Distribution of intestinal HLA-DR expression was not affected Number of CD25 and tryptase positive cells was not altered Distribution and intensity of ICAM-1 staining unchanged	No evidence of immune activation from oats supplementation
Kilmartin *et al.* (2006)	7 CD subjects	Immune response of gliadin-reactive mucosal T cell lines to wheat, barley, rye, and oat-related cereals	All 5 T cell lines demonstrated immunoreactivity to protein fractions from 4 related cereals Some cell lines reactivity to wheat, barley, and rye was evident only when cereal fractions were pretreated with tTG	Confirms similar T cell antigenic reactivity of 4 related cereals

(*continued*)

TABLE 6.2 (*continued*)

Reference authors/year	Number of subjects tested	Method of assessment	Results	Summary comments
		Measuring proliferation or cytokine production	Despite oats stimulation of T cell lines, it did not activate a mucosal lesion in most subjects	
***In Vitro*—other**				
Silano *et al.* (2007)	10 children with verified CD	Peripheral lymphocyte samples exposed to avenins from 4 different oat varieties: Lampton, Astra, Ava, and Nave Lymphocyte proliferation and IFN-γ release in the culture medium were measured as indexes on immune activation	All varieties of oats were immunogenic, with Lampton and Ava avenins inducing lymphocyte activation similar to gliadin Astra and Nave avenins showed less immunogenicity	More evidence is needed to show the safety of oats and varieties of low toxicity
Silano *et al.* (2007)	N/A	3 varieties of oats were tested by 2 assays based on the known ability of PT digests of celiac-active	Avenins from Italian variety Astra and the Australia variety Mortlook were more active than the	Results indicate that some varieties of oats may be potentially harmful

		proteins to agglutinate K562 cells and to disrupt lysosomes	Australian variety Lampton. Gliadin, digested in the same way, had more activity than all 3 avenins	to individuals with CD
Serology				
Guttormsen *et al.* (2008)	136 CD subjects 82 CD subjects oats (24 g/day (mean); 6 months minimum) and 54 CD subjects GFD without oats Control: 139 non-CD subjects	Serum samples were tested for levels of IgA to wheat gliadin, oats avenin, and tTG	Gliadin, avenin, and tTG did not differ among CD subjects	Findings support the notion that most adult CD patients can tolerate oats Ingestion of oats does not cause increased levels of IgA against oats in CD patients on GFD

Abs, Antibodies; CD, celiac disease; EM, endomysial antibodies; GFD, gluten-free diet; HLA-DQ2, human leukocyte antigen; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; Lab, laboratory; tTG, tissue transglutaminase antibodies.

A. Pivotal *in vivo* clinical studies on the effect of oats in patients with celiac disease and dermatitis herpetiformis (Table 6.1)

Studies were grouped by subject (adults vs. children) and organized chronologically by publication date. The following parameters were tabulated from each publication: reference (authors and year); study design (randomized controlled trials, observational changes from baseline in a single group, cohort study); number of subjects tested (newly diagnosed patients vs. those in remission on a gluten-free diet prior the oats challenge); study duration; amount of oats added to the gluten-free diet; purity of test material verified (if the oats used in the challenge were tested for gluten contamination); withdrawals; clinical and laboratory tests, biopsy, and histopathology findings after the oats challenge; and summary comments.

B. Nonpivotal studies testing the effect of oats in patients with celiac disease by *in vitro* methods or serology (Table 6.2)

Studies were grouped according to the *in vitro* method used (duodenal mucosal cultures, other, serology) and organized chronologically by publication date. The following parameters were tabulated from each publication: reference (authors and year); number of subject tested (subjects with celiac disease vs. controls); method of assessment; results; and summary comments.

III. RESULTS

A. Pivotal *in vivo* clinical studies on the effect of oats in patients with celiac disease and dermatitis herpetiformis

Table 6.1 summarizes the clinical dietary challenge studies in both adults and children that assess the effect of oats on patients with celiac disease and dermatitis herpetiformis. These studies are considered pivotal based on the use of biopsy results (intestinal/skin) as the key endpoint following the oats challenge. Within this parameter, 11 oats-challenge studies in adults and 3 in children were identified. Some of the pivotal challenge studies had clinical follow-up studies reported in separate publications. These follow-up publications are identified (*) in Table 6.1 given that the same pool of patients from the original challenge studies were evaluated using other laboratory methods to assess their biological response to oats. Further details of each study are provided in Appendix 1.

Janatuinen *et al*. (1995) were the first to evaluate the possible toxicity of oats in a large controlled study. Since then, a number of studies (Table 6.1) have assessed the safety of oats consumption by individuals diagnosed with celiac disease and dermatitis herpetiformis. Most studies were conducted on adults, with a smaller number of studies performed on children.

Of the 14 studies identified as pivotal in adults and children, five were randomized controlled trials, five were observational changes from baseline in a single group, and three were cohort studies. Furthermore, one study conducted with children utilized three study designs (randomized controlled, observational changes from baseline in a single group, and a cohort study follow-up) with different groups in the study population (refer to Table 6.1 or Appendix 1 for further details). The duration of all the studies within the database, ranged from 12 weeks to 5 years in adults and 6 months to 7 years in children (Table 6.1). In adults, there was one study with a 5-year follow-up period (Janatuinen *et al*., 2002) and an additional follow-up analysis of the local immunological response of the small-intestinal mucosa (Kemppainen *et al*., 2007). In children, one study had a 7-year follow-up period (Holm *et al*., 2006).

The amount of oats included in the gluten-free diets ranged from 30 to 93 g for adults and from 15 to 45 g for children (Table 6.1). In a small number of adult subjects, the median amount of oats tested over a 2-year period was 93 g (up to 100 g/day) (Størsrud *et al*., 2003a,b) and in one study (Holm *et al*., 2006) children ingested a median of 45 g (up to 81 g/day) oats daily for 2 years.

In this review, emphasis has been placed on the purity of the oats tested because the contamination of commercial oats by wheat, barley, and rye cereals has been reported as an intermittent problem that can potentially skew trial results (Gelinás *et al*., 2008; Hernando *et al*., 2008; Thompson, 2004). Cross contact of oats with these cereals is considered a key reason for apparent oat intolerance reported in the past and continues to be an issue of concern for commercially available oats (Dickey, 2008; Ellis and Ciclitira, 2008; Garsed and Scott, 2007). Hence, information about the purity of oats included in the gluten-free diets of study participants was identified in all the publications included in the database (Table 6.1). All of the pivotal studies reported the purity of oats tested with only two exceptions (Janatuinen *et al*., 1995; Peräaho *et al*., 2004b). However, many of the studies failed to indicate the basis upon which the oat samples were deemed to be gluten-free. It was assumed that unless otherwise specified, the oat samples that were reported as free of contamination with wheat, barley, and rye had gluten levels that were below the detection limit of the applied testing method(s). However, some studies did not specify the cut-off values used to determine whether the oat samples were considered gluten-free. Størsrud *et al.* (2003a,b) reported a 20 parts per

million (ppm) limit of detection and Holm *et al.* (2006) reported that 29 out of 30 oat samples tested contained gliadin levels below 28 ppm.

1. Safety of oats in adults

Among the studies conducted with adults (Table 6.1), there was a combined total of 170 patients with either celiac disease or dermatitis herpetiformis, either in remission or newly diagnosed, who were challenged with oats while consuming an otherwise gluten-free diet. Of the 170 adults who had an intestinal biopsy after they were challenged with oats, only one (1/170) case in one study (Lundin et al., 2003) showed histological evidence of intestinal mucosal injury associated with exposure to oats. Although some individuals in these studies reported some adverse gastrointestinal symptoms, the symptoms were considered mild and transient and there was no evidence to associate oats with the development of histopathological lesions of the small-intestinal mucosa, the definitive diagnostic criterion of celiac disease.

The majority of adults included in these studies were following a gluten-free diet and reported to be in remission as evidenced by the normal small-intestinal mucosa histology at the initiation of the trial. One study (Janatuinen *et al.*, 1995) included 40 newly diagnosed patients of which 21 were used as control and 19 were challenged with oats. Of these newly diagnosed patients, one control subject of the 21 did not enter remission after 1 year of consuming a gluten-free diet. The inclusion of oats in the gluten-free diet of 19 newly diagnosed patients did not prevent symptomatic and mucosal healing (Janatuinen *et al.*, 1995).

A total of 22 patients with celiac disease (22/170) had evidence of abnormal bowel histopathology (Kemppainen *et al.*, 2008; Størsrud *et al.*, 2003a,b) prior to a dietary challenge with oats (baseline). These patients were on a gluten-free diet for at least 6 months prior to entering the trial. Patients with a normal bowel biopsy at baseline remained normal after the inclusion of oats into their gluten-free diet and patients with an abnormal bowel biopsy at ($n = 22$) baseline showed either no evidence of further histopathological deterioration after the oats challenge or histopathological improvement. Furthermore, the results of a 5-year follow-up study with adult patients with celiac disease who included oats in their diet indicated that exposure to oats did not alter the small-intestinal mucosa morphology or stimulate an immunological response locally in the mucosa of the small intestine (Janatuinen *et al.*, 2002; Kemppainen *et al.*, 2007).

2. Safety of oats in children

Among the studies conducted with children (Table 6.1), there were 89 children with celiac disease, either in remission ($n = 13$) or newly diagnosed ($n = 76$), who were challenged with oats added to an otherwise gluten-free diet. All of the children who were in remission at the time of

entering the trial responded well to the test diet. Follow-up results for these children were comparable to those of the corresponding controls and there was no histological evidence of deterioration in the intestinal mucosa after the introduction of oats into their gluten-free diet.

Similarly, newly diagnosed children recovered well on a gluten-free diet that included oats (Hoffenberg *et al.*, 2000; Högberg *et al.*, 2004; Hollén *et al.*, 2006b; Holm *et al.*, 2006). A combined total of 76 newly diagnosed patients were challenged with oats in these studies. Children were placed on the test diet (gluten-free diet including oats) after diagnosis and were assessed for disease remission at the end of the study. A randomized controlled study (Högberg *et al.*, 2004) conducted with children who were newly diagnosed with celiac disease showed that the consumption of an oat-containing gluten-free diet for 1 year did not interfere with clinical, serological, or small-intestinal mucosal recovery. In another study, 22 children with celiac disease, 9 newly diagnosed and 13 in remission consumed a gluten-free diet plus oats for 7 years without clinical or serological evidence of relapse (Holm *et al.*, 2006).

3. Withdrawal of adults and children from pivotal clinical studies

Within the adult database (Table 6.1), 41 out of 170 individuals who were challenged with oats withdrew from the trials. However, only 11 of these 41 individuals reported adverse side effects associated with the consumption of oats. Therefore, of the 170 adults who were challenged with oats, only 6% reported adverse effects (11/170). Of the 89 children who were challenged with oats, 17 individuals withdrew from the trials and only 9% reported adverse side effects from the consumption of oats (8/89). In both adults and children, the side effects reported were mostly gastrointestinal in nature. Information regarding the reasons for the withdrawal of the other cases was limited.

4. Gastrointestinal symptoms in adults and children

As reported in the clinical trials (Appendix 1 and Table 6.1), some patients with celiac disease, both adults and children, experienced gastrointestinal symptoms more often when consuming an oat-containing diet than with a standard gluten-free diet. However, in general, such symptoms were reported as transient, mild, and were explained as the effect of an increased intake of fiber from oat products rather than the reoccurrence of clinical manifestations of celiac disease. Except for the withdrawals noted above, most patients continued to participate in the trials. The reports of mild gastrointestinal symptoms, without clinical or serological evidence of relapse, were not considered sufficiently adverse to warrant the exclusion of oats from the gluten-free diet of patients with celiac disease.

B. Nonpivotal studies testing the effect of oats in patients with celiac disease using *in vitro* methods or serology

In addition to the pivotal studies, several publications (Table 6.2) used other methods to test the response of individuals with celiac disease who were introduced to oats. These studies did not fulfill the selection criteria of pivotal studies namely an *in vivo* oats challenge with an intestinal/skin biopsy to assess the biological response to the introduction of oats into an otherwise gluten-free diet. Instead, they used various *in vitro* techniques to assess the immune response to avenin, or serology without an intestinal mucosal biopsy. Most of the methods used duodenal mucosal cultures prepared from biopsies or intestinal T cell lines obtained from individuals with celiac disease. Other studies measured the immunogenic reaction in peripheral lymphocytes or measured the presence of various antibodies in individuals with verified celiac disease who included oats in their diet, in comparison with a reference group (Table 6.2). Some of these studies used patients that were previously included in pivotal studies. These studies are identified with an asterisk (*) in Table 6.2.

1. *In vitro*—duodenal mucosal cultures

Despite the diversity of the methodology used, the overall results from these *in vitro* nonpivotal studies are consistent with the opinion that oats are well tolerated by the majority of patients with celiac disease. However, there is some evidence that avenin from oats can elicit an *in vitro* immunogenic response. One study that reported an *in vitro* immunogenic response to avenin (Arentz-Hansen *et al.*, 2004) established oat avenin-specific and reactive intestinal T cell lines from three patients who had clinical symptoms while consuming an oat-containing diet, as well as from two other patients who appeared to tolerate oats. Some of these patients were recruited from the same pool of patients participating in a designated pivotal study. Of these participants, one patient was known to be intolerant to oats with associated intestinal pathology (Lundin *et al.*, 2003). In contrast, other *in vitro* studies (Kilmartin *et al.*, 2003, 2006) report that purified avenin from oats is not immunogenic to the intestinal mucosa of patients with celiac disease.

2. *In vitro*—other

Recent studies using a peripheral lymphocyte method to test the immunogenic properties of oats (avenin) reported that avenins from different oat cultivars have different levels of toxicity when tested *in vitro* (Silano *et al.*, 2007a,b). All the varieties of oats tested (Lampton, Astra, Ava, and Nave) by these investigators were immunogenic with differences in their capacity to induce a response. All avenin from oat cultivars had less activity than gliadin (wheat), but more activity than rice prolamins.

3. Serology

Guttormsen *et al.* (2008) assessed several serological parameters (IgA against oat avenin and wheat gliadin, and tissue transglutaminase) in samples collected from 136 adult patients, who had a biopsy-confirmed diagnosis of celiac disease and eating a strict gluten-free diet for at least 2 years. Results were compared to 139 nonceliac controls. Although patients were not challenged with oats, 82 patients with celiac disease had been taking oats as part of their gluten-free diet for 6 months or more and recorded their oats intake during a 3-week period. Patients were instructed to consume only ecologically grown oats specifically produced for celiac disease patients. Other than this, there was no report of testing results of the purity of oats. No significant differences were found in IgA against oats in oats-eating and non-oats-eating patients with celiac disease.

C. Other studies relevant to the effect of oats in patients with celiac disease

One study (Peräaho *et al.*, 2004b) conducted a retrospective evaluation, beginning in 1997, on the inclusion of oats within a gluten-free diet by patients with celiac disease and dermatitis herpetiformis. The inclusion of oats, the effect of oats on symptoms of illness, and the quality of life were investigated in 1000 randomly selected members of the Finland Celiac Society. Altogether, 710 patients responded to the questionnaire: 423 (73%) with celiac disease and 70 (55%) with dermatitis herpetiformis were currently consuming oats. Subjects reported an appreciation of the taste, the ease of use, and the low cost of oats. Of the respondents, 94% believed that oats diversified the gluten-free diet. This study provides support to the beneficial effects of oats for individuals with celiac disease. However, 15% of patients with celiac disease and 28% of patients with dermatitis herpetiformis reported that they had stopped eating oats. The most common reasons reported for avoiding oats were the fear of an adverse effect or of contamination of the oats with cereals excluded from the gluten-free diet, for example, wheat, barley, and rye.

IV. DISCUSSION

A. Evidence based on pivotal and nonpivotal studies

An overall assessment of the studies published since 1995 (Tables 6.1 and 6.2) supports the opinion that the majority of adults and children who have celiac disease or dermatitis herpetiformis, regardless of whether the condition is newly diagnosed or in remission, can tolerate moderate amounts (20–25 g/day dry rolled oats for children; 50–70 g/day for adults) of pure

oats, uncontaminated with gluten from wheat, rye, and barley. The following issues were taken into consideration when determining the safety of introducing oats into the gluten-free diet of individuals with celiac disease:

Comparisons of the various studies are complicated by the different study designs, the different conditions used in the testing, and in the reporting of the purity of the oat products used in the clinical trials. All the studies reviewed had some limitations; however, regardless of these limitations most studies indicated that subjects could tolerate the inclusion of oats in a gluten-free diet, providing it was free of contamination with gluten-containing cereals.

The key limitations of the study designs are the duration of the study and the number of subjects. Since most studies conducted are short term, the potential effect of a lifetime exposure to oats requires further investigation. Long-term compliance to a gluten-free diet is one of the major difficulties in the management of celiac disease, and the collection of long-term data related to the consumption of oats is also very challenging.

The number of subjects included in each challenge study was limited, most likely due to the difficulty of recruiting enough subjects willing to comply with the long and laborious study protocols including endoscopic biopsies. More specifically, in studies involving children, particularly those including very young children, an additional challenge was the possible reluctance of the children and/or their parents to participate, which would interfere with compliance and the desire to continue until the end of the study. Training the families of the children to maintain compliance to the study protocol is an additional essential requirement to ensure accurate results. Furthermore, patients with celiac disease willing to participate in challenge studies are aware that they may face reactivation of the clinical symptoms or a delay in their recovery. For ethical reasons, most studies that recruited newly diagnosed patients had exclusion criteria to eliminate patients with the most severe pathologies. It is important to recognize the limitations that are encountered in celiac disease challenge clinical trials, as the information gleaned from these trials must be interpreted and evaluated within such limitations.

The differences in the oat products used, the testing, and the reporting of the purity of oats further limited a comprehensive safety assessment. Evaluation of the purity of the oats used in each study is paramount to being able to distinguish between possible toxicity of oats versus adverse effects induced by cross contamination. A standardization of the reporting of the purity of oats utilized within the studies would increase the accuracy of safety assessments and assist in the establishment of a threshold for the tolerance to gluten from wheat, barley, and rye. Cross validation, standardization, and international agreement on the test methodologies to assess the purity of oats will also need reevaluation as further information becomes available.

In the current database, there is no evidence that the inclusion of oats in the gluten-free diet induces adverse effects in newly diagnosed patients. Using the baseline biopsy and clinical assessment for comparison, clinical studies in both adults and children that examined newly diagnosed individuals, showed that patients either improved or did not deteriorate after the inclusion of oats to their gluten-free diet. It was noted within the database that 22 out of 170 adult patients had abnormal bowel mucosal histology consistent with active disease at baseline. This observation is of particular concern because these patients were identified as being in remission and on a gluten-free diet prior to their inclusion into the oats-challenge trial. Although no explanation was given for these findings, the following considerations were taken into account: (a) compliance to gluten-free diet (Martin, 2008), (b) cross contamination of gluten-free diet and threshold of gluten tolerance (Akobeng and Thomas, 2008; Gelinas *et al.*, 2008; Troncone *et al.*, 2008b), (c) lack of response to gluten-free diet or refractory celiac disease (Abdallah *et al.*, 2007), and (d) histopathology criteria for diagnosis (Upton, 2008).

From currently available data, one adult patient (1/170) challenged with oats was reported to have a severe adverse reaction to oats. Approximately 6% of adults and 9% of children withdrew from clinical trials due to reported adverse effects from the inclusion of oats in their diet. This evidence, along with the indication from some *in vitro* studies of an immunological response to avenin in the absence of clinical manifestations of celiac disease and the limitations of the database (previously discussed), supports a cautionary approach for the introduction of oats into a gluten-free diet until the prevalence of oats intolerance among people with celiac disease is well established (Dickey, 2008; Garsed and Scott, 2007).

Despite the noted challenges of conducting a comprehensive evaluation of the most recent clinical trials, the majority of the evidence indicated that most people with celiac disease could tolerate the inclusion of a moderate amount (20–25 g/day; 65 ml or 1/4 cup) dry rolled oats for children and (50–70 g/day; 125–175 ml or 1/2–3/4 cup dry rolled oats) for adults of pure oats (uncontaminated with other gluten-containing cereal grains) in a gluten-free diet (Table 6.1, Appendix 1). Based on some evidence that a possible sensitivity to pure oats exists, most investigators in the field recommend a clinical follow-up when introducing pure oats to the gluten-free diet (Haboubi *et al.*, 2006; Rashid *et al.*, 2007). This includes both initial and long-term assessments.

B. Evidence based on other reviews on the safety of oats

Earlier published reviews on the safety of oats for patients with celiac disease were included in this evaluation (Dor and Shanahan, 2002; Kumar and Farthing, 1995; Schmitz, 1997; Thompson, 1997, 2003). Two

systematic reviews were identified and compared to our review (Garsed and Scott, 2007; Haboubi *et al.*, 2006).

Haboubi *et al.* (2006) conducted a systematic review of the literature related to the inclusion of oats in the gluten-free diet for patients with celiac disease in order to assess whether oats can be recommended to these patients. In this report a computerized search of the scientific literature up to 2005 was carried out using specific search and selection criteria. Haboubi *et al.* (2006) identified 17 primary studies, six of which met their inclusion criteria. We compared our database to that of Haboubi *et al.* (2006) and identified the following differences: (a) the time period evaluated, (b) selection criteria, and (c) data analysis. A key difference between databases was that although Haboubi *et al.* used rigorous selection criteria, whether or not the purity of oats used in the studies were tested was not taken into consideration in their evaluation. By contrast, our analysis of the literature included all the studies identified by Haboubi *et al.* (2006) as well as relevant studies published up to November 2008. Attention was specifically given to the purity of the oats used in the studies because this parameter is considered paramount to the ability to distinguish between possible toxicity of oats versus adverse effects induced by cross contamination. Another noted difference between databases was that Haboubi *et al.* (2006) excluded all studies where the same patient served as control. It is, however, well accepted that in patients with celiac disease there is high variability in the susceptibility to gluten injury. Studies using the same subjects as controls provide valuable data. Hence, we included them in our assessment, but identified them as ''observational changes from baseline in a single group'' in the Table 6.1 under the study design column.

None of these six studies selected by Haboubi *et al.* (2006) found any significant difference in the serology between the groups consuming oats and the control groups. However, they observed that two of the six studies reported significant differences ($p < 0.001$; $p = 0.039$) in intraepithelial lymphocyte counts (IELs) between the oat-consuming and control groups (Peräaho *et al.*, 2004a; Reunala *et al.*, 1998). Although we concur with the findings, it is important to note that the purity of oats was not tested in one of the selected publications (Peräaho *et al.*, 2004a). Hence, it is possible that the difference between oats-challenged and control individuals was due to cross contamination. In the other study, Reunala *et al.* (1998) reported differences in the expression of $\gamma\delta$ T cell receptors ($p < 0.001$) when comparing oat-consuming to control groups, but these biomarkers were elevated at baseline and improved after the oats challenge. Furthermore, other biomarkers assessed by Reunala *et al.* did not show a difference between oat-consuming and control groups. Moreover, an additional study by Kemppainen *et al.* (2007) did not find evidence of local immune activation after an oats challenge using similar immunohistochemical biomarkers.

The systematic review conducted by Garsed and Scott (2007) included uncontrolled studies published prior to 1995, their cut-off point was February 2006, and the data are presented all in one table. Our review established a starting point of 1995 based on the publication by Janatuinen *et al.* (1995) which was the first clinical trial to evaluate the possible toxicity of oats in a large controlled study. We also segregated the studies conducted in adults versus children and grouped them as pivotal and nonpivotal. The rationale for grouping the studies as pivotal and nonpivotal was that the evidence provided by the pivotal studies (i.e., studies that used biopsy results (intestinal/skin) as the key end point following the oats challenge) contributed more strongly to the overall weight of evidence. We compared our database with all studies published after 1995 that were identified by Garsed and Scott (2007) and found that we had selected the same studies.

Garsed and Scott (2007) identified a total of 165 patients in the database as being challenged with oats and having a biopsy as an end point, of these patients only one individual (1/165) had histological evidences of intestinal mucosal injury associated to oats exposure. In our review, we reported a total of 170 adults with celiac disease challenged with oats and having a biopsy as an end point. Our tabulation includes challenge studies published after the Garsed and Scott (2007) cut-off point of February 2006 and separated children from the tabulation. Despite these differences, we identified the same individual as the only subject (1/170) to have a histological response to the inclusion of oats in a gluten-free diet. The results and conclusions of the Garsed and Scott (2007) systematic review are comparable to our review.

C. Biochemistry and taxonomy of oats relevant to its potential toxicity

The biochemistry and taxonomy of cereals is relevant to their potential differential toxicity. The proteins in the cereal grains known as ''gluten'' activate celiac disease. Gluten is a complex mixture of hundreds of related but distinct proteins. The grains considered capable of producing adverse effects in individuals with celiac disease include different species of wheat (e.g., durum, spelt, kamut), barley, rye, and their cross-bred hybrids (e.g., triticale, which is a cross between wheat and rye) (Ciclitira *et al.*, 2005; Cornell *et al.*, 2002; Dewar *et al.*, 2006; Howdle, 2006; Koning, 2008; Lester, 2008; Moron *et al.*, 2008; Thompson, 2000, 2001; Troncone *et al.*, 2008a; Vader *et al.*, 2003; Wieser and Koehler, 2008).

Gluten includes two major protein types, the gliadins and glutenins, both of which contain activating toxic peptides. Gliadins can be subdivided into α/β-gliadins, γ-gliadins, and ω-gliadins, whereas the glutenins consist of low molecular weight and high molecular weight glutenins.

Several gluten proteins are involved in the pathogenesis of celiac disease (Ciclitira *et al.*, 2005) and both prolamins (i.e., gliadins and glutenins) have been implicated (Londei *et al.*, 2005; Piper *et al.*, 2002; Shan *et al.*, 2005; Troncone *et al.*, 2008a; Vader *et al.*, 2003; Wieser and Koehler, 2008). Prolamins are the alcohol-soluble protein fractions in wheat and other related cereals and are of the most concern to individuals with celiac disease. Prolamins are typically rich in glutamine and proline making it resistant to the normal digestive breakdown (Shan *et al.*, 2002; Wieser and Koehler, 2008). After the digestion of prolamins by gastric, duodenal, and pancreatic enzymes, a 33-amino acid peptide molecule (33mer) and other immunogenic peptides remain. It is this nondigested fragment that is considered the key in eliciting the immune response in susceptible individuals with celiac disease (Shan *et al.*, 2002).

Wheat, rye, and barley have a common ancestral origin in the grass family. Oats are more distantly related to the analogous proteins in wheat, rye, and barley and the oat prolamins (avenin) have substantially lower proline content. Avenin accounts for 5–15% of the total protein in oats, whereas in wheat, barley, and rye, prolamins constitute 40–50% of the total protein (Kilmartin *et al.*, 2006). Some investigators believe that there are similarities between the protein structure of oats and some wheat-like sequences, which may indicate that large amounts of oats could potentially be toxic to patients with celiac disease. However, the putative toxic amino acid sequences are less frequent in avenin than in other prolamins, which explains the less toxic nature of oats (Arentz-Hansen *et al.*, 2004; Ellis and Ciclitira, 2001, 2008; Shan *et al.*, 2005; Vader *et al.*, 2002, 2003).

Recent studies have shown that avenins from different varieties of oats show different levels of toxicity when tested *in vitro* (Silano *et al.*, 2007a,b). All the varieties of oats tested (Lampton, Astra, Ava, and Nave) by these investigators were immunogenic, but some varieties were less immunogenic than others. All avenins from various oat cultivars had less activity than gliadin, but more activity than rice prolamins (Silano *et al.*, 2007a,b). The authors considered the possibility that contamination could have been a factor and indicated that the less toxic variety (Lampton oats) was obtained from an isolated farm where only this crop and buckwheat (nontoxic to individuals with celiac disease) were grown (Silano *et al.*, 2007a,b). Overall, the significance of these results needs further clarification, as the immunogenic ability of avenins does not necessarily mean that oats are toxic for patients with celiac disease when added to the gluten-free diet in moderate amounts. In the *in vitro* experimental models, the encounter between prolamins and lymphocytes is abnormal and the testing of only the alcohol-soluble protein fraction of oats does not reflect the *in vivo* situation (Troncone *et al.*, 2008a,b). Factors within oats that may exert a protective effect against avenin toxicity or interfere with avenin absorption have not been accounted for in these experimental models. More studies

are needed to assess the impact of possible differential toxicities of oat cultivars in the overall safety of oats for individuals with celiac disease.

D. Benefits of the consumption of oats

Avena santiva L. is the traditional oat of choice around the world, and the agricultural properties of hulless or naked oat seeds have recently been reviewed in detail (Burrows, 2005). The interest in oats for human consumption has increased in recent years because of their more widely recognized nutritional and health benefits. The nutrient composition of oats and its potential health benefits have been the subject of extensive investigation and recent reviews (Andon and Anderson, 2008; Food And Drug Administration, 2008; Maki *et al*., 2007a,b; Mantovani *et al*., 2008; Ryan *et al*., 2007; Sadiq Butt *et al*., 2008). Oat and its by-products are a good source of dietary fiber, especially β-glucan, and nutrients, including proteins, minerals, and B complex vitamins. A gluten-free diet can be low in fiber, and oats will help to provide the much needed fiber and nutrients (Malandrino *et al*., 2008; Størsrud *et al*., 2003a). Other beneficial effects of oats include attenuation of postprandial plasma glucose and insulin responses, increased transport of bile acids toward lower parts of the intestinal tract, increased excretion of bile acids, and decreased serum cholesterol levels. The incorporation of oats into a gluten-free diet would not only improve the nutritional value of the diet but for many also improve compliance as it increases palatability, and provides a greater variety of food choices in a restrictive diet (Janatuinen *et al*., 2002; Peräaho *et al*., 2004a,b) resulting in better health and overall quality of life.

V. CONCLUSIONS

Available scientific data evaluating the introduction of pure oats in the gluten-free diet of patients with celiac disease and dermatitis herpetiformis indicates that moderate amounts of pure oats are well tolerated by the majority of these individuals who are either in remission or newly diagnosed. The term ''pure oats'' is used to indicate oats uncontaminated with gluten from other cereal grains, like wheat, barley, and rye, as detected by current test methods. Based on pivotal clinical trials in the published literature, the amount of pure oats considered within safe limits is 50–70 g/day for adults and 20–25 g/day for children.

The benefit of the introduction of oats into a gluten-free diet for adults and children with celiac disease outweighs the possible risk to the few individuals with celiac disease who may exhibit intolerance for oats. However, the previously discussed limitations of the database require the need for some caution when introducing oats into a gluten-free diet.

Although the majority of evidence supports the inclusion of oats in a gluten-free diet, the evidence that some people with celiac disease cannot tolerate even pure oats requires further investigation and cautious progress. Individuals with celiac disease or dermatitis herpetiformis interested in introducing oats to their diet are advised to consult their physician and dietician. It is also advisable to add oats into a gluten-free diet only when such a diet is well established, so that any possible adverse reactions can be readily identified (Rashid *et al.*, 2007). Enhanced education and information for the consumer with celiac disease, industry, health care providers, regulators, and public at large are essential for an increased awareness of celiac disease and dietary management of this increasingly recognized sector of the population (Lohi *et al.*, 2007). Availability of pure uncontaminated oats minimizes the risks and maximizes the food choices for individuals with celiac disease and dermatitis herpetiformis.

VI. APPENDIX I

A. Summary of pivotal *in vivo* clinical studies testing the safety of oats in patients with celiac disease or dermatitis herpetiformis (Table 6.1)

1. Adults

1. Janatuinen *et al.* (1995) were the first to evaluate the possible toxicity of oats in a large controlled study in adult patients with celiac disease. In this randomized trial, which lasted up to 12 months, they compared the effects of gluten-free diets with and without oats. They studied two groups of patients: those with previously diagnosed celiac disease who were in remission and those newly diagnosed. The previously diagnosed group was selected based on records of the recovery of the small-intestinal mucosa while on a gluten-free diet for at least 12 months. For the newly diagnosed, endoscopy with a duodenal biopsy was performed and the diagnosis of celiac disease was based on the presence of the subtotal or total villous atrophy of the duodenal mucosa prior to the introduction of a gluten-free diet. Fifty-two adults with celiac disease in remission were followed for 6 months and 40 adults with newly diagnosed celiac disease for 12 months. Patients were randomly assigned according to sex to either the oats or the control group. The oats group included 26 patients in remission and 19 newly diagnosed. The control group included 26 patients in remission and 21 newly diagnosed. The controls received a gluten-free diet containing 0.74 mg of gluten/g of foodstuff. The oat groups supplemented the same basic gluten-free diet with 50–70 g/day of oats in the form of wheat starch flour mixed with an equal amount of oats, muesli-

containing 60% oats, and rolled-oat breakfast cereal. The authors neglected to report whether the oats added to the gluten-free diets were tested for purity. The mean (± SD) intake of oats in the oat group was 49.9 ± 14.7 g/day for 6 months among patients in remission and 46.6 ± 13.3 g/day for 12 months among newly diagnosed patients. The oat and control groups did not differ significantly in nutritional status, symptoms, or laboratory measures. The authors reported that patients in remission, regardless of diet, did not have a worsening of the duodenal villous architecture or increased mononuclear cellular infiltration. All the patients with a new diagnosis were in remission within 1 year, except for one subject in the control group. The rate of withdrawal was comparable: six patients in the oat group and five in the control group withdrew from the study. The investigators concluded that moderate amounts of oats could be included in a gluten-free diet for most adult patients with celiac disease without adverse effects. In this study, the basic gluten-free diet consumed by participants contained 0.74 mg of gluten/g of foodstuff, and although the oats were not tested for purity, there were no significant differences between the control and the test group in remission as per the duodenal biopsies and clinical findings.

2. Srinivasan *et al*. have several publications relevant to the safety of oats in patients with celiac disease (Srinivasan *et al*., 1996, 1999, 2006). In 1996, they studied the safety of oats in 10 adult patients with celiac disease in clinical and histological remission. Each patient consumed 50 g of oats (as porridge) daily for 12 weeks while maintaining a strict gluten-free diet. The oat cereal used in the study was tested for evidence of gluten contamination using reverse-phase high-performance liquid chromatography (HPLC), enzyme linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) techniques. The oats were determined to be entirely gluten-free. Details were not provided as to the levels of detection for these techniques or the cut-off values used to determine whether the oat samples were considered gluten-free. The patients were assessed clinically at 0, 1, 4, and 12 weeks. At each assessment, the following laboratory investigations were performed: full hematological and biochemical profiles and serological tests for antibodies to gliadin and endomysium. Duodenal biopsies were obtained before the start of the oats challenge and after the 12-week trial period. All patients complied fully with the study protocol. Throughout the oat challenge, all patients remained asymptomatic with normal hematological and biochemical indices. Endomysial and gliadin antibody values were unaltered by the oats supplementation and no morphological damage was evident using a standard histological evaluation. Quantitative histological examinations showed no

significant changes. Subsequently, two patients were given a gluten ''microchallenge'' consisting of 500 mg of gluten daily for 6 weeks: both developed histological evidence of relapse and one patient tested positive by antibody production tests. These investigators further assessed the toxicity of oats in patients with celiac disease by immunohistochemical techniques detecting the presence of lactase enzyme in the intestinal biopsy. This enzyme is lost in active celiac disease, but was unaffected by the oats challenge (Srinivasan *et al.*, 1999).

3. Hardman *et al.* (1997) studied seven men and three women (mean age: 58 years) with biopsy-confirmed dermatitis herpetiformis. The subjects had followed a strict gluten-free diet for a mean of 15.8 years and had controlled the rash and enteropathy. The subjects added oats to their diets for 12 weeks (mean (± SD) daily intake, 62.5 ± 10.8 g). The purity of the oats was tested by ELISA and PCR reaction. Details were not provided as to the levels of detection of these techniques, or the cut-off values used to determine whether the oat samples were considered gluten-free. All patients underwent duodenal and skin biopsies at the beginning and end of the study. None of the patients reported any adverse effects. Serologic tests for anti-gliadin, anti-reticulin, and anti-endomysial antibodies were negative both before and after the trial period. Villous architecture remained normal after the 12-week period: the mean (± SE) ratio of the height of villi to the depth of crypts was 3.59 ± 0.11 before the diet and 3.71 ± 0.09 afterward (normal, 3–5), and the mean enterocyte heights were 31.36 ± 0.58 and 31.75 ± 44 μm, respectively (normal range, 29–34). Duodenal IEL counts all remained within normal limits (mean, 13.8 ± 1.03 per 100 enterocytes before the diet and 14.2 ± 1.2 per 100 enterocytes afterward; normal range, 10–30). Dermal IgA in skin biopsies showed no significant changes.

4. Reunala *et al.* (1998) conducted an oat challenge study with 11 patients who had dermatitis herpetiformis. Another 11 patients with dermatitis herpetiformis were used as a control group. At diagnosis all patients had skin and duodenal biopsies. All patients were in remission on gluten-free diets for at least 5.5 years and free of a rash for 14 months. Test subjects consumed 50 g oats/day for 6 months within an otherwise gluten-free diet. The oats used in this challenge were free of gluten contamination as tested by ELISA and PCR. In this study, the specifications for the amount of gluten allowed to be consumed from wheat starch flours, was up to 0.3 g protein, equivalent to 50 mg of gluten per 100 g flour which was in accordance with Codex Alimentarius 118-1981. Clinical symptoms, serum, skin, and small bowel biopsies were assessed before and after the oat challenge. Eight patients

challenged with oats remained asymptomatic, two developed transient rashes, and one withdrew because of the appearance of a more persistent but mild rash. Three of the 11 controls also developed transient rashes. IgA endomysial antibodies remained negative in all patients and the small-intestinal villous architecture remained unaltered after the oat challenge. The densities of intraepithelial CD3 and αβ and γδ T cell receptor positive lymphocytes and crypt epithelial cell DR expression were assessed by immunohistochemistry. These biomarkers are considered to be sensitive indicators of immune response to gluten. Except for γ T cell receptor, these biomarkers were not altered by oats challenge. The expression of γδ T cell receptor showed a significant difference ($p < 0.001$) when comparing oat challenged to controls. However, it should be noted that this biomarker was elevated at baseline and improved after the oats challenge.

5. Janatuinen *et al*. (2000) conducted a randomized controlled intervention study over a 6–12-month period with 40 adults who were newly diagnosed with celiac disease and 52 adults who were in remission. Patients were randomized by sex into either the oat-consuming or the control groups. The control groups received gluten-free cereal. The oat-consuming groups received products supplemented with oats: two types of gluten-free wheat starch flour including 50% oats, muesli including 60% oats, and rolled-oat breakfast cereal. Some of the oat products used in this study were commercially available. The daily intake of oats was 50–70 g. The purity of the oats was regularly monitored. Gluten was analyzed by a quantitative enzyme immunoassay using a specific monoclonal antibody to α-gliadin. This antibody detects all prolamins in wheat and rye, only some of the prolamins in barley, and none of the prolamins in oats. All oat samples were considered gluten-free but no specification was provided with regard to the limit of detection. All patients were evaluated using serum levels of gliadin and reticulin antibodies. In the intestinal biopsies, the number of IELs in the intestinal mucosa was examined before and after the intervention. The rate of disappearance of gliadin and reticulin antibodies did not differ between the diet groups of the patients with newly diagnosed celiac disease. Oats also had no effect on gliadin or reticulin antibody levels in the patients who were in remission. The number of IELs decreased similarly regardless of the diet of newly diagnosed patients and no increase in the number of IELs was found in the patients who were in remission and consuming diets with or without oats. In summary, there were no significant differences between the clinical symptoms, laboratory measures, and histology of duodenal biopsies among the test groups who received oats or

those who did not receive oats. The authors concluded that this study further strengthens the view that adults with celiac disease can tolerate moderate amounts of oats.

6. Janatuinen *et al*. (2002) assessed the safety of the long-term ingestion of oats in the diet of patients with celiac disease. In a previous study, the effects of a gluten-free diet and a gluten-free diet including oats were compared in a randomized trial involving 92 adult patients with celiac disease (45 in the oats group, 47 in the control group). Oat products were obtained from commercial sources. The mean amount of oats added to the gluten-free diet was 34 g/day. The purity of the oats and the gluten-free products were monitored during the 6–12-month intervention (Janatuinen *et al.*, 2000). After the initial phase of 6–12 months, patients in the oats group were encouraged to eat oats freely in conjunction with an otherwise gluten-free diet. However, there was no systematic monitoring of the purity of these oat supplements. A follow-up was conducted after a 5-year period; there were 23 patients still on an oats diet. Of the original oat-consuming group, 12 subjects dropped out for reasons including uncertainty about safety, flatulence, and rash. In the control group, 28 patients on a conventional gluten-free diet were examined. In addition to the clinical and nutritional assessment, the following parameters were evaluated: duodenal biopsies histopathology and histomorphometry, and measurement of antiendomysial, antireticulin, and antigliadin antibodies. There were no significant differences between controls and those patients consuming oats with respect to duodenal villous architecture, inflammatory cell infiltration of the duodenal mucosa, or antibody titers after the 5-year follow-up. The authors reported that in both groups, histological and histomorphometric indexes improved equally over time. Despite the high withdrawal rate and the fact that neither the oats nor the gluten-free products were monitored for gluten, making it impossible to assess the level of gluten contamination in the diets, 23 out of 35 patients remained in the study for 5 years and were without signs of disease relapse as evaluated by clinical symptoms, serology, or histopathology.

7. Lundin *et al*. (2003) conducted an oat challenge study in 19 adult patients with celiac disease who were in remission on gluten-free diets. The gluten-free diets were supplemented with 50 g/day of oats for 12 weeks. Oat samples were obtained from a single manufacturer who upheld strict practices to avoid contamination with wheat and other gluten-containing grains. Twenty-five oat samples from this manufacturer were analyzed in-house and a reference laboratory analyzed an additional 120 samples. No contamination was detected in any of these samples using ELISA and mass spectrometry (MS) with a limit of detection for gluten contamination reported as 20 ppm. Six oat

samples were later analyzed by western blot, an ELISA test using a cocktail of antibodies, and MALDI-TOF MS with a limit of detection reported as 5 ppm. The level of gluten detected in five of these samples was estimated to be between <1.5 and 23 ppm and considered negative for gluten contamination. The sixth sample was considered contaminated (>400 ppm by western blot, ELISA, and MS). MS was unable to determine the source of the contamination due to the large amount of oat avenins in the sample and the fact that barley and oats are not distinguishable by MS. An additional sample was analyzed from the bottom of the same bag of contaminated oats and <1.5 ppm of gluten was detected. The authors assumed that a single or a few seeds of barley might have been present in the bag but determined that the oats used in the study were sufficiently pure and adhered to the limitations by the revised suggested Codex standard limit of 20 ppm for natural gluten-free products. All patients were evaluated by serological testing including IgA antigliadin, antiendomysium, and anti-tissue transglutaminase antibodies. Endoscopic small-intestinal biopsies were obtained before and after the oat challenge. Biopsies were scored using Marsh score and levels of mRNA specific for interferon (IFN) were determined by reverse transcription-PCR analysis. IFN was tested as a marker for T cell activation. Oats were well tolerated by most patients but several reported transient abdominal discomfort and bloating at the beginning of the challenge. This could have resulted from increased fiber consumption in patients not used to large intakes of fiber in their diets. One patient withdrew due to gastrointestinal symptoms and another patient developed partial villous atrophy and a rash during the first oats challenge. This patient subsequently improved on an oat-free diet but developed subtotal villous atrophy and dramatic dermatitis during a second challenge with oats. There was no evidence of contamination in the oats consumed by the patient exhibiting intolerance. Five of the subjects had positive levels of IFN-mRNA but no corresponding histological abnormalities after the challenge. The significance of this finding is not fully understood but the authors' suspect that T cell activation might not be directly responsible for the villous atrophy seen in celiac disease. The major difference observed in this study is that one of the patients was intolerant to oats. The authors reported that after the completion of this challenge study they became aware of other patients identified as clinically intolerant to oats (dermatitis, abdominal pain, and general anaphylactoid-like reactions) but not further confirmed by small-intestinal biopsy. However, none of the subjects were willing to ingest oats again. Therefore, the authors concluded that despite the ability of most patients to tolerate the incorporation of oats into their diets, some individuals might be intolerant to oats.

8. Størsrud *et al.* (2003a,b) studied the effects of adding uncontaminated rolled oats to the daily diets of 20 adult patients with celiac disease who were in remission. Although there are two reports, they represent one main study. The core results are based on the 15 patients who completed the study. These subjects added oats to their gluten-free diet for 2 years. The median intake of oats was 93 g/day. The oats were free from wheat, rye, and barley as tested by ELISA that detects gliadin. This method detects high molecular weight proteins in wheat, rye, and barley, but not oats (avenin). The method is quantitative with a detection limit of 20 ppm. The examinations of the subjects were performed four times during the study period and included endoscopic small-intestinal biopsies, blood samples (nutritional status, serological analysis), height and body weight, gastrointestinal symptoms, and dietary records. Histopathology of duodenal biopsies was assessed at baseline ($n = 20$), 6 months ($n = 17$) and 2 years ($n = 14$). One patient refused the biopsy. Villous architecture and inflammatory infiltrate were assessed. Evidence of partial atrophy and inflammation of the small intestine was present in some patients at baseline and remained after oats exposure ($n = 3$). None of the patients with normal histology deteriorated after exposure to oats ($n = 11$). None of the parameters evaluated indicated any evidence of reactivation of the disease in response to the introduction of pure oats into the gluten-free diet after the 2-year trial period. Five patients withdrew from the study, two due to gastrointestinal symptoms, and the other three for nonmedical reasons. Examinations of the patients after their withdrawal did not reveal any deterioration in small-intestinal histology, nutritional status, or raised levels of antibodies. The other report that was based on the subjects who completed this 2-year trial study focused on the benefits and the nutritional status of the participants. The mean intakes of iron and dietary fiber increased ($p < 0.001$) with the consumption of oats, as well as the intakes of thiamine and zinc ($p < 0.02$). Temporary increased flatulence was also experienced during the first few weeks of consuming oats, as well as improved bowel function. All participants who completed the study period reported a desire to continue to eat oats after the study because they found that the addition of oats to the gluten-free diet gave more variety, better taste, and satiety. The consumption of oats improved the nutritional value of the gluten-free diet, did not have negative effects on nutritional status and was appreciated by the subjects. The authors suggested that including oats could help people improve their compliance to a strict gluten-free diet.

9. Peräaho *et al.* (2004a) studied 39 patients with celiac disease who consumed gluten-free diets. Patients were randomized to a gluten-free diet with 50 g oats/day (23 patients) or without oats (16 patients)

for 1 year. The purity of the oats used in this study was not specified in the report. The following parameters were evaluated: quality of life, gastrointestinal symptoms, small-intestinal histopathology, and serum endomysial and tissue transglutaminase antibodies. The quality of life did not differ between the groups, but there were more gastrointestinal symptoms in the oats-consuming group. Patients consuming oats suffered significantly more often from diarrhea, but there was also a simultaneous trend toward more severe constipation symptoms. Three patients on the oats diet dropped out of the study due to gastrointestinal symptoms. The villous structure did not differ between the groups, but the density of IELs was slightly significantly higher in the oat group. The severity of the gastrointestinal symptoms did not appear to be dependent on the degree of inflammation. Antibody levels did not increase during the study period. The authors concluded that the oat-containing gluten-free diet caused more gastrointestinal symptoms than the traditional diet. Although the mucosal integrity was not disturbed, there was more inflammation evident in the oat group. However, the sources and purity of the oats used in this study are unknown so it is possible that the gastrointestinal symptoms and the increased inflammation among the oat-consuming group were due to gluten contamination from other cereal grains such as wheat, barley, and rye. Despite the limitations of this study and of the effects observed in the oat group, the authors suggested that oats can provide an alternative within a gluten-free diet but that patients should be aware of the possible effects on the gastrointestinal system. Furthermore, these investigators (Peräaho *et al.*, 2004b) also conducted an analysis on the effect of oats on symptoms and quality of life in 1000 randomly selected members of their Celiac Society. Altogether, 710 patients responded: 423 (73%) with celiac disease and 70 (55%) with dermatitis herpetiformis were currently consuming oats. Patients appreciated the taste, the ease of use, and the low costs; 94% believed that oats diversified the gluten-free diet; 15% of patients with celiac disease; and 28% of patients with dermatitis herpetiformis had stopped eating oats. The most common reasons for avoiding oats were fear of adverse effects or contamination. The authors suggest that there is a market demand for oats, and celiac societies and dieticians should make efforts to promote the development of products free of gluten contamination.

10. Kemppainen *et al.* (2007) presented additional data from their 5-year follow-up study on the safety of oats in patients with celiac disease (Janatuinen *et al.*, 2002). In the present study, they assessed the local cellular immunological responses using immunohistochemical biomarkers. Forty-two patients with celiac disease took part in an earlier

oats intervention study for 6–12 months. Twenty-two of these patients originally consumed oats as part of their gluten-free diet. During the 5-year follow-up, 10 patients had felt uncertain about the safety of long-term consumption of oats and gave up this part of their diet. Finally, 12 of the 22 patients consumed oats for the whole 5-year period. The control group consisted of the remaining 20 patients with celiac disease using a strict, conventional, gluten-free diet without oats. Intraepithelial CD3, γδ T cell receptors (γδ IEL), and αβ T cell receptors (αβ IEL) T cells were counted after specific staining of small-intestinal biopsy specimens. There were no differences in the densities of CD3, αβ IELs, and γδ IELs between the oat and the control groups. This study provides additional evidence that long-term use of oats included in the gluten-free diets of patients with celiac disease does not stimulate an immunological response locally in the mucosa of the small intestine. The high number of dropouts demonstrates the difficulties of conducting long-term studies in patients with celiac disease as the main reason for giving up was feeling of uncertainty about the long-term safety of oats. Further studies in this regard will help to increase patients' compliance and confidence about the safety of oats.

11. Kemppainen *et al*. (2008) conducted a randomized clinical trial to investigate the suitability of large amounts of unkilned oats in comparison with kilned oats in adult patients with celiac disease. Kilning generally processes oats, which in principle may change the antigenic properties and may be the reason that kilned oats, are tolerated by patients with celiac disease. The study included 32 patients (19 F, 13 M) who previously consumed oats as part of their gluten-free diet. Patients were randomized to two groups: group A was started on kilned oats and group B on unkilned oats. After 6 months, the patients changed the treatment groups. Patients had followed a gluten-free diet for 8.3 years and had used oats for approximately 5 years. The purity of the oats was controlled during the 12-month follow-up. Electrophoresis, immunoblotting of the prolamins, and the total protein extracts of the oat product samples by polyclonal anti-gliadin antibody did not show any contaminants. At the end of the study no marked differences were reported in the histopathology of small-intestinal biopsies between the two groups using either unkilned or kilned oats. At baseline, 10 patients had partial villous atrophy and 9 had mild mucosal inflammation. Biopsies from the remaining patients were interpreted as normal. In both groups, duodenal villous architecture and mucosal inflammation did not worsen, but rather improved during the first 6 months exposure to oats. At 12 months, five patients had partial villous atrophy and four had mild inflammation. All patients had negative endomysial antibodies,

at the beginning and during the follow-up. Only 1 woman out of 32 subjects withdrew from the study because of abdominal symptoms after 1 week. After 6 months, another female patient withdrew because of pregnancy. The authors concluded that the inclusion of large amounts of either kilned or unkilned oats in the diet did not cause a worsening of the healed duodenal mucosa, an increase in normalized antibody levels or symptoms, or affect the general well-being of the celiac patients in remission indicating that the kilning of oats by food processing is not a prerequisite for oats to be tolerated.

2. Children

1. Hoffenberg *et al*. (2000) conducted a self-controlled, open-label, 6-month trial of the consumption of a commercial oat breakfast cereal among children newly diagnosed with celiac disease. The children were placed on a gluten-free diet plus commercially available oats. Over 6.6 $\pm$ 0.7 months, they consumed 24 g oat cereal/day, or 1.2 $\pm$ 0.9 g/kg/day. The gliadin contamination of the oat cereal used in this study was tested by an ELISA, which detects gliadin. This method was chosen because it detects high molecular weight proteins in wheat, rye, and barley, but not oats avenin. The samples were considered negative at a level of <0.01% (100 ppm). The 10 children who completed the study were 6.8 $\pm$ 4.0 (mean $\pm$ SD) years of age. The children who are included in the study had at least one sign or symptom suggestive of celiac disease at the beginning of the study. Patients were evaluated clinically, including small bowel histology and anti-tissue transglutaminase IgA antibody titer. Compared with the start of the study, there was a significant decrease in biopsy score ($p < 0.01$), IELs ($p < 0.005$), anti-tissue transglutaminase IgA antibody titer ($p < 0.01$), and number of symptoms ($p < 0.01$) at the completion of the study. This study was limited because the children were newly diagnosed and the families were still learning how to manage a gluten-free diet so hidden sources of gluten may still have been present in the diet. However, the overall results showed improvement with the test diet.

2. Högberg *et al*. (2004) reported the results from a double blind multicenter study, which included 116 children with newly diagnosed celiac disease. Children were randomized into two groups, 59 received a gluten-free diet and 57 received a gluten-free diet plus oats. The study period was 1 year. The oats used in the study were specially grown, milled, and packaged to avoid contamination with wheat, rye, or barley. The oat products were tested by an ELISA assay to ensure absence of gluten contamination. The daily oat intake was 25–50 g. Small-intestinal biopsies were performed at the beginning and end of the study. Serum

IgA antigliadin, antiendomysium, and anti-tissue transglutaminase antibodies were monitored at 0, 3, 6, and 12 months. Ninety-three patients completed the study, 42 on gluten-free diet plus oats, and 50 gluten-free diet controls. The remaining subjects withdrew from the study, 15 in the oats group and 7 in the standard gluten-free diet and a majority of these children were from the youngest age groups in the study. However, all patients were in clinical remission after the study period and there were no significant differences between control and test groups for all of the above-noted parameters. It should be noted that avenin antibodies were also tested in the same group of patients and were reported separately by Hollén *et al.* (2006a,b).

3. Hollén *et al.* (2006a) reported the results from the serology tests in the double blind study described above (Högberg *et al.*, 2004). The focus of the study was to evaluate the antibodies to oat prolamins (avenins). Sera were obtained from the study participants. IgA and IgG antiavenin antibodies were monitored at 0, 3, 6, and 12 months. Nitric oxide metabolites were measured in seven patients with deviating antibody results. There was a significant decrease in antiavenin antibodies in both groups (standard gluten-free diet and gluten-free diet plus oats) at the end of the study as compared to the beginning of the study ($p < 0.001$) but no difference was found between the two groups, suggesting that oats was not producing a humoral immune reaction. IgA titers declined after 3 months. IgG titers, although significantly decreased, remained high in the majority of patients in both groups. Nitric oxide levels were high in four of the analyzed samples. The authors indicated that this study does not exclude the possibility that some patients with celiac disease are susceptible to oats based on the sero-positive results and evidence of high levels of nitric oxide metabolites in some subjects.

4. Hollén *et al.* (2006b) reported the results from the urinalysis test in the double blind study described above (Högberg *et al.*, 2004). Urine samples were collected from 87 children and urinary nitrite/nitrate concentrations were monitored at 0, 3, 6, 9, and 12 months. There was a rapid decline in urinary nitrite/nitrate concentrations in both groups as early as 3 months. No differences were seen between the study groups at any of the checkpoints. However, at the end of the study, the nitrite/nitrate values of nine children in the gluten-free diet including oats group and eight children in the standard gluten-free diet group had not normalized. Children with celiac disease on a gluten-free diet with oats display a similar reduction in urinary nitrite/nitrate as those on a traditional gluten-free diet. Some children, however, still demonstrate high nitrite/nitrate excretion after 1 year on either diet, indicating that long-term follow-up studies of children on an oats-containing diet are needed.

5. Holm *et al*. (2006) conducted a 2-year controlled clinical trial. A total of 36 children who were over 7 years of age and were either previously diagnosed or had newly detected celiac disease were recruited for this study. Of these children, 32 consented and 4 refused because they found the protocol too laborious. In all patients, the diagnosis of celiac disease was based on the presence of small-intestinal mucosal severe, partial, or subtotal villous atrophy with crypt hyperplasia, and initially all had been serum endomysial antibody (EMA) positive. Twenty-three out of the 32 children were previously diagnosed with celiac disease and had been treated with a conventional strict gluten-free diet (avoiding wheat, rye, barley, and oats) for at least 2 years before they were included in the study. All children exhibited disease remission. These 23 patients were randomized either to undergo an open oat challenge or a gluten challenge which allowed the consumption of wheat, rye, and barley in addition to oats. The intake of oats was 50 g/day and patients in the gluten-challenge group ingested 20 g gluten/day. The purity of the oats (gluten-free) was confirmed by ELISA and PCR. In addition to the patients in remission, nine newly diagnosed children with celiac disease were included in the trial consuming a comparable gluten-free diet including oats. In all patients the following parameters were assessed: small-intestinal mucosal morphology, IELs, human leukocyte antigen D-related (HLA-DR) expression, and celiac serology. During the first 2 years on an oat-containing diet, clinical, nutritional, and serological assessments were carried out at 0, 1, 3, 6, 12, 18, and 24 months. Small bowel mucosal biopsies were evaluated at baseline and after 6 and 24 months. If the small bowel mucosal biopsy confirmed a relapse among the gluten-challenge group, the patients reverted to a gluten-free diet (avoiding wheat, rye, and barley) including the consumption of oats. Follow-up examinations of the gluten-challenge group were carried out similarly to the oats group until small bowel mucosal histological relapse was evident. After the relapse and commencement of an oat-containing gluten-free diet, examinations continued to be carried out in the same way as in the oats-challenge group. After the 2-year trial, patients continued to supplement their diets with commercially available oat products. The purity of these products was previously analyzed: 29 out of 30 tested samples had gliadin levels below 28 mg/kg (= 28 ppm) and only one was clearly wheat contaminated in excess of 200 ppm gluten. Follow-up visits after the 2-year trial included nutritional and serological assessments once a year or every other year for 7 years. In the long-term follow-up, small-intestinal biopsies were considered only if the patient's clinical condition or serology implied a relapse of the disease. The authors reported that oats had no detrimental effect on intestinal histology or serology of the children with celiac disease who were

in remission, during the 2-year trial. In contrast, the gluten-challenge group relapsed after 3–12 months. Complete recovery from the disease was accomplished in all patients on gluten-free diet plus oats. After the 2-year trial, 86% of the children preferred to continue to consume oats and they all remained in remission for the duration of the 7-year follow-up. This study permitted higher levels of oat ingestion (median 43 g/day and up to 81 g/day) than other studies conducted in children (median 15–24 g/day). The trial was conducted for 2 years and patients on the gluten-free diet plus oats were followed-up clinically for 7 years thereafter. The authors concluded that uncontaminated oats could be safely included in a gluten-free diet in the majority of children suffering from celiac disease. In their view, oats diversifies the gluten-free diet and children preferred it in their diet.

B. Summary of nonpivotal studies testing the effect of oats in patients with celiac disease by *in vitro* methods or serology (Table 6.2)

1. *In vitro*—duodenal mucosal cultures

1. This study was a continuation of the previous study (Srinivasan *et al.*, 1996). Duodenal biopsies from 26 patients were stained for lactase expression using an indirect immunoperoxidase method. Eleven disease control patients had normal architecture and nine had features of active celiac disease. Ten patients, who had celiac disease in clinical and histological remission, underwent oats challenge for 12 weeks. Confluent expression of lactase was observed in the 11 control patients with normal histology, whereas staining was absent in the nine patients with active celiac disease. All 10 patients with treated celiac disease had normal lactase expression after exposure to oats. The preservation of lactase enzyme after oats challenge further supports their previous findings of lack of oats toxicity.

2. Picarelli *et al.* (2001) used an *in vitro* model to test whether oats induce endomysial antibody production in the supernatant fluid of cultured duodenal mucosal specimens which were collected from 13 treated patients with celiac disease. The biopsy specimens were cultured with and without a peptic-tryptic digest (PT) of gliadin and avenin (from oats) and in medium alone. Samples from 5 of the 13 patients were cultured with the C fraction of PT-avenin. Indirect immunofluorescence was used to detect EMAs. These antibodies were detected in specimens from all 13 patients after the challenge with gliadin but not

after culture in medium alone or with PT-avenin or its C fraction. The authors concluded that PT-avenin and its C fraction did not induce EMAs in patients with celiac disease.

3. Kilmartin *et al*. (2003) investigated the immunogenicity of avenin using cytokines IFN-gamma (IFN-γ) and interleukin (IL)-2 as markers of immunological activity. In this study, duodenal biopsies from patients with celiac disease were cultured with 5 mg/ml of PT gliadin ($n = 9$) or 5 mg/ml of PT-avenin ($n = 8$) for 4 h. These biopsies were compared against control biopsies cultured with the medium alone and biopsies from nonceliac patients cultured with PT gliadin ($n = 8$) or avenin ($n = 8$). Cytokine mRNA was quantified by TaqMan PCR. Secreted cytokine protein was measured in the culture supernatant by ELISA. The authors found that after culture with PT gliadin, an increase in IFN-γ mRNA was observed in all nine patients with celiac disease. Increased IFN-γ protein was also found in four of these patients and smaller increases in IL-2 mRNA were detected in six of the subjects with celiac disease with a corresponding increase of IL-2 protein found in two of these patients. In contrast, the biopsies of patients with celiac disease cultured with PT-avenin did not show a significant response of IFN-γ or IL-2. Similarly, the biopsies from normal controls did not respond to either gliadin or avenin stimulation. The authors suggest that the immunogenic sequences in gliadin are not present in avenin. This suggestion supports the results of *in vivo* studies reporting that oats are safe for consumption by patients with celiac disease.

4. Arentz-Hansen *et al*. (2004) conducted an *in vitro* study using intestinal T cell lines. The study included nine adults with celiac disease who had a history of oats exposure. The oats were derived from a quality control production line and were shown to be free of contamination from other cereals. The selection of the study participants was not random. Five of the patients had also participated in a clinical challenge study consisting of 19 adults with celiac disease who ate 50 g of oats for 12 weeks (Lundin *et al.*, 2003). Four of the patients had clinical symptoms on an oat-containing diet and three of these four patients had intestinal inflammation typical of celiac disease at the time of oats exposure. The investigators established oats avenin-specific and -reactive intestinal T cell lines from these three patients, as well as from two other patients who appeared to tolerate oats. The avenin-reactive T cell lines recognized avenin peptides in the context of HLA-DQ2. These peptides have sequences rich in proline and glutamine residues closely resembling wheat gluten epitopes. The authors concluded that some patients with celiac disease have avenin-reactive mucosal T cells that

can cause mucosal inflammation. The authors point out that the T cell response to the avenin epitopes were found in T cell lines derived from intestinal biopsies of patients with celiac disease that were stimulated with gliadin (Vader *et al.*, 2003). It is unknown whether any of the patients from whom these T cells were isolated had clinical symptoms or mucosal inflammation related to ingestion of oats. The authors suggest that it will only be possible to establish the frequency of intolerance and possible complications with extended clinical follow-up of patients with celiac disease consuming oats. The authors indicate that their observations demonstrate that even if oats seem to be well tolerated by many patients with celiac disease, there are patients who have intestinal T cell responses to oats. They suggest that until the prevalence of oats intolerance in patients with celiac disease is well established, clinical follow-ups of patients eating oats is advisable.

5. Srinivasan *et al.* (2006) evaluated the response of the small intestine to oats by assessing the activation of the gastrointestinal immune system. This study involved 10 adults who ingested 50 g of oats daily in conjunction with an otherwise gluten-free diet for a 12-week period (Srinivasan *et al.*, 1996). The oat cereal used in this study was reported to be entirely free of contamination as tested by various methodologies, including reverse-phase HPLC, ELISA, and PCR. Patient compliance, clinical symptoms, and serology (IgG antigliadin and IgA anti-endomysial antibodies) were monitored throughout the study period. Duodenal biopsies were obtained by endoscope at the beginning and end of the study. After the 12-week trial, four of the patients were challenged with 500 mg gluten/day and another two patients with 10 g gluten/day for a period of 6 weeks. Duodenal biopsies obtained before and after the inclusion of oats in the diet were stained with a series of antibodies directed against the following molecules: HLA-DR, Ki67, CD25, and CD54 (intercellular adhesion molecule 1 (ICAM-1)) and mast cell tryptase. These detailed immunohistological studies of the biopsies did not reveal evidence of immune activation or morphological damage following the consumption of oats. On the other hand, all patients challenged with gluten (500 mg or 10 g/day) showed evidence of reactivation of the disease with various degrees of gastrointestinal symptoms, serological responses, and evidence of morphological changes in the duodenal biopsy. The authors concluded that under these trial conditions, the ingestion of oats did not show evidence of immunogenic or toxic effects on the duodenal mucosa of individuals with celiac disease and supported the viewpoint that oats is well tolerated by the majority of patients with celiac disease.

6. Kilmartin *et al*. (2006) conducted investigations on the etiological role of the wheat-related cereals, barley, rye, and oats, by examining the immune response of gliadin-reactive mucosal T cell lines from patients with celiac disease to fractions from all four cereals. The oats used in this study were free from wheat contamination. Cell stimulation was determined by measuring proliferation (employing 3H-thymidine incorporation) or cytokine (IL-2, IFN-γ) production. All five T cell lines demonstrated immunoreactivity to protein fractions from the four related cereals. In some cell lines, reactivity to wheat, barley, and rye was evident only when these cereal fractions had been pretreated with tissue transglutaminase. The authors concluded that this study confirms the similar T cell antigenic reactivity of these four related cereals, which has implications for their exclusion from gluten-free diets. However, they also indicated that despite oat stimulation of T cell lines, this cereal does not activate a mucosal lesion in most patients with celiac disease. The authors indicate that in this study, even when 5 mg/ml of avenin was added to the biopsy culture, there was no evidence of cytokine production. An equivalent amount of gliadin activated the celiac mucosa. Because avenin accounts for only 5–15% of the total protein in oats (whereas wheat, barley, and rye prolamins constitute 40–50%), it has been suggested by other investigators that large amounts of oats still may be toxic to patients with celiac disease. The authors argued against this and demonstrate that purified avenin is not immunogenic to the intestinal mucosa of patients with celiac disease. The authors noted from their results that the immunogenic sequences of gliadin were not present in avenin, which further supports the conclusion that oats are safe for consumption by patients with celiac disease.

2. *In vitro*—other

1. Silano *et al*. (2007a,b) investigated the immunogenic effect of avenins from four different cultivars of oats (Lampton from Australia, and Astra, Ava, and Nave from Italy), using peripheral lymphocytes from 10 children with celiac disease. Results were compared with the immunogenic response induced by wheat gliadin and rice prolamins. Lymphocyte proliferation and IFN-γ release in the culture medium were measured as indices of immune activation. The authors report that all the varieties of oats tested were immunogenic, with Lampton and Ava avenins inducing lymphocyte activation similar to that activated by wheat gliadin, while Astra and Nave avenins showed less immunogenicity, but still with a measurable effect. Sliano *et al*. (2007a) had previously tested some of these oat cultivars using an *in vitro* model

based on the ability of PT digests of celiac-active proteins to agglutinate K562 cells and to disrupt lysosomes, respectively. As discussed in these publications, the significance of these results to patients with celiac disease needs further clarification. The authors further indicate that the immunogenic ability of avenins does not necessarily mean that the oat-containing foods are toxic for patients with celiac disease and that in fact, in these experimental models, the gliadin encounter with lymphocytes is abnormal and only the alcohol-soluble protein fraction of oats has been tested. Therefore, the study cannot rule out that factors exerting a protective effect against avenin toxicity or interfering with avenin absorption have not been tested. The authors conclude that until there is more evidence to show the safety of oats and varieties of low-toxicity oats, patients with celiac disease consuming oats-containing foods should be carefully monitored.

3. Serology

1. In a study by Guttormsen *et al*. (2008) serum was collected from 136 adult patients with treated celiac disease and 139 controls. Patients were recruited from the Norwegian Celiac Disease Association and had a definite diagnosis of celiac disease, which was confirmed by small-intestinal biopsy. The study participants had been eating a strict gluten-free diet for at least 2 years. Eighty-two of the patients with celiac disease had been consuming oats as part of their gluten-free diet for 6 months or more. The participants with celiac disease completed a questionnaire that focused on: diagnosis, dietary habits, and clinical symptoms. Patients with celiac disease, who were eating oats, registered their intake of oats during a 3-week period. Patients were instructed to consume only ecologically grown oats specifically produced for patients with celiac disease. Otherwise there is no report of testing of the purity of oats. In addition to the questionnaire, IgA against oats avenin, wheat gliadin, and tissue transglutaminase were tested with ELISA. The authors report that no significant differences were found in IgA against oats in oats-eating and nonoats-eating patients with celiac disease. Both groups had increased levels of IgA against wheat, oats, and tissue transglutaminase compared to healthy controls. A significant positive correlation was found between antiavenin and antigliadin IgA ($p < 0.0001$), and between antiavenin and anti-tissue transglutaminase IgA ($p = 0.0012$). The authors conclude that ingestion of oats does not cause increased levels of IgA against oats in adult patients with celiac disease on a gluten-free diet. The findings support the notion that most adult patients with celiac disease can tolerate oats.

C. Other studies relevant to the effect of oats in patients with celiac disease

1. Peräaho *et al.* (2004b) conducted a retrospective evaluation beginning in 1997, on the consumption of oats within a gluten-free diet by patients with celiac disease and dermatitis herpetiformis in Finland. The use of oats, the effect of oats on symptoms of the illness, and quality of life were investigated in 1000 randomly selected members of the Finnish Celiac Society. Altogether, 710 patients responded to the questionnaire: 423 (73%) with celiac disease and 70 (55%) with dermatitis herpetiformis were currently consuming oats in their diets. Patients reported appreciating the taste, the ease of use, and the low cost of oats. Of the respondents, 94% believed that oats diversified the gluten-free diet. However, 15% of the patients with celiac disease and 28% of the patients with dermatitis herpetiformis reported that they had stopped eating oats. The most common reasons for avoiding oats were fear of adverse effects or contamination.

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REFERENCES

Abdallah, H., Leffler, D., Dennis, M., and Kelly, C. P. (2007). Refractory celiac disease. *Curr. Gastroenterol. Rep.* **9**, 401–405.

Abenavoli, L., Proietti, I., Leggio, L., Ferrulli, A., Vonghia, L., Capizzi, R., Rotoli, M., Amerio, P. L., Gasbarrini, G., and Addolorato, G. (2006). Cutaneous manifestations in celiac disease. *World J. Gastroenterol.* **12**, 843–852.

Akobeng, A. K. and Thomas, A. G. (2008). Systematic review: Tolerable amount of gluten for people with coeliac disease. *Aliment. Pharmacol. Ther.* **27**, 1044–1052.

Alaedini, A. and Green, P. H. (2005). Narrative review: Celiac disease: Understanding a complex autoimmune disorder. *Ann. Intern. Med.* **142**, 289–298.

Alaedini, A. and Green, P. H. (2008). Autoantibodies in celiac disease. *Autoimmunity* **41**, 19–26.

Andon, M. B. and Anderson, J. W. (2008). The oatmeal-cholesterol connection: 10 years later. *Am. J. Lifestyle Med.* **2**(1), 51–57.

Arentz-Hansen, H., Fleckenstein, B., Molberg, O., Scott, H., Koning, F., Jung, G., Roepstorff, P., Lundin, K. E., and Sollid, L. M. (2004). The molecular basis for oat intolerance in patients with celiac disease. *PLoS Med.* **1**, e1.

Barton, S. H. and Murray, J. A. (2008). Celiac disease and autoimmunity in the gut and elsewhere. *Gastroenterol. Clin. N. Am.* **37**, 411–428.

Bianchi, M. L. and Bardella, M. T. (2008). Bone in celiac disease. *Osteoporos. Int.* **19**, 1705–1716.

Briani, C., Samaroo, D., and Alaedini, A. (2008). Celiac disease: From gluten to autoimmunity. *Autoimmun. Rev.* **7**, 644–650.

Buchanan, R., Dennis, S., Gendel, S., Acheson, D., Assimon, S. A., Beru, N., Bolger, P., Carlson, D., Carvajal, R., Copp, C., Falci, K., Garber, E., *et al.* (2008). Approaches to establish thresholds for major food allergens and for gluten in food. *J. Food Prot.* **71**, 1043–1088.

Burrows, V. D. (2005). Hulless Oats. *In* ''Specialty Grains for Food and Feed'' (E-S. M. Abdel-Aal and P. Wood, eds.), pp. 223–251. American Association of Cereal Chemists, St. Paul, MN.

Case, S. (2008). Gluten-free diet. *In* ''A Comprehensive Resource Guide'' pp. 19–72. Case Nutrition Consulting, Regina, Canada.

Catassi, C. (2005). The world map of celiac disease. *Acta Gastroenterol. Latinoam.* **35**, 37–55.

Catassi, C. and Fasano, A. (2008). Is this really celiac disease? Pitfalls in diagnosis. *Curr. Gastroenterol. Rep.* **10**, 466–472.

Catassi, C., Fabiani, E., Iacono, G., D'Agate, C., Francavilla, R., Biagi, F., Volta, U., Accomando, S., Picarelli, A., De, V. I., Pianelli, G., Gesuita, R., *et al.* (2007a). A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. *Am. J. Clin. Nutr.* **85**, 160–166.

Catassi, C., Kryszak, D., Louis-Jacques, O., Duerksen, D. R., Hill, I., Crowe, S. E., Brown, A. R., Procaccini, N. J., Wonderly, B. A., Hartley, P., Moreci, J., Bennett, N., *et al.* (2007b). Detection of Celiac disease in primary care: A multicenter case-finding study in North America. *Am. J. Gastroenterol.* **102**, 1454–1460.

Ciclitira, P. J., Ellis, H. J., and Lundin, K. E. (2005). Gluten-free diet—what is toxic? *Best Pract. Res. Clin. Gastroenterol.* **19**, 359–371.

Collin, P., Maki, M., and Kaukinen, K. (2007). Safe gluten threshold for patients with celiac disease: Some patients are more tolerant than others. *Am. J. Clin. Nutr.* **86**, 260–261.

Cornell, H. J., McLachlan, A., and Cullis, P. G. (2002). Extraction of cereal prolamins and their toxicity in coeliac disease. *J. Biochem. Mol. Biol. Biophys.* **6**, 151–158.

Cranney, A., Zarkadas, M., Graham, I. D., Butzner, J. D., Rashid, M., Warren, R., Molloy, M., Case, S., Burrows, V., and Switzer, C. (2007). The Canadian Celiac Health Survey. *Dig. Dis. Sci.* **52**, 1087–1095.

Dewar, D. H., Amato, M., Ellis, H. J., Pollock, E. L., Gonzalez-Cinca, N., Wieser, H., and Ciclitira, P. J. (2006). The toxicity of high molecular weight glutenin subunits of wheat to patients with coeliac disease. *Eur. J. Gastroenterol. Hepatol.* **18**, 483–491.

Dickey, W. (2008). Making oats safer for patients with coeliac disease. *Eur. J. Gastroenterol. Hepatol.* **20**, 494–495.

Dickson, B. C., Streutker, C. J., and Chetty, R. (2006). Coeliac disease: An update for pathologists. *J. Clin. Pathol.* **59**, 1008–1016.

Dor, R. and Shanahan, D. J. (2002). Oats and coeliac disease. *Gut* **51**, 757–758.

Ellis, H. J. and Ciclitira, P. J. (2001). *In vivo* gluten challenge in celiac disease. *Can. J. Gastroenterol.* **15**, 243–247.

Ellis, H. J. and Ciclitira, P. J. (2008). Should coeliac sufferers be allowed their oats? *Eur. J. Gastroenterol. Hepatol.* **20**, 492–493.

Fasano, A., Berti, I., Gerarduzzi, T., Not, T., Colletti, R. B., Drago, S., Elitsur, Y., Green, P. H., Guandalini, S., Hill, I. D., Pietzak, M., Ventura, A., *et al.* (2003). Prevalence of celiac

disease in at-risk and not-at-risk groups in the United States: A large multicenter study. *Arch. Intern. Med.* **163**, 286–292.

Fasano, A., Araya, M., Bhatnagar, S., Cameron, D., Catassi, C., Dirks, M., Mearin, M. L., Ortigosa, L., and Phillips, A. (2008). Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition consensus report on celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **47**, 214–219.

Food And Drug Administration (2006). USA. Threshold Working Group On ''Approaches To Establish Thresholds For Major Food Allergens And For Gluten. March 2006. Http://Www.Cfsan.Fda.Gov/~Dms/Alrgn.Html.

Food And Drug Administration (2008). USA. Food Labelling:Health Claims;Soluble Fiber from Certain Foods and Risk of Coronary Disease. Federal Register 73, No. 85.

Food and Drug Administration., U.D.o.H.a.H.S. (2007). USA. Food Labeling, Gluten-free labelling of foods. Federal Register, Vol. 72, No 14.

Freeman, H. J. (2008). Neurological disorders in adult celiac disease. *Can. J. Gastroenterol.* **22**, 909–911.

Garsed, K. and Scott, B. B. (2007). Can oats be taken in a gluten-free diet? A systematic review. *Scand. J. Gastroenterol.* **42**, 171–178.

Gelinas, P., McKinnon, C. M., Mena, M. C., and Mendez, E. (2008). Gluten contamination of cereal foods in Canada. *Int. J. Food Sci. Technol.* **43**(7), 1245–1252.

Gianfrani, C., Troncone, R., and La, C. A. (2008). Autoimmunity and celiac disease. *Mini Rev. Med. Chem.* **8**, 129–134.

Green, P. H. and Cellier, C. (2007). Celiac disease. *N. Engl. J. Med.* **357**, 1731–1743.

Guandalini, S. (2007). The influence of gluten: Weaning recommendations for healthy children and children at risk for celiac disease. *Nestle Nutr. Workshop Ser. Pediatr. Program.* **60**, 139–151.

Guttormsen, V., Lovik, A., Bye, A., Bratlie, J., Morkrid, L., and Lundin, K. E. (2008). No induction of anti-avenin IgA by oats in adult, diet-treated coeliac disease. *Scand. J. Gastroenterol.* **43**, 161–165.

Haboubi, N. Y., Taylor, S., and Jones, S. (2006). Coeliac disease and oats: A systematic review. *Postgrad. Med. J.* **82**, 672–678.

Haines, M. L., Anderson, R. P., and Gibson, P. R. (2008). Systematic review: The evidence base for long-term management of coeliac disease. *Aliment. Pharmacol. Ther.* **28**, 1042–1066.

Hardman, C. M., Garioch, J. J., Leonard, J. N., Thomas, H. J., Walker, M. M., Lortan, J. E., Lister, A., and Fry, L. (1997). Absence of toxicity of oats in patients with dermatitis herpetiformis. *N. Engl. J. Med.* **337**, 1884–1887.

Harrison, M. S., Wehbi, M., and Obideen, K. (2007). Celiac disease: More common than you think. *Cleve. Clin. J. Med.* **74**, 209–215.

Hernando, A., Mujico, J. R., Mena, M. C., Lombardia, M., and Mendez, E. (2008). Measurement of wheat gluten and barley hordeins in contaminated oats from Europe, the United States and Canada by Sandwich R5 Elisa. *Eur. J. Gastroenterol. Hepatol.* **20**(6), 545–554.

Hill, P. G. and Holmes, G. K. (2008). Coeliac disease: A biopsy is not always necessary for diagnosis. *Aliment. Pharmacol. Ther.* **27**, 572–577.

Hoffenberg, E. J., Haas, J., Drescher, A., Barnhurst, R., Osberg, I., Bao, F., and Eisenbarth, G. (2000). A trial of oats in children with newly diagnosed celiac disease. *J. Pediatr.* **137**, 361–366.

Högberg, L., Laurin, P., Falth-Magnusson, K., Grant, C., Grodzinsky, E., Jansson, G., Ascher, H., Browaldh, L., Hammersjo, J. A., Lindberg, E., Myrdal, U., and Stenhammar, L. (2004). Oats to children with newly diagnosed coeliac disease: A randomised double blind study. *Gut* **53**, 649–654.

Hollén, E., Forslund, T., Högberg, L., Laurin, P., Stenhammar, L., Falth-Magnusson, K., Magnusson, K. E., and Sundqvist, T. (2006a). Urinary nitric oxide during one year of

gluten-free diet with or without oats in children with coeliac disease. *Scand. J. Gastroenterol.* **41**, 1272–1278.

Hollén, E., Holmgren, P. K., Sundqvist, T., Grodzinsky, E., Hogberg, L., Laurin, P., Stenhammar, L., Falth-Magnusson, K., and Magnusson, K. E. (2006b). Coeliac children on a gluten-free diet with or without oats display equal anti-avenin antibody titers. *Scand. J. Gastroenterol.* **41**, 42–47.

Holm, K., Maki, M., Vuolteenaho, N., Mustalahti, K., Ashorn, M., Ruuska, T., and Kaukinen, K. (2006). Oats in the treatment of childhood coeliac disease: A 2-year controlled trial and a long-term clinical follow-up study. *Aliment. Pharmacol. Ther.* **23**, 1463–1472.

Hopman, E. G., von Blomberg, M. E., Batstra, M. R., Morreau, H., Dekker, F. W., Koning, F., Lamers, C. B., and Mearin, M. L. (2008). Gluten tolerance in adult patients with celiac disease 20 years after diagnosis? *Eur. J. Gastroenterol. Hepatol.* **20**, 423–429.

Hopper, A. D., Hadjivassiliou, M., Hurlstone, D. P., Lobo, A. J., McAlindon, M. E., Egner, W., Wild, G., and Sanders, D. S. (2008). What is the role of serologic testing in celiac disease? A prospective, biopsy-confirmed study with economic analysis. *Clin. Gastroenterol. Hepatol.* **6**, 314–320.

Howdle, P. D. (2006). Gliadin, glutenin or both? The search for the Holy Grail in coeliac disease. *Eur. J. Gastroenterol. Hepatol.* **18**, 703–706.

Janatuinen, E. K., Pikkarainen, P. H., Kemppainen, T. A., Kosma, V. M., Jarvinen, R. M., Uusitupa, M. I., and Julkunen, R. J. (1995). A comparison of diets with and without oats in adults with celiac disease. *N. Engl. J. Med.* **333**, 1033–1037.

Janatuinen, E. K., Kemppainen, T. A., Pikkarainen, P. H., Holm, K. H., Kosma, V. M., Uusitupa, M. I., Maki, M., and Julkunen, R. J. (2000). Lack of cellular and humoral immunological responses to oats in adults with coeliac disease. *Gut* **46**, 327–331.

Janatuinen, E. K., Kemppainen, T. A., Julkunen, R. J., Kosma, V. M., Maki, M., Heikkinen, M., and Uusitupa, M. I. (2002). No harm from five year ingestion of oats in coeliac disease. *Gut* **50**, 332–335.

Jolobe, O. (2008). The changing face of coeliac disease and shared genetic traits. *Br. J. Hosp. Med. (Lond.)* **69**, 299.

Kemppainen, T., Janatuinen, E., Holm, K., Kosma, V. M., Heikkinen, M., Maki, M., Laurila, K., Uusitupa, M., and Julkunen, R. (2007). No observed local immunological response at cell level after five years of oats in adult coeliac disease. *Scand. J. Gastroenterol.* **42**, 54–59.

Kemppainen, T. A., Heikkinen, M. T., Ristikankare, M. K., Kosma, V. M., Sontag-Strohm, T. S., Brinck, O., Salovaara, H. O., and Julkunen, R. J. (2008). Unkilned and large amounts of oats in the coeliac disease diet: A randomized, controlled study. *Scand. J. Gastroenterol.* **43**, 1094–1101.

Kilmartin, C., Lynch, S., Abuzakouk, M., Wieser, H., and Feighery, C. (2003). Avenin fails to induce a Th1 response in coeliac tissue following *in vitro* culture. *Gut* **52**, 47–52.

Kilmartin, C., Wieser, H., Abuzakouk, M., Kelly, J., Jackson, J., and Feighery, C. (2006). Intestinal T cell responses to cereal proteins in celiac disease. *Dig. Dis. Sci.* **51**, 202–209.

Koning, F. (2008). Celiac disease: Sandwiched between innate and adaptive immune responses induced by gluten. *J. Pediatr. Gastroenterol. Nutr.* **46**(Suppl. 1), E8–E9.

Kumar, P. J. and Farthing, M. G. (1995). Oats and celiac disease. *N. Engl. J. Med.* **333**, 1075–1076.

Kupper, C. (2005). Dietary guidelines and implementation for celiac disease. *Gastroenterology* **128**, S121–S127.

Lester, D. R. (2008). Gluten measurement and its relationship to food toxicity for celiac disease patients. *Plant Methods* **4**, 26.

Lohi, S., Mustalahti, K., Kaukinen, K., Laurila, K., Collin, P., Rissanen, H., Lohi, O., Bravi, E., Gasparin, M., Reunanen, A., and Maki, M. (2007). Increasing prevalence of coeliac disease over time. *Aliment. Pharmacol. Ther.* **26**, 1217–1225.

Londei, M., Ciacci, C., Ricciardelli, I., Vacca, L., Quaratino, S., and Maiuri, L. (2005). Gliadin as a stimulator of innate responses in celiac disease. *Mol. Immunol.* **42**, 913–918.

Losowsky, M. S. (2008). A history of coeliac disease. *Dig. Dis.* **26**, 112–120.

Lundin, K. E., Nilsen, E. M., Scott, H. G., Loberg, E. M., Gjoen, A., Bratlie, J., Skar, V., Mendez, E., Lovik, A., and Kett, K. (2003). Oats induced villous atrophy in coeliac disease. *Gut* **52**, 1649–1652.

Makelainen, H., Anttila, H., Sihvonen, J., Hietanen, R. M., Tahvonen, R., Salminen, E., Mikola, M., and Sontag Strohm, T. (2007). The effect of beta-glucan on the glycemic and insulin index. *Eur. J. Clin. Nutr.* **61**(6), 779–785.

Maki, K. C., Davidson, M. H., Witchger, M. S., Dicklin, M. R., and Subbaiah, P. V. (2007a). Effects of high-fiber oat and wheat cereals on postprandial glucose and lipid responses in healthy men. *Int. J. Vitam. Nutr. Res.* **77**, 347–356.

Maki, K. C., Galant, R., Samuel, P., Tesser, J., Witchger, M. S., Ribaya-Mercado, J. D., Blumberg, J. B., and Geohas, J. (2007b). Effects of consuming foods containing oat beta-glucan on blood pressure, carbohydrate metabolism and biomarkers of oxidative stress in men and women with elevated blood pressure. *Eur. J. Clin. Nutr.* **61**, 786–795.

Malandrino, N., Capristo, E., Farnetti, S., Leggio, L., Abenavoli, L., Addolorato, G., and Gasbarrini, G. (2008). Metabolic and nutritional features in adult celiac patients. *Dig. Dis.* **26**, 128–133.

Mangione, R. A. (2008). Celiac disease and osteoporosis. *Am. J. Health Syst. Pharm.* **65**, 1601–1602.

Mantovani, M. S., Bellini, M. F., Angeli, J. P. F., Oliveira, R. J., Silva, A. F., and Ribeiro, L. R. (2008). β-Glucans in promoting health: Prevention against mutation and cancer. *Mutat. Res. Rev. Mutat. Res.* **658**, 154–161.

Martin, S. (2008). Against the grain: An overview of celiac disease. *J. Am. Acad. Nurse Pract.* **20**, 243–250.

Moron, B., Cebolla, A., Manyani, H., Alvarez-Maqueda, M., Megias, M., Thomas Mdel, C., Lopez, M. C., and Sousa, C. (2008). Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide. *Am. J. Clin. Nutr.* **87**, 405–414.

National Institute of Health (2004). NIH consensus development conference on celiac disease. *NIH Consens. State Sci. Statements* **21**, 1–23.

Pastore, L., Carroccio, A., Compilato, D., Panzarella, V., Serpico, R., and Lo, M. L. (2008). Oral manifestations of celiac disease. *J. Clin. Gastroenterol.* **42**, 224–232.

Pellicano, R., Astegiano, M., Bruno, M., Fagoonee, S., and Rizzetto, M. (2007). Women and celiac disease: Association with unexplained infertility. *Minerva Med.* **98**, 217–219.

Peräaho, M., Collin, P., Kaukinen, K., Kekkonen, L., Miettinen, S., and Maki, M. (2004a). Oats can diversify a gluten-free diet in celiac disease and dermatitis herpetiformis. *J. Am. Diet. Assoc.* **104**, 1148–1150.

Peräaho, M., Kaukinen, K., Mustalahti, K., Vuolteenaho, N., Maki, M., Laippala, P., and Collin, P. (2004b). Effect of an oats-containing gluten-free diet on symptoms and quality of life in coeliac disease. A randomized study. *Scand. J. Gastroenterol.* **39**, 27–31.

Picarelli, A., Di, T. M., Sabbatella, L., Gabrielli, F., Di, C. T., Anania, M. C., Mastracchio, A., Silano, M., and De, V. M. (2001). Immunologic evidence of no harmful effect of oats in celiac disease. *Am. J. Clin. Nutr.* **74**, 137–140.

Piper, J. L., Gray, G. M., and Khosla, C. (2002). High selectivity of human tissue transglutaminase for immunoactive gliadin peptides: Implications for celiac sprue. *Biochemistry* **41**, 386–393.

Pope, R. and Sheiner, E. (2009). Celiac disease during pregnancy: To screen or not to screen? *Arch. Gynecol. Obstet.* **279**, 1–3.

Presutti, R. J., Cangemi, J. R., Cassidy, H. D., and Hill, D. A. (2007). Celiac disease. *Am. Fam. Physician* **76**, 1795–1802.

Rashid, M., Cranney, A., Zarkadas, M., Graham, I. D., Switzer, C., Case, S., Molloy, M., Warren, R. E., Burrows, V., and Butzner, J. D. (2005). Celiac disease: Evaluation of the diagnosis and dietary compliance in Canadian children. *Pediatrics* **116**, e754–e759.

Rashid, M., Butzner, D., Burrows, V., Zarkadas, M., Case, S., Molloy, M., Warren, R., Pulido, O., and Switzer, C. (2007). Consumption of pure oats by individuals with celiac disease: A position statement by the Canadian Celiac Association. *Can. J. Gastroenterol.* **21**, 649–651.

Reunala, T., Collin, P., Holm, K., Pikkarainen, P., Miettinen, A., Vuolteenaho, N., and Maki, M. (1998). Tolerance to oats in dermatitis herpetiformis. *Gut* **43**, 490–493.

Reyna-Villasmil, N., Bermudez-Pirela, V., Mengual-Moreno, E., Arias, N., Cano-Ponce, C., Leal-Gonzalez, E., Souki, A., Inglett, G. E., Israili, Z. H., Hernandez-Hernandez, R., Valasco, M., and Arraiz, N. (2007). Oat-derived beta-glucan significantly improves HDLC and diminishes LDLC and non-HDL cholesterol in overweight individuals with mild hypercholesterolemia. *Am. J. Ther.* **14**, 203–212.

Ryan, D., Kendall, M., and Robards, K. (2007). Bioactivity of oats as it relates to cardiovascular disease. *Nutr. Res. Rev.* **20**(2), 147–162.

Sadiq Butt, M., Tahir-Nadeem, M., Khan, M. K., Shabir, R., and Butt, M. S. (2008). Oat: Unique among the cereals. *Eur. J. Nutr.* **47**, 68–79.

Schmitz, J. (1997). Lack of oats toxicity in coeliac disease. *BMJ* **314**, 159–160.

Shan, L., Molberg, O., Parrot, I., Hausch, F., Filiz, F., Gray, G. M., Sollid, L. M., and Khosla, C. (2002). Structural basis for gluten intolerance in celiac sprue. *Science* **297**, 2275–2279.

Shan, L., Qiao, S. W., Arentz-Hansen, H., Molberg, O., Gray, G. M., Sollid, L. M., and Khosla, C. (2005). Identification and analysis of multivalent proteolytically resistant peptides from gluten: Implications for celiac sprue. *J. Proteome Res.* **4**, 1732–1741.

Silano, M., Dessi, M., De Vincenzi, M., and Cornell, H. (2007a). *In vitro* tests indicate that certain varieties of oats may be harmful to patients with coeliac disease. *Eur. J. Gastroenterol. Hepatol.* **22**, 528–531.

Silano, M., Di Benedetto, R., Maialetti, F., De Vincenzi, A., Calcaterra, R., Cornell, H. J., and De Vincenzi, M. (2007b). Avenins from different cultivars of oats elicit response by coeliac peripheral lymphocytes. *Scand. J. Gastroenterol.* **42**, 1302–1305.

Srinivasan, U., Leonard, N., Jones, E., Kasarda, D. D., Weir, D. G., O'Farrelly, C., and Feighery, C. (1996). Absence of oats toxicity in adult coeliac disease. *BMJ* **313**, 1300–1301.

Srinivasan, U., Jones, E., Weir, D. G., and Feighery, C. (1999). Lactase enzyme, detected immunohistochemically, is lost in active celiac disease, but unaffected by oats challenge. *Am. J. Gastroenterol.* **94**, 2936–2941.

Srinivasan, U., Jones, E., Carolan, J., and Feighery, C. (2006). Immunohistochemical analysis of coeliac mucosa following ingestion of oats. *Clin. Exp. Immunol.* **144**, 197–203.

Størsrud, S., Hulthen, L. R., and Lenner, R. A. (2003a). Beneficial effects of oats in the gluten-free diet of adults with special reference to nutrient status, symptoms and subjective experiences. *Br. J. Nutr.* **90**, 101–107.

Størsrud, S., Olsson, M., Arvidsson, L. R., Nilsson, L. A., Nilsson, O., and Kilander, A. (2003b). Adult coeliac patients do tolerate large amounts of oats. *Eur. J. Clin. Nutr.* **57**, 163–169.

Thompson, T. (1997). Do oats belong in a gluten-free diet? *J. Am. Diet. Assoc.* **97**, 1413–1416.

Thompson, T. (2000). Questionable foods and the gluten-free diet: Survey of current recommendations. *J. Am. Diet. Assoc.* **100**, 463–465.

Thompson, T. (2001). Case problem: Questions regarding the acceptability of buckwheat, amaranth, quinoa, and oats from a patient with celiac disease. *J. Am. Diet. Assoc.* **101**, 586–587.

Thompson, T. (2003). Oats and the gluten-free diet. *J. Am. Diet. Assoc.* **103**, 376–379.

Thompson, T. (2004). Gluten contamination of commercial oat products in the United States. *N. Engl. J. Med.* **351**, 2021–2022.

Troncone, R., Auricchio, R., and Granata, V. (2008a). Issues related to gluten-free diet in coeliac disease. *Curr. Opin. Clin. Nutr. Metab. Care* **11**, 329–333.

Troncone, R., Ivarsson, A., Szajewska, H., and Mearin, M. L. (2008b). Review article: Future research on coeliac disease—A position report from the European multistakeholder platform on coeliac disease (CDEUSSA). *Aliment. Pharmacol. Ther.* **27**, 1030–1043.

Tully, M. A. (2008). Pediatric celiac disease. *Gastroenterol. Nurs.* **31**, 132–140.

Tye-Din, J. and Anderson, R. (2008). Immunopathogenesis of celiac disease. *Curr. Gastroenterol. Rep.* **10**, 458–465.

Upton, M. P. (2008). ''Give us this day our daily bread''—Evolving concepts in celiac sprue. *Arch. Pathol. Lab. Med.* **132**, 1594–1599.

Vader, L. W., de, R. A., van der, W. Y., Kooy, Y. M., Benckhuijsen, W., Mearin, M. L., Drijfhout, J. W., van, V. P., and Koning, F. (2002). Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J. Exp. Med.* **195**, 643–649.

Vader, L. W., Stepniak, D. T., Bunnik, E. M., Kooy, Y. M., de, H. W., Drijfhout, J. W., Van Veelen, P. A., and Koning, F. (2003). Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology* **125**, 1105–1113.

Wieser, H. and Koehler, P. (2008). The biochemical basis of celiac disease. *Cereal Chem.* **85**(1), 1–13.

Zarkadas, M. and Case, S. (2005). Celiac disease and the gluten-free diet. *Clin. Nutr.* **20**, 127–138.

Zarkadas, M., Cranney, A., Case, S., Molloy, M., Switzer, C., Graham, I. D., Butzner, J. D., Rashid, M., Warren, R. E., and Burrows, V. (2006). The impact of a gluten-free diet on adults with coeliac disease: Results of a national survey. *J. Hum. Nutr. Diet.* **19**, 41–49.