Is porridge still on the menu?
Clarifying the safety of oats in coeliac disease

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15th March 2018

Coeliac UK Research Conference

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Coeliac Australia

ImmusanT
DOMINANCE IN IMMUNOTHERAPY

Disclosure: Consultant & Inventor on patents

Coeliac disease: an immune-mediated systemic disorder elicited by gluten in genetically susceptible people

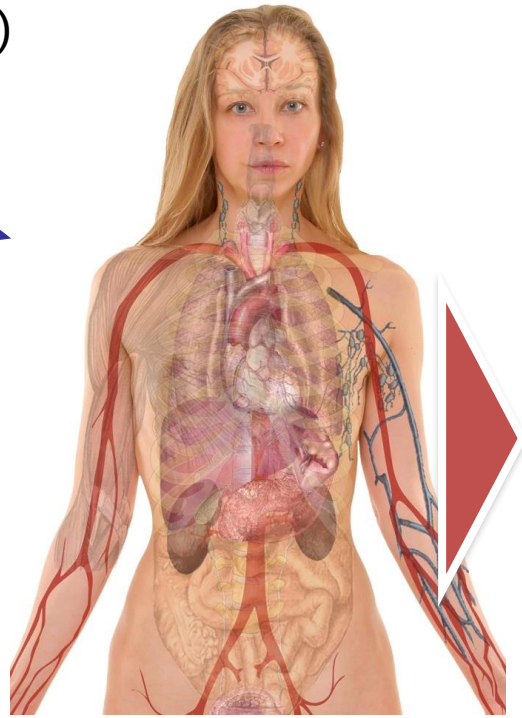
Dietary gluten

•Wheat, rye, barley (? oats)

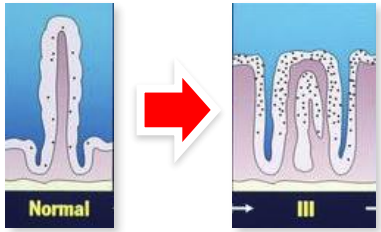
HLA-DQ2/8 and other genes

T cells to gluten

Environmental factors

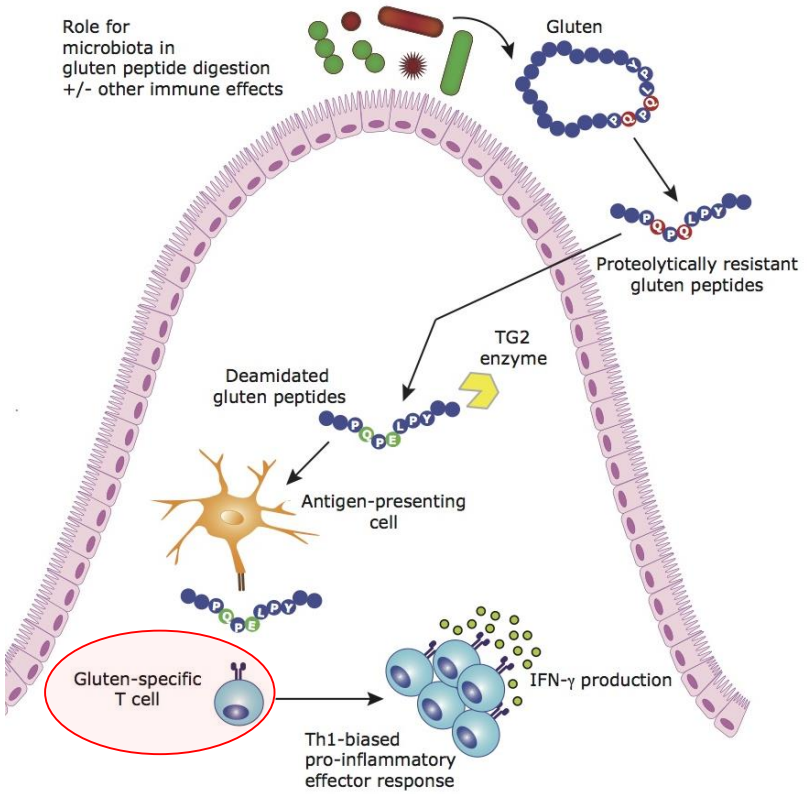


- Transglutaminase-IgA
- Villous atrophy:



- **Elevated morbidity and mortality**
- **Impaired quality of life**

Pathogenic CD4+ T cells targeting specific gluten peptides drive disease



Hardy and Tye-Din, Clinical & Translational Immunology 2016

- The gluten-specific T cell is a key player in coeliac pathogenesis

- HLA-restricted CD4+ gluten-specific T cells can be found:
  - in the small intestine of people with coeliac disease *(Lundin et al, JEM 1993)*
  - in the blood of people with coeliac disease following a 3-day oral gluten challenge *(Anderson et al, Nat Med 2000)*

- Detection of these T cells may provide a basis for novel diagnosis of coeliac disease *(Ontiveros Clin Exp Immunol 2014; Brottveit, Am J Gastro 2011; Sarna et al. Gut 2017)*

- Studies of T cells from the intestine and blood of coeliac patients has defined a series of immunogenic peptides (epitopes)
(Sjostrom 1998; van de Wal 1998, Arenzt-Hansen 2000, 2002; Vader 2002, 2003, Qiao 2005; Tollefsen 2006; Tye-Din 2010, Kooy-Winkelaar 2011; Bodd 2012)

- Three peptides from wheat, barley and rye account for most of the immune response to gluten in HLA-DQ2.5+ coeliac disease - basis for Nexvax2 *(Tye-Din et al, Science Transl Med 2010)*

Oats safety – why should we care?

- Oats are a desirable grain to add to the restrictive gluten free diet
- There remains some lingering uncertainty about its safety for some people with coeliac disease
- We now have the understanding and technology (immunology and cereal science) to conduct feeding trials that link a molecular understanding of oats with clinical endpoints
- In Australia and NZ, oats are still *excluded* from the gluten free diet (FSANZ code) – patients here are desperate to eat them!
    - In select situations, we may allow people to have oats following a 3 month oat challenge with pre- and post-biopsies – but this is cumbersome and unreliable

Benefits of oats in the gluten free diet

- Highly nutritious cereal
  - Quality protein with good amino acid balance
  - Good source of resistant starch and fiber (soluble and insoluble) e.g. beta-glucan
  - Good source B group vitamins, iron, zinc, magnesium, phosphorus and phytochemicals e.g. avenanthramide
- Provides palatability and diversity of the GFD
- Increases satiety and helps reduce the use of fat and sugar
- Reduces postprandial blood sugar and insulin response
- Can reduce LDL cholesterol
- Can help manage constipation
- May reduce risk of cardiovascular disease, obesity/metabolic syndrome, hypertension, and cancer

Rasane et al. J Food Sci Technol 2015

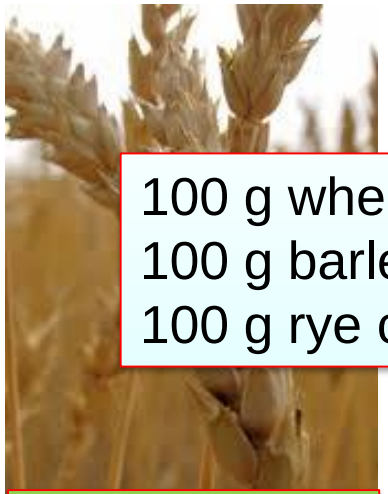
Same family as wheat, rye and barley but different tribes

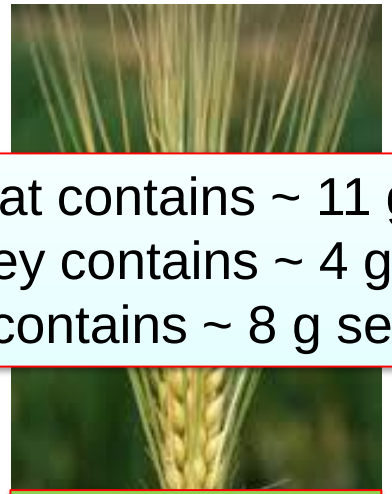
Rice, corn, millet

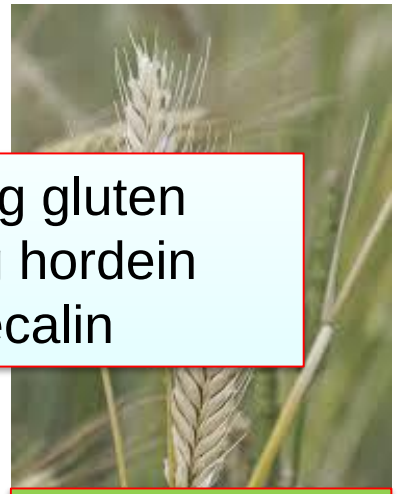
Pooideae

Triticeae

Aveneae







100 g wheat contains ~ 11 g gluten
100 g barley contains ~ 4 g hordein
100 g rye contains ~ 8 g secalin

Gluten

Hordein

Secalin

Triticum aestivum (Wheat)

Hordeum vulgare (Barley)

Secale cereale (Rye)

100 g oats contains ~ 1 g avenin

Avenin

Avena sativa (Oats)

Clinical studies generally support safety

- Toxicity concerns first raised with the work of Dicke, Weijers and van de Kamer *(Acta Paediat 1953)*

- Early studies did not control for potential oat contamination so may be unreliable - a single grain of wheat in 200 gm of oats can result in a wheat gluten level of > 100 ppm

Controlled trials n=661 patients

Observational studies

Study , Yr	Country	Yr	Ref	Study design	Age category	N	Amount of oats (g)	Source of oats	Length of treatment (months)	Outcomes				
										GI	Serology	Histology	IELs	DH
RCTs														
Gatti	Italy	2013	24	1	Children	306	**unclear**	GF	6					
Hogberg	Sweden	2004	23	1	Children	116	moderate	pure	12					
Holm K	Finland	2006	25	1	Children	32	moderate	GF	24					
Janatuinen	Finland	1995	26	1	Adults	92	moderate	GF	12					
Kemppainen	Finland	2008	27	1	Adults	32	large	GF	12					
Peraaho	Finland	2004	11	1	Adults	39	moderate	GF	12					
Non-RCTs														
Reunala	Finland	1998	28	2	Adults	23	moderate	GF	6					
Srinivasan	Ireland	1999	29	2	Adults	21	**unclear**	**unclear**	3					
Baker	UK	1976	7	3	11 adults 1 children	12	moderate	pure	1					
Cooper	Ireland, UK	2012	34	3	Adults	54	moderate	pure	12					
Hardman C.	UK	1987	33	3	Adults	10	moderate	pure	3					
Hoffenberg	US	2000	37	3	Children	10	moderate	GF	6					
Lundin	Norway	2003	38	3	Adults	19	moderate	pure GF	3					
Sev	Canada	2011	39	3	Adults	15	moderate	pure	3					
Srinivasan	Ireland	1996	35	3	Adults	10	moderate	Pure	3					
Srinivasan	Ireland	2006	36	3	Adults	10	moderate	Pure	3					
Storsrud	Sweden	2003	40	3	Adults	20	large	GF	24					
Storsrud1	Sweden	2003	41	3	Adults	20	large	GF	24					
Guttormsen	Norway	2008	44	4	Adults	170	moderate	pure	**unclear**					
Kaukinen	Finland	2013	45	4	Adults	110	small	**unclear**	60					
Tapsas 2	Sweden	2014	29#	4	Children	316	**unclear**	**GF and no GF**	NA					
Tuire	Finland	2012	46	4	Adults	177	**unclear**	**unclear**	NA					
Janatuinen	Finland	2000	30	5	Adults	92	moderate	GF	12					
Koskinen	Finland	2009	31	5	Children	23	moderate	GF	24					
Sjoberg	Sweden	2014	32	5	Children	28	moderate	pure	12					
Janatuinen	Finland	2002	43	6	Adults	63	moderate	**unclear**	60					
Kemppainen	Finland	2007	42	7	Adults	44	moderate	**unclear**	60					

NOTE. Study design: 1 = Randomized controlled trial; 2 = Non-randomized controlled trial, 3 = Before and after comparison; 4 = Cross-sectional; 5 = Post hoc from RCT; 6 = Cohort; 7 = Post hoc cohort.
Green: no change in outcome after oats consumption, *yellow:* change of outcome in low proportion of patients; *red*: significant worsening after oat consumption.
GF, gluten-free; GI, gastrointestinal symptoms.

Pinto-Sanchez et al. Gastroenterology 2017

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Aaltonen et al. Nutrients 2017

- 82% Fins with CD consuming oats. 10 year follow-up.
- Clinical outcomes no different to non-oat consumers. ? Better QOL scores.

Lionetti et al, Oats in children: DBPCRCT. J Pediatrics 2018

- 79 oats vs 98 placebo-oats; 15 month; crossover design
- No change in symptoms, serology, intestinal permeability scales

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Pinto-Sanchez et al. Gastroenterology 2017

Controlled trials n=661 patients

Observational studies

- **Some studies have shown intestinal deterioration with oats:**
  - Villous atrophy/rash in 1 patient of 19 adults having oats 50g/d for 12 weeks; inflammatory markers elevated in intestine in 5 *(Lundin et al, Gut 2003)*
  - Intraepithelial lymphocytosis *(Peraaho et al. Scand J Gastroenterol 2004; Ilus et al. Am J Gastroenterol 2012; Tuire et al. Am J Gastroenterol 2012)*
  - Inflammatory cytokine (mRNA) profile *(Sjoberg et al. Clin Transl Gastroenterol 2014)*

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Pinto-Sanchez et al. Gastroenterology 2017

Systematic review (Pinto-Sanchez et al. *Gastroenterology* 2017)

- Most studies support clinical safety of oats ingestion…*however*,
- Quality of RCT rated as “very low” - attrition bias, imprecision
- 2 of the 6 “before and after” comparison studies reported more frequent gastrointestinal symptoms after oats intake
- **Limitations:**
  - small sample sizes; no RCTs outside Europe – cannot extrapolate
  - unclear assessment of compliance to a GFD
  - unclear if symptoms related to oats avenin or fibre
  - oats cultivar usually not described - several *in vitro* studies suggest cultivars can vary substantially in toxicity *(Maglio et al. Scand J Gastroenterol 2011; Silano et al, J Gastroenterol Hepatol 2007; Silano et al Scand J Gastroenterol 2007; Silano et al. Eur J Nutr 2014; Comino et al, Gut 2011)*
- Study withdrawals, often due to adverse gastrointestinal symptoms and the inability to maintain the oats diet, may have underestimated the true adverse impact of oats
- Recruitment bias may lead to oats sensitive participants avoiding oats feeding studies

Consensus: more data is needed

- “Our confidence is limited by the low quality and limited geographic distribution of the data…Rigorous double blind, placebo controlled, randomized controlled trials, using commonly available oats sourced from different regions, are needed.”

  (Safety of adding oats to a gluten free diet for patients with celiac disease: systematic review and meta-analysis of clinical and observational studies. Pinto-Sánchez MI, Causada-Calo N, Bercik P, Ford AC, Murray JA, Armstrong D, Semrad C, Kupfer SS, Alaedini A, Moayyedi P, Leffler DA, Verdú EF, Green P. **Gastroenterology** 2017)

- “…incorporating oats into a GFD is a more complex issue than previously suggested. Further work is required on development of oat cultivars with low avenin content or low immunogenicity…accurate assays to detect avenins in oat products…and also identification and follow-up of those with ‘oat intolerance’.”

  (The gluten-free diet and its current application in coeliac disease and dermatitis herpetiformis. Ciacci C, Ciclitira, P, Hadjivassiliou, M, Kaukinen, K, Ludvigsson, JF, McGough, N, Sanders, DS, Woodward, J, Leonard, JN, Swift, GL **United European Gastroenterol J** 2014)

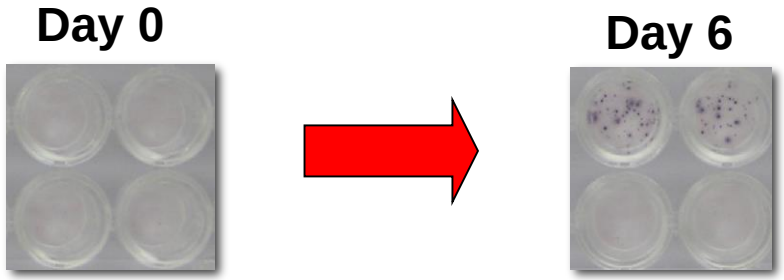
Important insights from immune studies

- Several oat avenin peptides PYPEQEEPF (DQ2.5-ave-1a) and PYPEQEQPF (DQ2.5-ave-1b) predicted to immunogenic based on their sequence homology to known wheat immunogenic peptides *(Vader et al. Gastroenterology 2003)*

- Intestinal T cells from people with coeliac disease recognise these avenin peptides

  (Arentz-Hansen et al. PloS Medicine 2004)

- What about T cells from blood?
  - Gluten-specific T cells can be detected in blood in people with coeliac disease after wheat, rye or barley is consumed



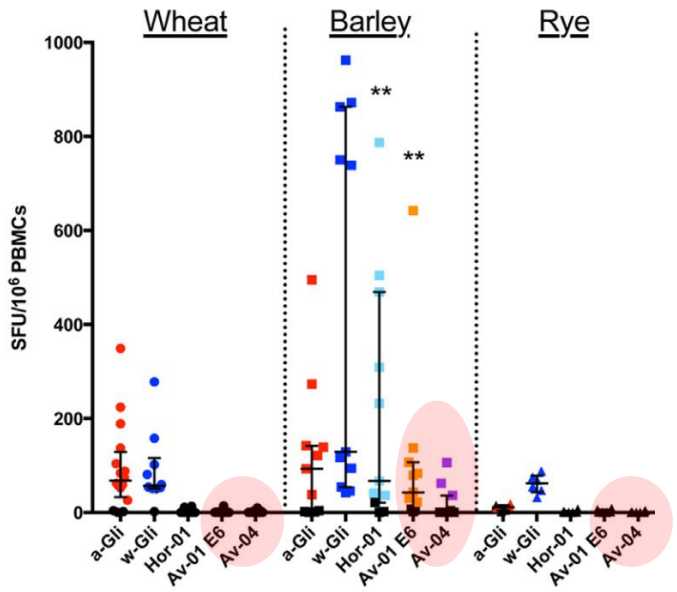
(Anderson, Nat Med 2000)

  - ***What happens after oats is consumed?***

- 100 g pure dry oats (cooked into porridge) consumed daily for 3 days
- Blood assessed for oat specific responses on day 6
- **Findings**:
  - 6 out of 73 (8%) CD participants had significant T cell responses to specific oats peptides (including DQ2.5-ave-1a and DQ2.5-ave-1b)
  - 50% of CD participants had GI symptoms but no correlation with T cell responses
  - These stimulatory oat peptides were very similar to barley peptides

Oats: PYPEQEQPI
Barley: P**I**PEQ**P**QP**Y**

…but were more susceptible to digestion and bound less stably to HLA-DQ2

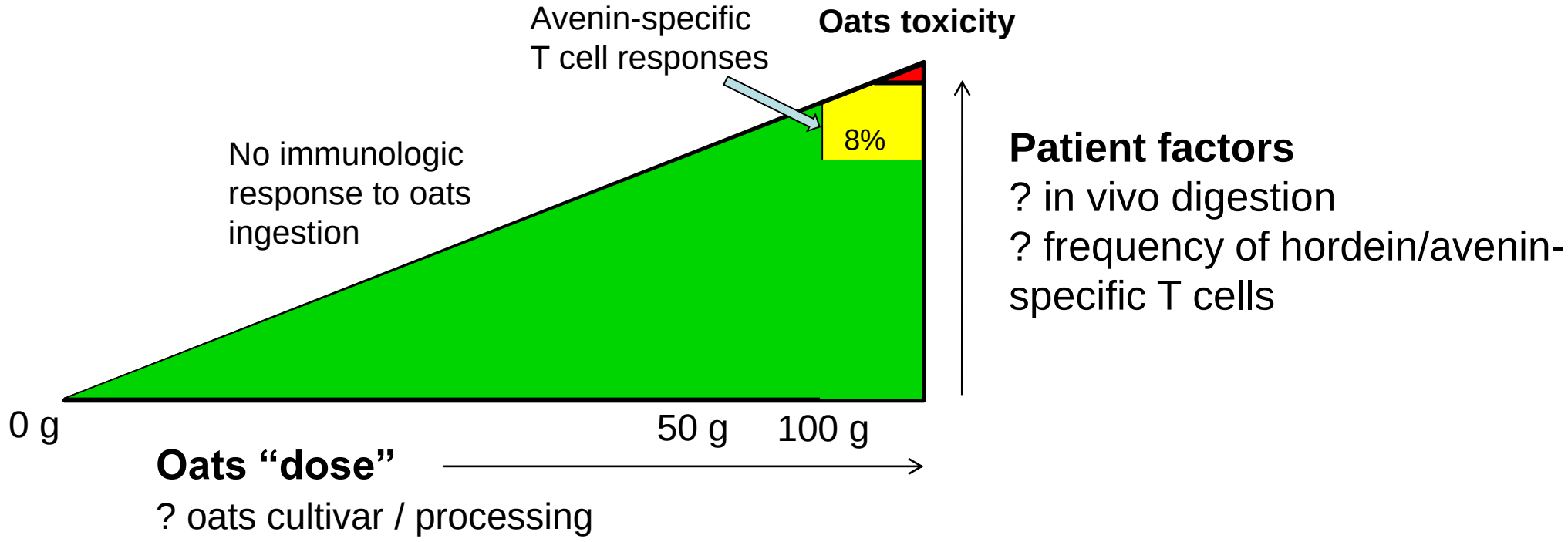


Hardy et al. J Autoimm 2015

Conclusions:

- Pure oats induce a T cell response to avenin in a proportion of people with coeliac disease
- Avenin is less immunogenic and has reduced bioavailability
- Most HLA-DQ2.5+ CD patients harbor T cells capable of being activated by avenin peptides, but ingestion of oats itself provides rather weak antigenic stimulation for these T cells

Hypothesis: Avenin-specific T cells are ubiquitous - not idiosyncratic; all people with coeliac disease could potentially respond to oats, but at the amounts typically consumed this is unlikely



Questions to resolve

- What is the clinical significance of avenin-specific T cells?
  - Do they mediate pathogenic responses to oats?
  - If so, can their presence be exploited to develop a simple diagnostic for ‘oats sensitivity’?
  - ➢ Compare clinical outcomes in “responders” (avenin-specific T cell positive) and non-responders (avenin-specific T cell negative) volunteers

- Can a safe oat threshold be defined for everyone with coeliac disease?

➢ Assess a variety of doses of oats with immune/serologic/histologic outcomes

- What is the impact of different oat cultivars?

➢ Assess a variety of cultivars

- What is the significance of symptoms after oats?
  - Is it related to a true avenin effect or something else e.g. fibre?

➢ Undertake a blinded placebo-controlled randomised feeding trial (control for fiber load)

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The challenge - Only 8% of volunteers with coeliac disease have T cell responses….

THE PLACE OF OATS IN THE COELIAC DIET

BY

A. LINDSAY C. MOULTON

From the Department of Paediatrics and Child Health, University of Birmingham, and Birmingham Children's Hospital

(RECEIVED FOR PUBLICATION JUNE 21, 1958)

Since the discovery by Dicke (1950) that remarkable benefit followed the withdrawal of wheat and rye from the diet of the coeliac child, much work has been done on the significance of these and other foodstuffs in the coeliac syndrome. Few of these studies, however, involved oats, a food containing a prolamin similar to the gliadin of wheat which was proved to be injurious by van de Kamer, Weijers and Dicke (1953).

In 1955, van de Kamer and Weijers suggested that the effects of various foodstuffs on the coeliac patient are related to their amide:non-amide nitrogen ratios and they compiled a list of foods arranged according to this ratio. In many respects their series agrees with the known effects of the foods in coeliac disease. Oats and oatflakes are of interest as they are placed on the margin between the harmful and non-harmful foods.

Previous workers on oats, Dicke, Weijers and van de Kamer (1953), and van de Kamer *et al.* (1953), who fed the same child 140 g. oatmeal per day, and Hansted (1955), who gave one child 150 g. oatmeal per day, claim that such large intakes have some harmful effect. However, in another patient, 75 g. oatmeal per day produced only a slight increase in fat excretion (Hansted, 1955). The effect of still smaller amounts on two groups of three patients each was investigated by Sheldon (1955). The first group received 2 oz. (56 g.) oatmeal per day and the second 1 oz. (28 g.). Despite the short duration of his experiments, he concludes that oatmeal, at these more normal levels, has no ill effect on coeliac children. Frazer (1956) reports that his team found no deleterious effect of oats, but details are not given.

Hansted (1956a) suggests that either a high intake of oats for a short time or a small intake over an extende
equival
excretic
focused on this suggestion in the present investigation. Four cases only have been studied as the continuous fat balances necessary to demonstrate any resulting slight upward trend in fat excretion are very time consuming. A further problem is that high intakes of oats are not easily maintained, as the child becomes tired of the flavour and overwhelmed by the bulk of the food.

The possibility of using oat protein itself was explored but, although several methods of preparation were attempted, considerable difficulties were encountered. Oats do not yield a gluten, although they contain a gliadin and a glutelin similar to those found in the gluten of wheat. Thus preparation of protein by merely washing gluten free from starch is not feasible. No concentrated oats in the form of protein have been used in the present investigation, though dry separation of oat protein particles by means of a micronizer would appear to be the most suitable method of preparation. If in future experiments large quantities of oats prove too much for a child's appetite, replacement with equivalent amounts of this oat protein could be attempted.

Methods and Patients

Continuous fat balance studies over relatively long periods were carried out, fat being estimated daily in the stools by the method of van de Kamer, Huinink and Weijers (1949). Any uneaten food was weighed, analysed for fat and its oat content calculated. Three-day sliding means of the faecal fat excretion and the percentage fat absorption were calculated. In addition, seven-day means of fat intake, fat excretion, percentage fat absorption and oat intake were obtained as these figures show more clearly the general trend of the results. Both for this reason and because of the rather lengthy results in certain cases, only the seven-day means have been charted (Figs. 1-4).

Four coeliac children, diagnosed according to the criteria described by Smellie (1956), were fed on a diet which was gluten-free apart from the content of Three
Bears Oats (quick porridge oat flakes; fat content 6·3%;
s the nitrogen
ir, 1946), one
The quantities
of oatflakes fed were 46, 70, 163 and 169 g. per day, the lengths of the trials being 45, 96, 22 and 23 days respectively.

Archives of Disease in Childhood, 1958

Fructan, Rather Than Gluten, Induces Symptoms in Patients With Self-Reported Non-Celiac Gluten Sensitivity

Gry I. Skodje,[1,2,4] Vikas K. Sarna,[2,3] Ingunn H. Minelle,[4] Kjersti L. Rolfsen,[4] Jane G. Muir,[5] Peter R. Gibson,[5] Marit B. Veierød,[4,6] Christine Henriksen,[2,4] and Knut E. A. Lundin[2,3,7,8]

[1]Division of Cancer Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [2]K. G. Jebsen Celiac Disease Research Centre, University of Oslo, Norway; [3]Department of Immunology and Transfusion Medicine, Oslo University Hospital, Oslo, Norway; [4]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway; [5]Department of Gastroenterology, Monash University and Alfred Hospital, Melbourne, Victoria, Australia; [6]Oslo Centre for Biostatistics and Epidemiology, Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; [7]Department of Gastroenterology, Oslo University Hospital Rikshospitalet, 0424 Oslo, Norway; and [8]Centre for Immune Regulation, University of Oslo, Oslo, Norway

Research plan

1. Extract pure (uncontaminated) avenin for use in feeding studies

Extracting food-grade oat avenin (Jan-Feb 2018)

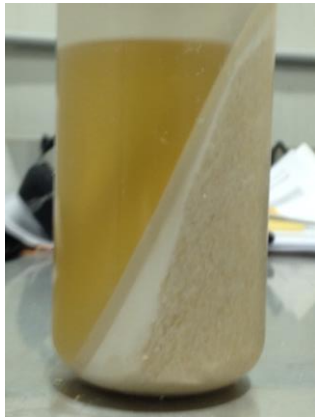
200kg pure, wheat-free oats (Western Australia)

Milled in a wheat free facility (Melbourne)

Wheat free processing facility (NSW)

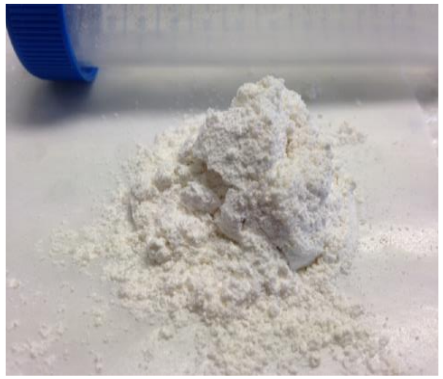
300L of 50% alcohol

Suspension stirred, decanted and centrifuged



Clear supernatant decanted, pooled then chilled at 4C for 2 days

Chilling at 4C precipitates the avenin; collected by centrifugation



- Freeze dried avenin (Melbourne) - 1.2 kg!
- Purity 90% based on Western blot
- Mass spec being run to determine avenin peptide content

Research plan

1. Extract pure (uncontaminated) avenin for use in feeding studies

2. Blinded RCT
   - Gluten free rice porridge +/- avenin spiked in
   - 6 week challenge
   - Avenin tested at - zero (placebo)
     - low dose (0.5g/day = 50g oats)
     - high dose (6g/day = 600g oats)
   - Clinical readouts: Daily CD symptom diary e.g. CeD-PRO, coeliac serology, duodenal histology
   - Immune readouts: ELISpot assay, avenin-specific tetramer, blood and intestinal cytokines
   - 15 cultivars of oats (UK, Europe, USA, Australian) will be assessed in avenin-specific T cell studies to compare immunogenicity

Conclusions

- Most studies supports clinical safety of oats for the majority of people with coeliac disease
- Reassure patients, but remind them that clinical follow-up remains important
- Some people with coeliac disease (a minority) may be more sensitive to oats
- A pure avenin controlled feeding study currently underway may help address some important outstanding issues regarding oats immunity and safety

Acknowledgements

Dr Melinda Hardy
Ms Amy Russell
Immunology Division
Clinical Translation Centre

Ms Cathy Pizzey
Dr Emma Halmos
Gastroenterology Department

Prof Don Cameron
Prof Katie Allen

Dr Greg Tanner
Dr Frank Bekes
Mr Kim Rintoul
Dr Amy Barrie and team (Manildra)

All the volunteers with coeliac disease who participated in our research