We can follow the monocots down to the Gramineae or the grass family.

Here, we have only wheat, rye and barley that are toxic. Triticale is a cross between wheat and rye, and so would be expected to be toxic.

Now, oats I have had to put in both columns, and I will explain why. There are many other grasses in which the grains do not have toxic proteins as far as we know.

There are only two grains that have been studied with modern methods and modern approaches to understanding their relationship to celiac disease, and that is wheat and oats.

These others have often been studied very minimally including rye and barley, but rye and barley do contain proteins that have sequences quite close to those in wheat.

We assume that rye and barley are probably toxic grains according to the early results of Dicke in the Netherlands back around 1950, where they considered rye, barley and oats as part of the toxic group.

Now about 15 years ago, I suggested that if there are only a few grasses that contain the toxic sequences, and they are closely related as you will see, and then there are many other grasses that do not contain the toxic sequences.

I suggested that if you get into the dicot group -- the buckwheat, quinoa, amaranth, and these other grains -- it would not be toxic, simply because of their distant taxonomic relationship to wheat.

I was a little bit apprehensive about suggesting this, but over the past 15 years since I suggested this, people have been eating these other grains. As far as I know, there hasn't been any serious indication that these do, in fact, have toxicity for celiac patients.

There have been some very fine studies from Finland and throughout the world, indicating that oats were safe for celiac patients. But towards the end of last year the Oslo, Norway, group under Knut Lundin and Ludvig Sollid. They have found -- well, I'm getting a little bit ahead

of myself. Let's deal with this slide first.

(Slide.)

DR. KASARDA: If we take a subfamily, festucoidiae, of the grass family and we look at the tribal level, and I made this slide before the results from Oslo were published. The hordeae -- which includes wheat, rye, and barley -- were one tribe. I thought that oats were probably non-toxic, so I put them in -- well, they belong in a separate tribe. It was only this one tribe that had the toxic sequences.

Now, it is pretty certain that the oats are toxic to a few probably rare individuals, but we don't really know how this works out. They found three celiac patients who definitely reacted to oats by the same mechanism that they reacted to gliadin peptides. This, I think, was pretty well demonstrated by the work from Norway.

Now, here I show the proteins that you find in wheat, gamma-type gliadins and related low-molecular-weight glutenin subunits, they are found in rye, barley, not in oats. Alpha-type

gliadins are found in wheat, but you don't find them in rye and barley or in oats, and so on down the line.

Now, the avenins are a small fraction of the total proteins in oats. These make up only about 10 percent of the protein. Most of the protein is oat globulin, which as far as we know if not harmful or toxic to celiac patients. There are low-molecular weight proteins related to the avenins in rye, barley, and wheat.

(Slide.)

DR. KASARDA: Now, this is another one of those sequence slides, but let me just try to make a few points here. This top sequence is a gliadin sequence. It starts here (indicating) and runs down to here.

The bottom sequence is an avenin, which shows a lot of homology with the C-terminal half of this gamma-gliadin molecule. This is also true for the alpha gliadins. Where the amino acids are the same, I have colored them in blue.

There is a lot of homology here in the

C-terminal half, but that is not where the toxicity lies. The Oslo group has shown that this particular sequence, which I have underlined, does have certain characteristics including glutamic acid at key positions that are important, as Dr. Murray pointed out, for binding to MHC proteins.

Consequently, most of the repeat region is absent from the avenins. There is just this sort of residual section here, which does have a lot of glutamine and a lot of proline and some key glutamic acid sequences or amino acids that seem to be responsible in these few patients that have been studied for the toxicity. This sequence is certainly capable of stimulating T-cell clones from these patients.

I think that the evidence is pretty good that there are at least a few -- it seems as though there are probably rare individuals who respond to oats and probably most celiac patients do not respond to oats.

This is a rather puzzling situation, because I have always thought of the proteins as

being pretty definitive for celiac disease, that all celiac patients reacted to wheat and probably to rye and barley, and that this was part of the definition of celiac disease.

Now we have a situation here where it appears that some patients react to oats and some don't. This is some ongoing research that needs some elucidation.

(Slide.)

DR. KASARDA: Just to jump back into the classification, this is another subfamily, panacoideae. Here we have maize and sorghum and millet. We have actually done a little bit of end-terminal sequencing on sorghum and millet proteins, and they do seem fairly close to the maze protein. This would explain to some degree why they are not toxic in celiac disease. As far as we know, it is still wheat, rye, and barley that are harmful.

(Slide.)

DR. KASARDA: Now, this is my last slide. The currently favored method for determination of

gluten in food is the R5 monoclonal antibody ELISA test developed by Mendez in Spain. This seems to be a pretty good test; it is not perfect.

The antibody reacts to monomeric wheat, rye and barley prolamins, but not the oat avenins, and it reacts weakly or not at all with the glutenin or glutelin, pretty good sensitivity, recognizes these particular motifs more strongly, although some others that are similar are recognized weakly.

The Codex Committee on Methods agreed in 2004 to endorse temporarily the R5 ELISA for the determination of gluten. Now, there are some possible problems. There is the failure to detect the glutenin proteins.

In some preliminary work from our laboratory, for example, when we look at wheat starch that is intended for use by celiac patients, we find that the gliadins are pretty well washed out, but we do find evidence of high-molecular-weight glutenin subunits attached to the starch surface. These would not be picked up

by the R5 ELISA.

Then, there is the question about small peptides from hydrolases. There has been some work described using a competitive assay, which might possibly solve the problem of the small peptides from hydrolases.

Also, there is a certain amount of data indicating differences in the results from different labs on the same sample. On the whole, it seems like a pretty good test that can be used. As I say, it is not perfect, but it is a pretty impressive test on the whole, and it may be as close as we are going to get.

Although, I certainly can think of some ideas for maybe improving it. At any case I think I will end my talk here and I thank you very much for your attention.

CHAIRMAN DURST: Thank you.

QUESTION AND ANSWER SESSION

CHAIRMAN DURST: Are there questions?

Okay. Margaret?

DR. BRILEY: Margaret Briley. Can you

give us any idea of the use of this ELISA test by industry in terms of the frequency and the acceptance and willingness to do it? Do you have any feel for that?

DR. KASARDA: Well, I think it is being used, and Dr. Collin can comment on this, extensively used in Europe and it is becoming used in the U.S. Susan Hefle, who spoke yesterday, I think that they have done a certain amount of work using this test for industry.

My impression is that not much testing is done in the U.S. Maybe Cynthia could comment on that. I think it is developing, but we are quite a bit behind the Europeans in terms of a willingness to test and desire to test products and make sure that they are as close to gluten-free as they possibly get. I can't give you a definitive comment on that. I haven't made any surveys.

CHAIRMAN DURST: Any further questions or discussion?

Yes?

DR. CALLERY: Pat Callery. Thank you for

the review of the relevant biochemistry. Could you relate the transglutaminase substrate specificity to these various glutamine-containing --

(Simultaneous discussion.)

DR. KASARDA: Not personally, but other people have. There are certain sequences that are susceptible to deamidation and probably transamidation as well. These have been described in some recent publications. There are many sites in the gliadins that are susceptible to transglutaminase.

DR. CALLERY: The transglutaminase I understood was an important feature in binding these proteins and causing the --

(Simultaneous discussion.)

DR. KASARDA: Well, you know, in the MHC proteins there is a positive charge in the binding pocket that binds well to a negatively charged glutamic acid. This does enhance the strength of the binding to the binding site of DQ2/DQ8.

Now, I didn't get into it, although possibly someone else will, there seems to be two

legs to the celiac disease situation. There is the adaptive immune system, which has been worked on quite a bit in terms of this presentation of gliadin peptides to T-cells, to the T-cell receptor, and stimulation along that leg.

However, one of the peptides I described that Mike Marsh had studied, that particular peptide is not immunoactive. It does not stimulate the T-cells, yet when instilled directly into the small intestine it produced changes that were characteristic of celiac disease.

In the last couple of years, there has been an interest in the role of the innate immune system in possibly triggering the first leg of celiac disease, which then progresses on to involve the adaptive immune system and the CD4 T-cells of the lamina propria.

I think that part is becoming pretty well understood. Ludvig Sollid and his co-workers, while he was on sabbatical at Stanford, they actually crystalized a DQ2 with the gliadin peptide in the binding site. They have defined a number of

characteristics of the peptides that are important for binding.

However, I think we still don't understand a good part of what is active or toxic about these peptides, and it may have to do with this triggering and innate immune response. That is research that is really developing right now.

CHAIRMAN DURST: Soheila.

DR. MALEKI: Soheila Maleki, USDA. Essentially, the substrate for the transglutaminase is the same peptide that is presented by the antigen-presenting cells?

DR. KASARDA: Well, after you deaminate a particular glutamine, then the binding strate goes up for the receptor site on the MHC protein.

DR. MALEKI: Essentially, it is the same substrate, just after deamination --

(Simultaneous discussion.)

DR. KASARDA: Well, there is also the question of, Why do you have antibodies to the transglutaminase? This may involve transamination reactions where you get a binding of gliadin in the

transglutaminase, and then this triggers the apparent autoimmune antibodies to transglutaminase.

DR. MALEKI: I see. Well, I just find it amazing that when you show the lineup of the peptides, the homology, that you had a two amino acid difference and went from immunoreactive to non-toxic. I'm sure by now they probably can explain that?

DR. KASARDA: No, they can't.

DR. MALEKI: Well, even in the fitting up to the transglutaminase or to the processing by antigen-presenting cells?

DR. KASARDA: Well, it is very puzzling, very interesting. I really can't answer your question.

DR. MALEKI: Thank you.

CHAIRMAN DURST: Any further discussion?

(No verbal response.)

CHAIRMAN DURST: All right. Thank you, Dr. Kasarda.

We are now scheduled for a break. We are just about on schedule, so we will take a brief

break and reconvene at 10:45.

(Thereupon, from 10:25 a.m. to 10:45 a.m., there was a pause in the proceedings.)

CHAIRMAN DURST: Our first speaker after the break is Dr. Alessio Fasano, professor of pediatrics, medicine and physiology and director of the Mucosal Biology Research Center, Center for Celiac Research, University of Maryland School of Medicine.

PROSPECTIVE STUDIES

DR. FASANO: Thanks so much. I've got to do this. I need really to thank the FDA, who has been so kind to invite me, but also to be so sensitive to use Italian candies.

(Laughter.)

DR. FASANO: This is very nice of you guys, and we appreciate that. I also want to tell you guys that because of the other speakers, I decided to reduce a little bit my talk, so a few slides have been taken out from the handouts to go straight to the point.

There has been a general perception that

this is a quite young disease, in other words, something that we are dealing with kind of recently. I want to put this in the right perspective and give you some of the background to justify this prospective study to decide what is threshold of tolerable gluten.

First of all, believe it or not the first trace of a description of this disease goes back to the Roman Empire. This is not something that has happened in the last few years.

(Slide.)

DR. FASANO: Who really put the disease on the map is this fellow here. Samuel Gee, at the end of the past century, around 1890, gave an historical lecture to a place where I had the privilege to study for a little while at Saint Bart's Hospital in London.

He really put celiac disease on the "scientific map." I took little sentences here and there from his lectures to give you the sense of how this guy got the story right more than 120 years ago.

He described these as a chronic indigestion that is met in every single age. Again, our misconception in the past was celiac disease was confined to a specific age group. He knew already that was not the case; it can affect at any age.

Of course, it is particularly more frequent in all kids between one and five years old, and that was the observation at the time. He spent time and effort to clarify the fact that everybody can be affected.

Now, symbiotics, hands-on, was the way to do a disease diagnosis at that time. We didn't have a lot of sophisticated tools, so it was really hands-on.

For a gastroenterologist, dealing with a problem like that means describing feces, stools, and that's what it is. He introduced with this description a very important concept about celiac disease in terms of classical GI presentation, malabsorption.

In other words, right before they would

know about the genetics, right before they would know about the grains, the eyes are telling us that the feces are loose, malformed, but not watery, definitely more bulky than the food taken seems to account for, i.e., malabsorption.

What is remarkable is this part here. He ventured also to understand what was the pathogenesis of the disease and introduced two concepts: the genetics and environmental trigger.

He said kids that suffer from it are not all weak in constitution, errors in diet. I want to clarify that the first time that the link between celiac disease and grains was made was soon after World War II. Until then we had no clue whatsoever what was the trigger leading to celiac disease.

The link was made during World War II because there was a higher rate of mortality among kids in Middle Europe that was not explained. During World War II, grains were not available anymore.

They were fed with potato starch, potato

flour, and the mortality dropped dramatically, to reappear after the end of the war when flour was again available. That is when the link was made.

This guy is already there. Errors in diet may be perhaps a cause, but whatever. Why, out of a family of kids all brought up in a much similar way, should only one suffer?

Again, he is trying to understand what is the genetic component, what is the environmental component, why some people have got it and some not from the same family eating the same stuff.

Then, he finished up by saying, "Okay, I think that I have a way to get to the bottom line in treatment. The treatment has to be regulating food in the main part of the treatment. It is amazing if you come already with this conclusion. The allowance of farinaceous food must be small. Again, I find this remarkable. Highly starchy food, rice, sorghum, corn-flour are unfit.

Now he is losing himself a little bit when he says malted food is better. Also, rusks or bread, provided it is cut thin and well-toasted on

both sides, will be all right.

Grant him the benefit of that. I believe that, again, in 1890 making this kind of statement, even if he [made] this little boo-boo here, I think that it is absolutely remarkable.

(Slide.)

DR. FASANO: Now, fast forward that 120 years later, and that is what we understand about celiac disease. You heard from Dr. Kasarda and Dr. Murray already that this is an immune-mediated reaction.

It is not an allergic reaction, but rather right now we really truly believe that this is an autoimmune condition. In other words, we are in the same kind of range as multiple sclerosis, type 1 diabetes, and so on and so forth.

Therefore, as such there are two key elements to develop the disease: You have to be genetically susceptible. I'm not going to spend more time about this DQ2/DQ8, but they are the docking station, the "eyes," of the autoimmune system to see the trigger from the environment

coming in.

It is unique because the only other autoimmune disease for which we know everything specifically is the only autoimmune disease for which we know the trigger, that is, gluten.

I wish that we had that kind of information for other autoimmune diseases, for which we will have a solution. Theoretically, we have on hand the possibility of treatment of this disease.

However, I will argue that unless we have a clear rule of engagement, i.e., a food labeling bill that will really clearly define what is "gluten-free," this is a theoretical solution but very difficult to put in practice.

(Slide.)

DR. FASANO: Again, it is pretty obvious that you have to have these two ingredients, you have the genes and you have to have the grains. When they interplay, you may end up developing celiac disease.

We heard already that variability in terms

of the timing, how long it is going to take, the outcome in terms of symptoms, and so on and so forth, is unbearable. However, they are all under the same kind of umbrella of celiac disease.

What are our treatment options at the moment? If these two elements are absolutely necessary to developing the disease, I believe it is a no-brainer, it is pretty simple, there are only two solutions.

First, we can remove the genes, and I don't think that we can do that. We are not quite there yet anyhow. As Dr. Murray explained, we know some of them but we don't know all of them. Or, secondly, we eliminate the grains. Those are the options that we have available. There is no other way to turn from this.

Don Kasarda went extensively into this. I didn't know that he was invited, by the way. However, the bottom line is the only treatment right now is strict, lifelong -- as you heard, you don't grow out of this, so you have got to endure it for the rest of your life -- avoidance of wheat,

rye and barley. The oats story, again, I am not going to go back because you heard about that.

(Slide.)

DR. FASANO: It is pretty obvious what are the major sources of gluten. This is the easy part when you have to deal with the patients freshly diagnosed. It is easy to say, "You know what? No bread, pasta, pizza, beer, cookies, muffins, and so on and so forth.

(Slide.)

DR. FASANO: This is a little bit more complicated, and that is where I believe a food labeling bill will help. Of course, it is not necessary to go and say, "You know what? This muffin that you buy at the bakery needs a label." We know that already whether it is gluten-free.

However, this stuff here (showing "Sources of Gluten" slide) definitely needs a label, some of them, because it is not clear if they have gluten or not because they can be processed with or without gluten.

Gluten is a formidable, extremely cheap

biological glue. Don told you the physical, chemical characteristics of the molecule. The reason why manufacturers use that is because when you have two elements of a processed food that does not stick together, the cheapest way to keep them together over time is to use gluten. Right now, the label can see just the nature of flavor but not gluten, not necessarily so.

Then, there are really the tough ones in which, this is not even food really, a source of gluten needs to be considered. I can't conceptualize enough how many times we've gotten E-mails of people asking, "Is my husband, who has celiac disease, going to be sick or whatever," or the Playdough for the kids in kindergarten, and so on and so forth. These are elements to keep in mind that we deal with all the time.

Of course, the big deal is right here -- medications, prescriptions. As for foods, processed foods, also medication they enjoy gluten as an additive to keep elements together.

Now, while I was saying adhere to a diet

is a pure theoretical no-brainer, but in a practical sense it is extremely complicated. It is a chronic intervention that you have to do, and you have to stick with it with full commitment for the rest of your life.

Every single individual in this room I am pretty sure that you have made some commitments here and there to go on a certain diet or to exercise or to decide to change your lifestyle. To keep that constantly for the rest of your life, it takes a lot of stamina. That is the reality of the story.

That is true particularly in the American society in which any chronic illness will require chronic treatment, whether it be diet or exercise or medication or whatever, will pose a problem of compliance. Definitely among different interventions, a diet compliance can be really a difficult aspect of treatment.

In my book, food is one of the few joys in life. How many times do we leave home and go to work, we drive, we don't think about it, and we

find ourselves at work without having to pay attention to directions, streets, and so on and so forth? We are used to it.

That is the same with food, we are used to it. However, that is not the case for celiacs because they have to think about this over and over and over again. It will become not a natural, spontaneous activity in life, but it will become a very, very demanding operation.

(Slide.)

DR. FASANO: Why don't people stick with diets? This is a survey that was done in Upstate New York. This statement, and this is just to paraphrase something that Cynthia was telling us:

"If I eat less gluten, I will have less intestinal damage."

Half of the people say, "You know what? I really don't have to stay a hundred percent gluten free. As far as I decrease this, I will have less problems. I will be all right."

"I've lived this long eating gluten, how

much will a gluten-free diet really help me now? I mean, you know, if it's not been a big deal so far, why should I just dramatically change my lifestyle? I've survived so far, I'm not going to die from it."

"It's not me, that I have to do this. It's my doctor who should tell me when I need follow-up testing or whether I need to stick with a diet, and so on and so forth." One-fourth of the people say that.

Again, you heard Dr. Murray, that unfortunately some of the confusion is generated by the professionals, the healthcare professionals. They don't know the rule of the game, and, therefore, they cannot transmit how to play the game.

It is pretty much the sense that you go to the doctor as an individual that has to teach you how to play chess, and this fellow has no clue whatsoever how to move the pieces. Patients have to learn how to play chess while playing against a professional player. How fair is that? It is an

ongoing process.

This is the one that disturbed me the most: "Scientists and doctors still haven't proven that gluten really hurts them." You know, there is no clear information that gluten is dangerous to celiacs, and that is quite disturbing.

(Slide.)

DR. FASANO: What are the current barriers in compliance? Again, you heard about the emotion of the person, anxiety. There is a tremendous reaction when you are diagnosed with a chronic illness, no matter how you want to put it. Now, grief and fear and denial are part of the story.

The ability to resist temptation and to be disciplined on a gluten-free diet is tough. There are feelings of deprivation. A few years ago I was with one of the patients, and he got the chance to drink a gluten-free beer. Soon after he started to drink the beer, I saw tears coming down his cheeks. His simple statement was, "I've waited 25 years for this." Imagine, 25 years to drink again beer.

He was disciplined, and he didn't touch

it. However, there are many others, particularly adolescents, in which that kind of discipline is really hard to obtain.

This is very much the heart of the problem, fear generated by inaccurate information. If we do not have clear ideas, we, as professionals, and one says black and the other one says white, and the other one says up and the other one says down, that creates a lot of confusion and a lack of trust.

(Slide.)

DR. FASANO: Other barriers to compliance are of course we live in a society that drives 150 miles an hour, and we don't have the time to seek to prepare our food to enjoy. My kids consider that the stove is the microwave. The stove does not exist.

Cynthia teaches us the fact that the new generation believes that cooking is just powder mixed with water, stick it in the microwave, and the only thing that you've got to do is read how long should that go on and that's it. What

sophistication.

Here, assessing gluten content in food and label reading is the most compelling change in lifestyle that these people go through. Right now, I don't know about you guys, but I don't enjoy food shopping. I really do not. I tend to go at midnight when nobody is there, because I want in and I want out.

That is not an option for celiacs. One thing that will take you, I don't know, half an hour will take four or five hours for celiacs because you've got to read every single label to the nitty-gritty and make decisions.

Many times now I see people with cell phones calling an 800-number right there on the spot saying, "I have your Box XYZ, is this gluten-free or not?" It is cumbersome.

(Slide.)

DR. FASANO: All of this to come to the heart of what I'm going to share with you guys. How much is too much? Unfortunately, I can't conceptualize and stress enough what Cynthia

already said. In biology, the absolute zero does not exist. If you really do believe that we can achieve zero as gluten-free, this is a pure theoretical concept that nobody will ever be able to achieve.

Assume, just for a moment, that we will have a sophisticated, super-duper sophisticated, monoclonal ELISA to really go down to zero. To manufacture food in that way, people in that particular factory should be dressed with spray suits, all antiseptic. A piece of bread will cost $250, because that is what that level of sophistication and controlled environments will take. Consequently, it is impossible.

At the same time we need to give industry, manufacturers, a parameter of what is tolerable and what is not. There have been many retrospective studies that Dr. Collin is going to tell us about, very few prospective studies because they are extremely challenging to do right.

This study that I am going to show you the data of has really been coordinated by

Dr. Carlo Catassi, who has been involved in this kind of topic for the past 15 years. He is a member of our center, and we have been doing this in coordination for the past four years.

Why do we need to do this? Because again this is a long-term, strict gluten-free diet. If we do a prospective study design, we can answer questions that a retrospective study was not able to answer.

How we did this? We did it in a way that the gluten-free diet, people that come in are already diagnosed on a gluten-free diet. We are monitoring this gluten-free diet in a blind fashion where a given amount of gluten is added to the diet, then, the clinical, serological and biopsy evaluation before and after the microchallenge.

The background noise, this is very important, is caused by possible contamination of the food was minimized by using a control group, in other words, to really do this by the book.

(Slide.)

DR. FASANO: Studies done in the past, for

example, from Dr. Catassi almost a decade ago, showed a linear relationship between the amount of gliadin -- that is the toxic part of the story here -- a daily dose, and it causes damage between 100 and 1,000 milligrams a day.

The intraepithelial lymphocytes -- and we are going to go back to what these intraepithelial lymphocytes are all about, the meaning -- was the most sensitive index, not the serology and not the symptoms.

What you heard already from Dr. Murray is that after all these red flags the antibodies may not be sensitive enough to uncover exposure to gluten. Indeed, even 10 years ago this was very clear.

(Slide.)

DR. FASANO: Why do this again? If it was done 10 years ago, why revisit this if we have already the information? Several reasons. The need, first of all, to investigate the effects of lower gluten doses. Because at that time they were using large doses, because that was the level of

sensitivity of the tests for the foodstuff.

There is a need for prolonging the duration of the microchallenge. In the past, the longest that we went was a month, and people would ask, "How about two months or three months?"

How about if the period, the lag period, between the exposure to gluten and when you react is longer? You believe it to be safe for one month, but you keep going, and eventually you react.

There is a need of a control group that was never used before, and, most importantly, you heard that gliadin is part of the story. They are the glutamines.

If you do the study just as done in the past, you may really not uncover what is really the story; in other words, what you leave out there is not pure gliadin but rather this mixture of proteins that Don Kasarda was telling us about.

(Slide.)

DR. FASANO: I don't want to spend too much time on this, but for a matter of

quantification, to give a sense of what we are talking about. In 200 grams of wheat-based products -- bread, pasta, so on and so forth -- you heard that the main proteic fraction in wheat is gluten. For 8 to 14 percent of the overall amount is wheat. Gluten is 75 percent of all the protein. Between gluten and glutamine, we can say that all of this 8 to 14 percent are these toxic proteins for celiacs. This 8 to 14 percent translates into 15 grams.

The real toxicity, the main toxic, is due to the gliadins. Again, glutamines contributed to toxicity. Of the 200 grams, 8 to 14 percent is equal to 15 grams. Half of it is gliadin. Gliadin has more than 50 toxic fragments, and so on and so forth.

If you go on a gluten-free diet, an adult that is on a gluten-free diet, roughly, consumes -- I mean, in a normal diet, roughly, the amount that you consume is this, 15 grams. Roughly, you consume 200 grams of wheat-based products.

If you are on a gluten-free diet, a

typical gluten-free diet, the subject consumes gluten-free flour-based, that is roughly 80 grams. The key element is how much of this 80 grams of gluten-free products can be contaminated with the toxic element, gluten? How much is the amount that you can tolerate? That is the heart of the problem here.

(Slide.)

DR. FASANO: That prompted the design of the study. It is a quite complicated study. The aim was to evaluate the consequences of the protracting just minimal intake, either 10 of 50 milligrams, a very small intake.

In a group of adult celiacs on long-term treatment with the gluten-free diet, why this amount? Because, again, 100 milligrams was already tested and proved to be dangerous 10 years ago.

How the study was designed was as a multicenter, prospective randomized, placebo-controlled, double-blind and was a three-year study. It was entirely sponsored by the Italian Celiac Society.

The reason why we did it in Italy, as I was mentioning before, is mainly because economical support of such a complicated and expensive study could be executed at this time only in a place other than the United States where we don't have that kind of resources.

(Slide.)

DR. FASANO: Who was eligible? Patients with biopsy-proven celiac disease had to be on a gluten-free diet for at least two years. These people that had been diagnosed with all of the criteria are accepted and have to be complying with the diet for at least two years.

If you are younger than 18 years old, poor compliance, abnormal results at the baseline evaluation or you have IgE deficiency, that will be an exclusion criteria.

(Slide.)

DR. FASANO: Now, how we did this? Well, again, these people were heroes to accept such a study, but this was the only way to do it. These people would come in to be scrutinized to see if

they were eligible.

If they were eligible, a consent form was obtained and there was an intense, strict monitoring of their gluten-free diets for a month before the beginning of the study was obtained. Baseline clinical serological and a biopsy was obtained. In other words, they underwent a endoscopy with a biopsy to show that they were fine.

They were blindly randomized in three groups, either no gluten, 10 milligrams of gluten or 50 milligrams of gluten. They were followed for three months. At a monthly interval there was a check with the serologist for symptoms.

At the end of the study, at the end of the three months, once again there was a clinical evaluation and a serological evaluation and a second intestinal biopsy under endoscopy.

This was the kind of study that this was the only way, given the fact that the we know symptoms and serology tests cannot be sensitive enough to do this right.

(Slide.)

DR. FASANO: The purified gluten was used for the challenge. Gluten -- or lactose-containing placebo -- capsules were randomly prepared. The lab tests were centralized. There was monthly monitoring of adherence to the protocol; it was checked by a nutritionist.

Measurement of gluten contamination in commercially available gluten-free food that they had during the challenge was checked by ELISA. The serum AGA and anti-tTG antibodies were checked; a biopsy was performed with morphometry; there was an intraepithelial lymphocytes count; and control biopsies from non-celiac patients were used.

(Slide.)

DR. FASANO: These are the foods that they had a gluten-free foods. You keep in mind that in Italy right now the food labeling policy is to be labeled as gluten-free you have to have 20 parts per million or less.

Indeed, with this simple exception, the vast majority of the foods that these people there

are eating was gluten-free, by definition of the 20 parts per million.

Consequently, the only gluten that these people were seeing was actually the ones that were dealing with the challenge, if they were in the group of gluten exposure.

(Slide.)

DR. FASANO: We were able to recruit 39 people, who were divided equally into three groups. There were a couple of things that were interesting to us.

Of all the parameters that we measure, two are extremely important to establish the health of the intestine and the exposure to gluten damage, one was the villous height/crypt depth ratio. It is very typical use of morphometric analysis that we do in clinical practice.

Typically, we want to see this: roughly, a ratio of 3:1. In other words, the height of the line has to be 3 times the depth of the crypt. That is what typically we consider to be normal.

Despite the fact that they were on a

gluten-free diet, despite that, they fulfilled the criteria. They were gluten-free, symptom-free, immunologically negative, and all the 9 yards. They went on a one-month controlled diet.

When we did the starting biopsy, there was a slight decrease of the villus-crypt ratio, meaning, the villi were a little bit shorter. That is what happens when you have an insult, the villi become short and the crypts go deeper.

The other parameter is the number of CD3-positive cells, the intraepithelial lymphocytes if you wish, was again 20 per hundred entrocytes and controls and 30 in the celiacs on a gluten-free diet.

Therefore, at baseline already something was going on. It is like there is a status of inflammation in which this is like a very well-trained athlete, ready to react to anything if it smells gluten coming through. It is really at the edge, ready to jump.

There was a strong correlation between the number of intraepithelial lymphocytes and the

villus/crypt ratio, meaning, that the more healthy is tissue, the less intraepithelial lymphocytes. The healthier the tissue -- when the crypts are elongated and the villa get short, the more intraepithelial lymphocytes are there. The intraepithelial lymphocytes are really soldiers that the immune system sensed at the forefront and ready to fight the battle. That is what it is.

(Slide.)

DR. FASANO: Now, what kind of symptoms after the three months these people experienced in the three groups? There were not really significant differences: abdominal distention, anemia, iron deficiency, loss of appetite, bloating, and so on and so forth.

There were equally distributed in all groups including the placebo, but two really stand out -- all in the 50 milligrams. This stomatitis and the mouth, there are the typical signs of mucosal involvement of the oral cavity in celiacs, well-described, it was present only in the 50 milligrams. Weight loss was experienced only in

the 50 milligrams. For the rest, we didn't see any major differences.

To revisit the concept that the antibodies were useless -- these are the antibodies, IgA and anti-tTG and IgG anti-gliadin antibodies -- before and after the challenge in placebo 10 and 50 milligrams, there was no difference. Pretty much there was no difference among the groups.

What we saw as the difference was the villus/crypt ratio, that all in the 50 milligrams started to decrease to a level of significance. After three months, we saw the crypts become a little bit deeper and the villi to become a little bit shorter. This translates in the fact that there was damage that started to occur, or possibly damage that started to occur.

The intraepithelial lymphocytes, there are these spots here (indicating). Again, these are lymphocytes under normal circumstances you see in a smaller quantity in between epithelial cells.

It came to be of a very increased number in people with 50 but not in 10, not reaching

statistical significance, but these are trends that I have the obligation to report. It is not significant that there are more of these cells in the 50 milligrams compared to the starting point, but it is a trend there.

(Slide.)

DR. FASANO: I believe the heart of all of this is this table. I believe this really cuts to the chase. It is extremely confusing, particularly to patients, when you talk about milligrams and parts per million. What the heck are you talking about? Why do we use this parameter of parts per million and not just straight milligrams?

Because, by the way, say, you do the study and you show that 50 milligrams could be dangerous, so how can it be 10 milligrams? How much is 10 milligrams? How much of a pizza is 10 milligrams? You say, "Well, let me give you the bad news. It's less than a fraction of a crumb of a piece of bread. That is what we're talking about.

Still, it doesn't give you clearly what is the magnitude of the stuff that we are talking

about. The reason why we prefer to express in part per million rather than in milligrams is because the amount that is tolerable really depends on how much you eat.

(Slide.)

DR. FASANO: As you see here, this is the daily intake of gluten-free flour or whatever products are based on gluten-free. If you eat 50 milligrams, of course you end up to ingest much less than 300 milligrams of the substance that you are eating.

Let's say that, for example, we set the parameter at 200 parts per million. If we want to accept the outcome of this study as something to keep in mind, 10 milligrams is safe for everybody, 50 milligrams start to be questionable.

If you set the threshold at 200 parts per million, if you eat a relatively small amount of the stuff a day, you are okay. If you eat a little bit more, you are in an area that we don't quite know, because again it is between 10 to 50 milligrams. You can argue, "Is 40 okay? Is 30

okay?" We don't know.

Definitely, if you eat 300 grams a day -- in other words, you eat large amounts of gluten-free products that is contaminated to the level of 200 parts per million -- you start to go into the red zone. That is dangerous.

If you go down this table, you see that if you set 20 parts per million, no matter how much your Italian lifestyle of eating like crazy food that is gluten-free based, no matter how far you go, you still are well below the threshold.

Therefore, at least based on this study, that I believe has been done really the way that it is supposed to be done, long enough, because three months is definitely a long period, a threshold of 20 parts per million should be safe for the vast majority of the people because it will keep you way below the cutoff that seems to be dangerous, i.e., the 10 milligrams.

(Slide.)

DR. FASANO: Now, this litany of names is just to explain that this was not a trivial study.

It was a multicenter study that involved a tremendous amount of work and a tremendous amount of dedication of people that have no business to undergo this, particularly two endoscopies with two biopsies. However, it is only because of the dedication and the commitment of these people that we have an answer and we have a chance to come to you today with something that is a little bit less foggy than so far we have had in terms of prospective studies.

I will stop there, and I will take any questions that you have.

CHAIRMAN DURST: Thank you very much.

QUESTION AND ANSWER SESSION

CHAIRMAN DURST: Questions?

Margaret.

DR. BRILEY: Margaret Briley. Can you tell me, I didn't understand, how often did they do the biopsies? Every month? Every three months?

DR. FASANO: No. No, no, no, that would kill us if we do it every month. No, the biopsy was done at the beginning of the study, at the

entrance, and at the end of the study, three months after, the idea being how much insult did you get in two months.

What was done at intermediate intervals was a survey of the diet, to make sure that they were complying with a gluten-free diet, survey compliance of taking the pill, and the serological tests for the antibodies. Those were done on a monthly basis.

DR. BRILEY: On a monthly basis?

DR. FASANO: That's right.

DR. BRILEY: Thank you. That was good.

CHAIRMAN DURST: Erica.

DR. BRITTAIN: Erica Brittain. If I'm understanding correctly, the conclusion of the study is that 10 milligrams daily would be safe, was shown at least to be fairly similar to your placebo group in this four-month exposure. How would you know how that would translate to four decades of exposure?

DR. FASANO: Only with decades of prospective study. You are a statistician, and you

know better than I do that you've got to start somewhere.

There is no question in my mind that the only way to do that is like when you put a new drug on the market, and you go to Phase I and you do 10 people. When you do Phase II, you do 100 people. You do Phase III, and 10,000 people. Everything is fine. Ten years later, because millions of people took it, it may be that something wrong will come up, a classical example.

I don't have an answer for you. How do I know in 10 years what's going to happen? But, you know, we have to have some way to start. I believe that this study is giving us a parameter, a justification, a scientific rationale to say, "Let's start here."

CHAIRMAN DURST: Okay. Soheila.

DR. MALEKI: Soheila Maleki. I was just wondering, this is probably not directly related to your topic, I heard earlier mention of wheat flour and exposure. How much is inhaled exposure involved in some of these reactions?

DR. FASANO: I don't think that anybody can answer with scientific confidence that inhaling is or is not a possible port of entry of gluten for people with celiac disease to react to.

What we know as a fact, an undisputable fact, is that the intestine is the port of entry, the key port of entry. I can tell you anecdotally that we have patients that react to inhalation of gluten leading to asthma as an allergic reaction to gluten rather than to celiac disease.

How confident am I that this could be an alternative to the other route? I'm not really confident, because I don't think that we have the scientific proof beyond any reasonable doubt, as we do with the other route, that it could be a possibility.

DR. MALEKI: Thank you.

CHAIRMAN DURST: Ciaran.

DR. KELLY: Yes.

Thank you, Alessio, I agree. Thank you for sharing the data with us, and I agree that at least it is a basis that we can begin to work from

and make some rational approaches to what is best for our patients with celiac disease.

A couple of questions: The first relates to the earlier question about the 40-year experiment. There is one that there is a 20 part-per-million threshold set already in Italy. Could you comment on how well that is tolerated by every or the vast majority of celiac patients in Italy?

DR. FASANO: Actually, it is much more than that. There are interesting natural experiments being done. Italy for many years now reinforced the 20 parts per million. England has this 20 to a hundred, and so on and so forth.

As far as I can tell you, this is something that in Italy the food labeling legislation setting it at 20 parts per million has been there for 7 years. It has been considered to be absolutely safe with very sporadical reports of reactions.

Now, I think it was telling you when you have a stomachache and you are a celiac, you tend

to go that way to the extreme that some people say, "Today's ache is because I had gluten."

I mean, this is the reality of the story. But if you want to, statistically speaking, work on the large numbers, I would say that 20 parts per million has been proved to be safe.

DR. KELLY: In your study, then, and this is something that we discussed a lot yesterday in the context of food allergy and challenge studies, is there the potential for bias in selection; in other words, individuals who are highly sensitive, in terms of symptomatically highly sensitive, to low levels of gluten would either be afraid or not choose to enter the study?

DR. FASANO: Absolutely. Absolutely, no question about it. The reality of the story is that if you are extremely sensitive to gluten, you would be less willing to expose yourself to something that you know is going to harm you.

The point is, What percentage of the population does that represent? Is it 10 percent, 20 percent, 50 percent, or a fraction of 1 percent

of the celiac population?

You know, I'm pretty sure everybody that is involved in the clinical care of people with celiac disease has run into people who are extremely, extremely sensitive to gluten out there. The exception are people where actually the problem is the opposite; these are people who can eat dangerous amounts of gluten and they do not react. That is a problem.

CHAIRMAN DURST: Dick Durst. Just to follow up on that, How did you recruit the people for these studies?

DR. FASANO: The method of recruitment is a major advantage of the Italian setting is that there is a single Celiac Society, and they are extremely committed. What we did was very simple. They have a national bulletin, both electronically and on paper, that is read pretty much by the vast majority of the members of the celiac community.

I believe that we originally asked for 45 volunteers. That is the number that the biostatistician told us to go for to have a

meaningful outcome. We got 470 volunteers, so we had to turn people down.

CHAIRMAN DURST: Did you at that point know which ones were the hypersensitive or the more sensitive versus other and select on that basis at all?

DR. FASANO: No. The way that these people were selected was completely random. In other words, the least that we had every "X," three or four up -- I don't know, to make the number -- were called to make that unbiased. We really wanted a representative portion of the population. This was done by also sex and age.

Yes?

DR. KELLY: Ciaran Kelly again. I do have one other question, and it has to do with the interpretation of the data on villus/crypt ratio and IEL counts in the controls versus the well-controlled celiacs.

You showed that there was a small difference at baseline, even though those individuals were doing well on a gluten-free diet.

Your interpretation is that there is an underlying immune activation. My question is, Is it possible or likely or relevant that the 20 parts per million that they are taking is perpetuating that?

DR. FASANO: What I am trying to convey is the difference is that the recovery -- even if you are completely, religiously gluten-free -- is not 100 percent. That is what I meant.

I don't know if this is due to an ongoing immune response. I believe that to not probably be the case. Because after all, after all with all of the machinery in the community, these people have been proved not to go back to normal. Whereas, again, the fact is that no matter how you push it, you can't really go back to normal.

I think that the fact that for three months, even if you were really "touched," so to speak, you did not react to 10 milligrams. For me it was a great level of confidence that this is the way to go -- together again with data with a retrospective study, that we are going to hear about in a moment, and on-the-field exercise in

Italy.

CHAIRMAN DURST: Jean?

MS. HALLORAN: Another question about the sample group. When you did the baseline study, how much variability did you find in the members of that group?

DR. FASANO: Let me see if I can go back on this.

MS. HALLORAN: You had two factors that you looked at, the villus height --

DR. FASANO: Can you put on the slide show for a second?

(Ms. Sylvia Smith complies.)

DR. FASANO: You will see that there was --

MS. HALLORAN: Slide 32.

DR. FASANO: Can you bring me over there, please?

(Slide.)

DR. FASANO: There is a fair amount of variability. You see that, and there is some overlapping at baseline.

If you go down -- keep going -- now, if you can go up to 28, please?

(Slide.)

MS. HALLORAN: It is 32, I think.

DR. FASANO: You want 32? I thought that you were talking about the variability of the villus/crypt ratio. Is that what you are talking about?

CHAIRMAN DURST: Yes.

MS. HALLORAN: Yes.

DR. FASANO: It is a little bit higher than that.

Can you go higher?

MS. HALLORAN: Ah.

DR. FASANO: Stop here. I need 26.

(Slide.)

DR. FASANO: All right. You see here, this is the variability. You see here that this is the variability. These are the single points. If there was somebody that was high right here (indicating), and someone like here, these are the celiacs. There was a continuum, so it is not that

there are people here, people there; it is a continuum.

This is the standard deviation, and this is the mean. Again, there is some variability but not huge. There is much more variability in the intraepithelial lymphocytes -- you can see this scatter -- that are being monitored.

CHAIRMAN DURST: Suzanne.

DR. TEUBER: Suzanne Teuber. I would assume, and this may be a completely incorrect assumption, that in the population that is following a gluten-free diet strictly, as those you indicated you recruited, would actually be a subset of patients who perceive themselves to be very sensitive, and thus would have a higher motivation to follow such a diet.

This would bring up in Italy what percent of patients do comply with the gluten-free diet? We heard about the extreme difficulties here and the poor compliance rate. Is it better in Italy? Would this mean that, perhaps, this population that you recruited from really might be a good sensitive

population?

DR. FASANO: I think, and I'm paraphrasing Joe Murray on this, that the compliance with the diet is the results of many factors, some of them diet there. Education I believe is at the top of all.

It is not that you feel it to be more sensitive or less sensitive. If you understand the facts, if you understand the rules of the game, no matter how you are perceived as being sensitive or not sensitive, you know that you can't cheat. You know that you need to start with that.

If you go to 10 doctors and they say all the same things, "I'm sorry, you don't have an alternative," then the level of confidence increases. We don't have that here. We don't have it, honestly.

Let's be honest. We have people, doctors, that will tell you, "You know, you need to go on a gluten-free diet." These are the teaching sheets that were printed 20 years ago. "After three months, go back on a regular diet. You're going to

grow out of it." What level of confidence do you have?

Definitely, a study like here, like this done here, will have a tremendous amount of bias. Because who is going to do that? It will be only the ones that are extremely compliant. The population in Italy that is compliant -- in Italy?

I should not say in Italy, in Europe -- because they are like 10 or 15 years ahead of us in this, because the level of awareness has been there for quite a long time -- is pretty high.

They understand exactly what is at risk. That is the reality of the story. It is more than to be the people with high cholesterol, high blood pressure and to be on medication because there is much more flexibility there.

These people they understand that if they don't comply the pay a price, and they do. The level of frustration, particularly here, is that they want to do that, the ones that understand the game, but they can't because there is no way in their current situation they can comply.

CHAIRMAN DURST: Doug.

DR. HEIMBURGER: Doug Heimburger. Would you go to the next slide, please, after this one?

DR. FASANO: Sure.

(Slide.)

DR. HEIMBURGER: Does this graph include the controls or only celiac patients?

DR. FASANO: These are only the celiacs.

DR. HEIMBURGER: Just out of interest, did you test for this correlation in the controls?

DR. FASANO: Yes, it is the same. We put it all together, yes. There is a strong correlation. Again, if you conceptualize this intraepithelial lymphocytes as, again, the first folks to go there -- just two weeks ago, for example, there was a paper in science in which they claimed that the lymphocytes, they are called gamma/delta, they are able to present antigens.

They can see gluten and they can start the entire reaction, at least to the adaptive immunity Th2 response to interferon-gamma, that will translate in damage, i.e., to make the villi short

and the crypt deeper. That makes a lot of sense. The more you have, the more cytokines you can use, the more damage you have.

CHAIRMAN DURST: Dr. Fasano, will you be around for the discussion this afternoon?

DR. FASANO: Yes. Yes, I will.

CHAIRMAN DURST: Because I think maybe we will stop the questions.

DR. FASANO: I have my candy so I can't leave you.

(General laughter.)

CHAIRMAN DURST: Okay. We will probably move on so we don't go too far into the lunch hour.

Our next speaker is Dr. Pekka Collin. He is a professor at the University of Tampere Medical School in Finland. He will discuss retrospective studies.

RETROSPECTIVE STUDIES

DR. COLLIN: Yes, good morning everyone. I come from Tampere. You probably know where Finland is and Tampere is a hundred miles north of our capitol, Helsinki.

(Slide.)

DR. COLLIN: We at least in Tampere think that is the celiac center of Finland, but maybe somebody disagrees with that. We have a half a million people around our hospital and now our clinical prevalence of celiac disease is approaching 1 percent. I think it is .7 at the moment, so we have 1,000 patients with celiac disease. Consequently, we have tried to examine both the symptoms and the diet.

(Slide.)

DR. COLLIN: I had some specific issues which I should address at this meeting, and they are here. I should explain why we carried out our retrospective analysis of the gluten content in our gluten-free products; then, also, calculate what is the significance of daily gluten exposure in this small amount of gluten; and then, also, to discuss is there some variability in the sensitivity of people with gluten intolerance which has been discussed here already many times. That should also include patients who are taking with

starch-based, gluten-free products and who are taking oats where we have a lot of experience.

(Slide.)

DR. COLLIN: I think that celiac disease has been described very well by previous speakers, so I will go straight into the point. However, I will emphasize that now we are talking about parts per million or 10 milligrams or 20 milligrams of gluten intake.

(Slide.)

DR. COLLIN: In real life, if you have 100 patients with celiac disease, I think 90 percent of them are taking 15 grams of gluten a day because they do not know that they have celiac disease.

Only 10 out of 100, for instance, in the U.S.A. I think know that they suffer from celiac disease. Of the remainder 10, maybe 3 or 4 do not follow a gluten-free diet strictly because they don't care, or it is more likely because there are not enough products when they are eating out or eating in restaurants, and so on.

I think that is very important, that we

have a good choice of products. That is more important than some parts per million in order to achieve a good percentage of compliance.

The amount of threshold, I think it started more than 10 years ago in Europe. The celiac societies were very, very active in these respects. From southern countries, some people say that we are in northern countries poisoning our people because they know that we are giving them wheat-starch-based, gluten-free products.

On the other hand, our society, I think they are very -- I don't find the right word -- but I admire them because they said, "Please make the study. Look at what we are now eating. Celiac patients are the last who will have some extra, unnecessary dietary restrictions, so please make a study where you show whether we are now eating safely or not." I think that was the background for our so-called "retrospective study."

At that time we were quite relaxed. We were not afraid that we are poisoning our people, because we published a study where we showed that

in our patients we did not have, in treating patients we did not have, any extra mortality and even we did not have any extra risk of malignant conditions at that time.

Then, we looked at what the Finnish celiacs are eating. As expected, the majority of them took wheat-starch-based, gluten-free products.

(Slide.)

We can also see that compliance was very good. These patients they were invited, after 5 or 10 years on a gluten-free diet they were invited, a cohort of those patients, both so-called "sensitive" and not sensitive, and we can see that only a small percentage of patients had dietary transgressions. Although there were a few who daily or twice a week or once a month had dietary lapses, most people preferred to follow a naturally gluten-free diet.

We also show that for these patients their quality of life is good, and they did not have any additional symptoms compared to the population. As has been mentioned many times earlier, symptom is

not a very reliable objective sign of gluten intolerance.

(Slide.)

DR. COLLIN: This is an example how symptoms can be misleading. This is maybe a little bit out of the topic, but I think this is very interesting.

We ask family doctors to send us all such patients who spontaneously reported that they get symptoms after taking wheat or rye. The majority of them had also on their own account tried to avoid or withdraw these products from their diets, and they experienced clear improvement in symptoms.

We thought that many of them had latent or overt celiac disease, but to our surprise only 10 percent of people with a clear history of intolerance to gluten had really celiac disease. Then, there are some which we thought that they maybe had wheat allergy.

When I was here yesterday I heard about that. Yes, the diagnosis is so difficult, so I hope that I don't have to discuss this in more

detail.

However, the majority of them, even with sophisticated methods, they did not have any signs of celiac disease and probably they have irritable bowel syndrome. Hence, we cannot trust symptoms even in the diagnosis of celiac disease.

(Slide.)

DR. COLLIN: Then, of course we have to go to small bowel biopsy as they did also earlier. We took a control biopsy after 5 to 10 years from these patients who had been diagnosed with celiac disease and who were asked to come to our hospital.

(Slide.)

DR. COLLIN: What we can see here is that this is the same villous height/crypt depth ratio which has been measured by, for instance, Alessio Fasano. Here is our reference value for people who have no suspicion of celiac disease. They have come to endoscopy because of suspected some gastrointestinal disorder, reflux symptoms or dyspepsia.

We can see that in our long-term treated

patients, there is a 95 confidence interval, so it was exactly the same as in our non-celiac people. I could show also a similar slide of intraepithelial lymphocytes, very similar. They did not have extra intraepithelial lymphocytes.

We did not either have any so-called "highly sensitive" patients with celiac disease. We had some here who had not a complete recovery in the mucosa.

After dietary inspection, it turned out that all of these people are taking occasionally gluten. Even once a month, I think that was in the data, the histological recovery was not complete Then, we had also here are the celiac patients where the ratio was of course low.

Then, we had some short-term treated patients, that means from half year to one year. We show that the healing was not complete at that time. From this slide we had two questions.

First, when we have a complete recovery, are those patients still taking some small amounts of gluten or are their products complete

gluten-free?

The second question was, When we have this incomplete recovery, does it depend on wheat starch or gluten contamination or is it normal life in celiac disease? In other words, would the healing be better if instead of wheat starch used, the use of naturally gluten-free products?

To the first issue, Are those products contaminated which have shown that our people are doing well and their mucosal is healthy? It was not surprising that most of naturally gluten-free products had less than 10 ppm gluten.

However, I think it is very important to realize that some of the so-called naturally gluten-free products, they may be contaminated with gluten, even quite high. All of these were fulfilling the current European Codex standard.

If we go to the wheat-starch-based, gluten-free flours, there were two with zero gluten, and as expected most of them contained trace amounts of gluten. Two had more than 100, but the majority has less than 100. That was our

idea that maybe we can set the limit to 100 ppm.

When I had this slide and my conclusion in Europe, one of the representatives of industry said that he was disappointed because I am talking about 100 ppm, and I should have talked about the limit of 200 ppm because it is much easier for them.

However, I said that we had too few products here to assert that 200 ppm would be recommendable. I think I will remind you that 90 percent of our celiacs have used this product for 40 years or even more, and we have biopsy-proven results from that so-called challenge from 5 to 10 years. The mucosal recovery, as I said, was perfect.

(Slide.)

DR. COLLIN: We also looked at how much they did use those flours. Maybe somebody who has taken gluten-free products can know that they are not necessarily as good as wheat, baking with wheat.

Nevertheless, here are how the patients used these products. There was no difference

between wheat-starch-based products or a naturally gluten-free diet. The average was 80 grams, and the majority took less than 150 grams as you can see here. There was no correlation between the villus damage and the amount of data used of loss.

(Slide.)

DR. COLLIN: From here we come to this conclusion, which maybe you have seen this kind of table in Alessio Fasano's presentation. Provided that we set the limit to 100 ppm, and provided that each of these products also contained the maximum amount allowed, when patients are taking 100 grams of those or 200 grams of those the gluten contamination is from 10 to 20 milligrams.

If you look at Fasano's results and if you look at some earlier, small studies -- even the Catassi study, which was referred to, and some smaller studies made by Sturgis and so on -- I think we are very, very safe here at the 100 ppm. I think also that our clinical experience will show that the same.

Of course, this is not a prospective

study, and we did not have any control group, and, unfortunately, we did not have many patients who have clear dietary restrictions, so we cannot make any statistical analogies between those who are -- what is the word -- cheating with their diet and who are not. However, I think with this kind of system, we can treat our patients and have good compliance.

(Slide.)

DR. COLLIN: If I can, go to the issue whether patients are more sensitive or hypersensitive patients with celiac disease. When we look at those patients, we can see that their mucosal recovery takes place in a different way in different people. That has been very well shown in some challenge studies. Where earlier it was customary to accept diagnosis, we have once again to challenge the patients to gluten-free diets and look at if there will emerge new villus atrophy.

We show that in some cases it took two months or one month to see a mucosal relapse, but in some cases it took two or three years. Our

record is 15 years. Fifteen years with normal diet and earlier diagnosed celiac disease, after 15 years a mucosal relapse occurred.

Here we can see that in the short-term some people do not respond, and you could think that these might be so-called "hypersensitive." If we give enough time and the patients are truly following the gluten-free diet, which means that we must be really accurate that they do not take wheat at the same time, I think in the long-term we have almost complete recovery. We did not have any so-called "hypersensitive."

I think patients with refractory sprue they can be very sensitive because they do not respond at all to celiac disease, but that is a different issue. It is probable that even zero gluten would not help them. There is something wrong in their gut. Probably the diagnosis has been made too late, and it does not recover any more. I think that refractory sprue is outside of the topic of this day.

Also, we were discussing with Peter Chen,

when he wrote to "Gastrointestinal Endoscopy" that complete mucosal recovery is not possible, and we had a very friendly, friendly discussion in the pages of that journal. However, we said that it is possible when we have a good choice of products and people also outside the home know what celiac disease is what this means for the patient with a gluten-free diet.

The second issue in my slide was that could it be that the mucosal healing would be more rapid in those who are on a naturally gluten-free diet than in those who are maintaining wheat-starch-based, gluten-free products?

Here, we carried out a randomized prospective study of one year in newly detected celiac disease patients. If we look at the villus healing here and here, villous height/crypt depth ratio, there were no differences between these two groups. We can also see that in one year, you cannot achieve the limit of three, which is considered normal.

Similarly, when we look at intraepithelial

lymphocytes, they decreased in a similar way in both patients. At that time, unfortunately, we could not measure what was the exact amount of gluten these patients were taking; we did not have methods. We can assume they took those same products which were mentioned in my last slide which contained trace amounts of gluten but not more than 100 ppm.

(Slide.)

DR. COLLIN: If I may say some words about oats. It was in Finland, the first publication. After that, very soon it was accepted for celiacs in Finland that they may use oats. At the beginning we were very careful. We followed up with them each month and looked at what to do, but now we do not do it anymore.

We made a question out, too. We sent a question out to members of the Celiac Society, how do they appreciate oats. As you can see, they like about the permission to eat oats.

Almost all said that it is a very significant part of every day gluten-free diet in

terms of tasty and low lost. They even thought that it is healthy, diversifies the diet, and we have a good availability in Finland of oat products. I understand that maybe in some countries oat is not so important.

Some might say that in Finland they are not eating good, so maybe people in Italy do not operate yet in the same manner as in Finland, but we can discuss it.

(Slide.)

DR. COLLIN: Here are how our people have now used oats, the majority of patients -- not great amounts, it is only 20 grams, 15 or 20 grams. There, most of the studies are about approximately 50 grams, so less than in those randomized studies.

Some people do not prefer oats, and that is the same thing in people in general not only in celiac patients. Some of them had stopped, and the reason is that they had developed symptoms. Some even got a rash, basically dermatitis herpetiformis. We do not have any proof that the reason for stopping would be that they

simultaneously had mucosal damage. Usually, the mucosa is good even though the patient has stopped the diet.

The rest, in dermatitis herpetiformis, we also saw that even in patients with no oat diet, so even they may have a temporary rash. There are some clinical relapses in patients with dermatitis herpetiformis also.

It is excellent to study these questions, because we can change the subjective symptoms quite rapidly to objective science, count the number of blisters, for instance.

(Slide.)

DR. COLLIN: We also looked at the quality of life in patients with oats. Actually, there was no change, difference, compared to patients with no oats. This also was a prospective, randomized study in treating celiac disease.

Interestingly, those patients who were taking oats, they reported more symptoms of diarrhea, which was statistically significant. They also reported more constipation, which was not

significant. Even in these patients, we did not have any mucosal deterioration.

From this we learned that if we start on a gluten-free diet with oats, we must inform the patient that "You may have symptoms after this. If you have symptoms, why continue. But it is improbable that we have done any harm to your small-bowel mucosa.

We also saw that those who were taking oats had a little bit more intraepithelial lymphocytes, not CD3 lymphocytes, which we have discussed today, but gamma/delta lymphocytes.

The gamma/delta lymphocytes were a little bit increased in the oat group. I cannot explain the reason for that, and that has not been published elsewhere -- but that is the fact.

(Slide.)

DR. COLLIN: Here are my conclusions to the questions which I was asked to answer. Maybe I also specific questions which you have, specific issues which you have to address in the final report.

If I may say something about the subpopulation, the most highly sensitive people, I think such people of course may be, but eventually they have good mucosal recovery, provided that they follow a gluten-free diet. The majority of these highly sensitive patients are probably such people who have advertent or inadvertent gluten intake.

We can also remember that even if it happens, the consequences are not disastrous, because they do not develop an anaphylaxis aspect as do people with peanut allergy as we heard today.

We can quite easily detect these highly sensitive, if we after the diagnosis, one year after the diagnosis, take a small-bowel biopsy and look at whether there is an improvement in the mucosal architecture. If there is not, we must consider that they may be very sensitive, but usually they do not follow the gluten-free diet.

About the risk of malignant diseases, I think the whole literature tells that those people who are at an increase risk of malignant lymphoma, their diagnosis has been made too late. They

already have lymphoma when the symptoms of celiac disease appear and when they get the diagnosis of celiac disease, or they have had dietary transgressions for a prolonged time.

Of over 1,000 patients I have seen during the 15 years, I have seen one patient who has developed lymphoma after being 5 or 10 years on an apparently gluten-free diet. The risk of these severe complications in those small daily intake is probably very low. Even our new data show the same, which is now published only in abstract.

Similarly, the mortality, it depends on those patients who come to the hospital together with the diagnosis of celiac disease and later, usually within six months, we can see that they also have lymphoma.

(Slide.)

DR. COLLIN: What about the oats? Here I summarized some studies. Those with plus signs they are those who have shown that oats have no adverse effect on the mucosa. I think nearly almost all of these studies are randomized,

open-randomized. They have a control group with non-oat. We have hundred of patients who seem to tolerate oats.

But I think I would be stupid if I did not see also those two papers and patients who are sensitive. I cannot close my eyes from the results, because Don Kasarda told the data very convincingly.

I don't know who they are. Maybe there are some who really develop villus atrophy after taking oats, but that must be an extremely rare condition. Because, as you see, we have so many, many patients who are taking oats, and we have not seen this phenomenon.

Still, we must be careful, and we must be careful because patients with oats may develop symptoms. If everything does not go well, of course we stop the use of oats. However, we must be aware of that, that maybe there are some rare patients where it acts the same as gliadin for most people with celiac disease.

I don't know whether these, my results and

recommendations, can be applied in the United States but that is how we are doing now. Our celiac society is very happy because we said that you can continue with starch-based, gluten-free products.

Thank you very much.

CHAIRMAN DURST: Thank you.

QUESTION AND ANSWER SESSION

CHAIRMAN DURST: Do we have questions? Suzanne.

DR. TEUBER: Suzanne Teuber. My question relates to the applicability of the diet parameters to the United States dealing with how much gluten-free flour do people in different parts of the world ingest, if they were to have the option of knowing that something was truly, truly gluten-free.

You talk about 100 parts per million. It was your data that came up with the 80 grams a day that people ingest. I'm wondering -- you know, we are not setting any level here today -- in terms of United States' folks, I have no idea how that would

apply. Would this be a safe level for them? Or, here, would people be preferring to adjust much more? Do you have any input on that?

DR. COLLIN: I think there is not much data on that, how much people really in different parts of the world are really using wheat or other flours which may be harmful to patients with celiac disease.

I think that this is a subject for further studies. Maybe somebody here knows how much celiac patients are here using gluten-free flours, but I don't know. I have not seen any publications about this issue.

CHAIRMAN DURST: Any other questions?

DR. McBRIDE: Margaret McBride. Did I understand correctly that gluten-free in Finland means 100 part per million?

I guess for me, as I'm thinking about it, maybe part of the difference between the two studies, aside from the obvious retrospective/prospective, et cetera, is that in Italy the gluten-free diet did contain some,

although very little at 20 parts per million gluten, in addition to what was administered.

I don't know if there is an estimation of how much that would be. I'm also thinking maybe there is more interest in pasta in Southern Europe than in Northern Europe.

DR. COLLIN: I think that today we have given the formal Codex standard which says that 200 ppm is okay, but of course we need to reconsider that.

I think that in the whole of Europe there will be two limits, that is the 20 milligram which can be used in the highly sensitive people, but in the majority of people it is 100 ppm.

Of course, there is a problem with labeling, how we should label that. We cannot say that it is "low gluten," because then people will use that. That is our problem.

What our recommendation is, is that maybe the majority of people with celiac disease can tolerate products which are under the limit of 100 ppm.

DR. KELLY: Ciaran Kelly. I wonder in terms of compliance with the diet and acceptance of the diet, is there a big difference between 20 parts per million or 100 parts per million from the perspective of the palatability of the food?

DR. COLLIN: I think the important thing is, at least the industry in Europe says, that, if we go to very low level, there are not so many alternatives for gluten-free products, which again may result in that general compliance will be worse than I have shown now.

How the products, how they--? I think that those wheat-starch products, I think they are very tasteful. Does it depend on the small milligrams of gluten or not? I don't know. But, as can be seen, most of the people are preferring those products instead of naturally gluten-free.

CHAIRMAN DURST: Anyone else?

(No verbal response.)

CHAIRMAN DURST: If not, thank you, Dr. Collin.

Our final speaker for this morning is

Rhonda R. Kane from the Consumer Safety Office of CFSAN, FDA, on international perspectives on gluten-free.

INTERNATIONAL PERSPECTIVES ON GLUTEN-FREE

MS. KANE: Good afternoon. My name is Rhonda Kane. I am with the Food and Drug Administration, and I was asked to present information to the Food Advisory Committee about how the term "gluten-free" is defined in other countries and the basis for those definitions.

(Slide.)

MS. KANE: My presentation today will focus on four examples of international or national definitions of the term "gluten-free" that apply to labeled packaged foods.

The first two examples I will be discussing pertain to Codex Alimentarius and they include, the first one, Codex Standard 118-1981, which pertains to the Codex standard for gluten-free foods that was established in 1981, was amended in 1983 and is in effect today; and, two, the Proposed Draft Revised Standard for Gluten-Free

Foods at Step 7 that is now under consideration by the Codex Committee on Nutrition and Foods for Special Dietary Uses as a replacement for the current standard.

For ease in my presentation, I am going to refer the Codex Committee on Nutrition and Foods for Special Dietary Uses simply as the "Codex Nutrition Committee."

In the early 1990s, members of the Codex Nutrition Committee agreed that developments in the characterization of gluten on studies on gluten tolerance warranted a revisiting of the current standard and an updating of it.

The current proposed standard has undergone several revisions and is now at Step 7 of an 8-step process pending resolution of certain issues including what method of detection is going to be used for gluten and the results of gluten threshold studies in celiac patients. The Codex Nutrition Committee will be meeting in November 2005, and will be discussing the proposed standard.

The third example of gluten-free that I

will be discussing is found in Canada's Food and Drug Regulations at Section B.24.018. It became effective on May 1, 1996.

Lastly, I will review the definitions of both "gluten-free" and "low-gluten" that are found in Clause 16 of Standard 1.2.8 of the Australia New Zealand Food Standards Code, and I will also discuss the definition of gluten found in Clause 1 of that same standard.

(Slide.)

MS. KANE: The current Codex standard that is in effect today defines "gluten" as "Those proteins commonly found in wheat, triticale, rye, barley or oats to which some persons are intolerant."

The current standard further defined the term "gluten-free" to mean that "The total nitrogen content of gluten-containing cereal grains used in the product does not exceed 0.5 gram nitrogen per 100 grams of the cereal grains on a dry weight basis."

(Slide.)

MS. KANE: The current standard states that it does not apply to foods which in their normal form do not contain gluten. Gluten-free foods are defined according to two categories, those that contain the cereal ingredients -- wheat, triticale, rye, barley or oats or their constituents, which have been rendered gluten-free -- or those foods in which any ingredients normally present that contain gluten have been substituted by other ingredients that do not contain gluten.

(Slide.)

MS. KANE: In comparison, the Codex Proposed Draft Revised Standard for Gluten-Free Foods at Step 7 defines "gluten" to be "The protein fraction from wheat, rye, barley oats or their crossbred varieties and derivatives to which some persons are intolerant and that is insoluble in water and 0.5 molar solution of sodium chloride."

You will see that in this definition and in others that are occurring in the proposed standard information within brackets is intended to indicate that that information is pending

additional discussion at the Codex Nutrition Committee. Their next session meets in November 2005.

The Proposed Standard also defines the term "Prolamin" to mean "The fraction from gluten that can be extracted by 40 to 70 percent aqueous ethanol." This definition specifically identifies the prolamins: gliadin from wheat, secalin from rye, hordein from barley, and avenin from oats.

(Slide.)

MS. KANE: The Proposed Standard also states that it applies to those foodstuffs and ingredients which have been especially processed or prepared to meet the dietary needs of persons intolerant to gluten.

Therefore, this parameter is similar to the one for the current standard in that neither of the two standards, the current and the proposed, would include foods that are naturally or inherently free of gluten.

The proposed standard also identifies three categories of gluten-free foods where their

definitions specify certain limits on their gluten content.

(Slide.)

MS. KANE: In the first proposed category, gluten-free foods consisting of ingredients which do not contain any prolamins from wheat or all Triticum species -- rye, barley, oats -- or their crossbred varieties cannot have a gluten level that exceeds 20 parts per million. Again, you will see that "20 parts per million" is within brackets, therefore, this number is pending.

(Slide.)

MS. KANE: This proposed definition also specifically cites three examples of grains within different species of Triticum, they are: spelt, kamut, and durum wheat.

Although triticale is not one of the grains that is identified within the definition by its name, it is included because it is a crossbred hybrid of wheat and rye.

In the second proposed category of gluten-free foods, they are those consisting of

ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties that have been rendered gluten-free and cannot have a gluten level that exceeds 200 parts per million. Again, "200 parts per million" is cited in brackets, and it is therefore pending.

(Slide.)

MS. KANE: In the third proposed category, gluten-free foods consisting of any mixture of the ingredients as described in the previous two categories, cannot have a gluten level that exceeds 200 parts per million. Again, "200 parts per million" is cited in brackets and it is pending.

(Slide.)

MS. KANE: Based upon my reading of the session reports for the Codex Nutrition Committee and related documents, it appears that the rationale for including two levels, the 20 and 200 parts per million, in the definition of gluten-free foods was to accommodate different points of view of the Codex member countries that thought there should be a different level of gluten based upon

their experience with their populations, what would be adequately protective.

There were some countries that believed either the lowest limits of detection or 20 parts per million would be most protective of those that are very sensitive to gluten.

Twenty parts per million was considered a practical limit to make it more feasible for industry to produce gluten-free foods in that category.

Other countries believed that the higher level of 200 parts per million would be appropriate, because they had experiences with citizens in their country that had celiac disease where they had been consuming wheat-starch-based products for years without harm, and they enjoyed them.

The 20 parts per million level would essentially prohibit the inclusion of those wheat-starch-based products. Therefore, it was a compromise, the low limit and the high limit, and they realized they could create some confusion on

the part of the consumer.

I also want to point out that the proposed definition of gluten-free foods specifically cites that whatever detection method is used it should have a detection limit of at least 10 parts per million gluten in the product on a dry weight basis.

(Slide.)

MS. KANE: The next definition I will discuss is that found in Canada's Food and Drug Regulations at Section B.24.018, and it states: "No person shall label, package, sell or advertise a food in a manner likely to create an impression that is a gluten-free food unless the food does not contain wheat, including spelt and kamut, or oats, barley, rye, triticale or any part thereof."

(Slide.)

MS. KANE: Canada's definition of gluten-free prohibits the use of derivatives or constituents of any of the cited grains. Therefore, wheat starch would not be allowed in a product that was labeled gluten-free.

It is my understanding based upon communication with staff who work with Health Canada and the Canadian Food Inspection Agency, that the definition that Canada is using was developed using a rule-making process, but they closely coordinated with the Canadian Celiac Association in the parameters for this definition.

Canada underwent a rule-making process similar to the one that we use in the United States where they reviewed the relevant scientific literature, they published a proposed rule, considered comments before it went final, and they determined that back in the mid-1990s that there was insignificant or insufficient I should say scientific evidence to support establishing a level that would be safe for all celiac patients.

(Slide.)

MS. KANE: In the last definitions of gluten-free that I will be discussing, in the Australia New Zealand Food Standards Code, first, in Clause 1 of Standard 1.2.8 of the "Code," which I will refer to simply as that rather than

repeating that long name, it defines "gluten" as "'The main protein in wheat, oats, barley, triticale and spelt relevant to the medical conditions, Coealic disease and dermatitis herpetiformis.'"

It also defines in Clause 16 of that same standard the terms "gluten-free" and "low gluten."

(Slide.)

MS. KANE: "Gluten-free" is defined as those foods that contain no detectable amount of gluten. They also cannot contain any oats or their products or any cereals containing gluten that had been malted or their products. It has to meet all of those three criteria not just one.

In addition, their Code defines the term "low-gluten foods" to mean those that contain no more than 20 milligrams of gluten per 100 grams of food. Now, although not stated in the Code as such, this level of gluten is equivalent to 200 parts per million.

(Slide.)

MS. KANE: It is my understanding based

upon communication with Food Standards Australia New Zealand's staff that they also underwent a rule-making process where they proposed these definitions for "gluten-free" and "low-gluten" before they went final.

They did a review of the relevant scientific literature. They considered public comment, and they also consulted with experts in the appropriate fields to develop the definitions that are in effect today.

In addition, the fair trading laws in both Australia and New Zealand were interpreted as prohibiting the term "gluten-free" from being used with any foods that contained any detectible amount of gluten.

(Slide.)

MS. KANE: Further, the definition of gluten-free was influenced by a lack of reliable analytical methods to detect gluten in oats and malted cereals. Essentially, their definition says not only no detectible amount of gluten, but no oats or other products, no malted cereals

containing gluten and their products because of this limitation of analytical methods.

(Slide.)

MS. KANE: The Code includes two definitions, "gluten-free" and "low gluten," to provide citizens who have celiac disease a choice between which level of gluten-containing foods they want to consume based upon their individual gluten tolerance level and the advice of their healthcare provider.

In closing, I would like to sincerely thank the staff that I consulted with at Health Canada, and the Canadian Food Inspection Agency, as well as Food Standards Australia and New Zealand.

With that, I will take any questions.

CHAIRMAN DURST: Thank you very much.

QUESTION AND ANSWER SESSION

CHAIRMAN DURST: Does the Committee have a question or comment?

Erica.

DR. BRITTAIN: Erica Brittain. I guess I find it appealing the idea of the two levels, just

as a comment, in the last one you cited. This might be applicable to the allergy situation as well.

CHAIRMAN DURST: Okay. Anything else?

Mark.

DR. NELSON: Mark Nelson. Did your contacts in Australia, New Zealand and Canada give any indication that they might change their definitions or their categorizations if there were more work done on thresholds, if that data based changed?

MS. KANE: That sort of conversation didn't occur between me and them, but I would think because they are government agencies, just like FDA is, if there were newer information on the horizon, they would probably consider it. Whether they would go through the rule-making process and change it, I guess they would base it on the needs of their own populations.

DR. NELSON: I guess the opportunity for -- this is Mark Nelson again -- two categories does have some attractiveness. I guess at Codex it is

going to be gluten-free and really gluten-free.

(General laughter.)

CHAIRMAN DURST: Suzanne.

DR. TEUBER: Suzanne Teuber. To your knowledge in talking with these folks, have their been any consumer-preference studies or behavior studies completed or underway with how the celiac disease patient is using these standards in terms of their overall intake?

MS. KANE: I don't have personal knowledge of that. However, the Canadian Celiac Association is very supportive of Canada's definition of gluten-free. Because they were instrumental in helping develop it, so they were very supportive of it.

CHAIRMAN DURST: Yes.

DR. McBRIDE: Margaret McBride. Do I understand from your slides about the Codex proposed changes that the term "gluten-free" would be applied both to those foods that contained none of the products in question and are lower than 20 parts per million, and to those foods that are