

Functional Dairy Products



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INTRODUCTION

What are functional foods?

Functional foods are foods, and in fact they are foods, that go beyond simple nutrition and have specific targeted actions. Various strategies have been adopted to develop functional foods. Some of the multiple ways to approach the development of functional foods follow.

1. First, we can use probiotics, which are specific live microorganisms that have a beneficial effect on the host. Specific probiotics are used for specific functions.
2. The second approach is based on prebiotics. Prebiotics are ingredients or compounds that have a beneficial effect on the microflora in the host itself.
3. A third possibility is a mixture of probiotics and prebiotics, called synbiotics.
4. The last approach to developing functional foods is based on the addition of ingredients that are very specific and have a very targeted action. Examples are conjugated linoleic acids or polyunsaturated fatty acids, and many others.

Based on this strategy, the domain of action of functional foods could be divided into two parts. First, there are functional foods that are used to **enhance a certain physiological function**, and second, there are functional foods that are used to **reduce the risk of disease**. These two strategies have been applied in the development of functional foods.

Fermentation is not only a means to provide, or to maintain, or to improve the keeping quality of a product. Fermentation has also been shown to have an important impact on food quality and acceptability worldwide. Yogurt fermentation involves two basic bacteria or microorganisms, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These bacteria ferment the milk into yogurt. During this process of fermentation, the basic milk nutrients, including high quality protein, calcium, phosphorus, and B vitamins are still available. But the benefits of yogurt go beyond these nutritive properties to a demonstrated physiological function. A major obstacle to consumption of milk is lactose intolerance and its associated symptoms. It has been demonstrated clearly that live yogurt will alleviate

those symptoms. Thus, there is a specific functionality that is associated with these probiotics.

The emphasis in this symposium is not just on yogurt, but to explore the broader range of functional dairy foods. This is an area where interest is growing, both in academia and in the private sector. The research that is going on is in line with major health issues. What are some of the research objectives and activities?

The first topic is digestive health. The role of functional foods in the maintenance of a healthy digestive tube is well recognized by all of us. However there are many questions that are important to answer. What are the specific roles of the prebiotics and the probiotic microorganisms? And how do they really influence the microflora balance, thereby changing metabolism?

The second topic is the whole domain of immune modulation and immune response systems. Research has been focusing for the past 10 to 15 years, not only on one single lactobacillus, but also on different lactobacilli, in a number of different fermented products. These include *Lactobacillus casei*, *paracasei* and *johnsonii*. The list is long, and research is very active and promising in this area.

A third domain where functional foods can play a role is in cardiovascular disease. We know that diet coupled with physical activity can have a tremendous positive effect on the cardiovascular system. Work that has been done in the past in Norway, Finland, and also in the DASH program has demonstrated clearly that the consumption of calcium, dairy foods, fruits and vegetables can have a positive effect on lowering blood pressure. We can go past this and see a recent product that was launched in Japan that is based on fermentation. The potential that this product will have to combat ulcers based on *Helicobacter pylori* is welcome news. So fermented products will have a potential wide range of impact on inflammatory bowel syndrome, ulcers and even Crohn's disease.

It is important to have a biological marker and also to be able to associate that biological marker with the quality of life. A few examples follow. We have demonstrated that a probiotic has an effect on certain factors of the immune system, for example the cytokine modulation or enhancement. Does it really reduce the frequency of colds? Does it really increase the resistance to different illnesses?

We need to identify domains where we need to do further research, to correlate the biological markers with the actual quality of life. These issues will be seriously discussed, and an ensuing rich debate will result in identifying the domains where solid data is available, and also the domains where research is needed.

Akram Fazel
Director of Research
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CHAPTER I

THE DEFINITION OF A FUNCTIONAL FOOD

What is a functional food?

The US and the EU not only have different definitions, they have different terms to describe an industry which is estimated to be growing at a rate of 15 to 20% per year and is now at 63 billion dollars. In the US the term “nutraceutical” is preferred, while in Europe the favored term is “functional foods”. There are huge differences between Europe and the US in terms of definitions, regulations, and approaches to developing functional foods.

US regulators have drawn a clear distinction between nutritionally enhanced foods and dietary supplements, with much looser restrictions on ingredients and claims for the latter. Europeans regulators, on the other hand, have drafter regulations that clearly define and favor functional foods as “food consumed as part of the normal diet”. There is definitely more engineering of functional foods in Europe than in the US. Also, Europe has lead Americans in the development of functional dairy foods. The market for probiotics is clearly underdeveloped in the United States, compared to both Europe and Japan.

US AND EUROPEAN PERSPECTIVES

US

A US expert offered the definition that “Nutraceuticals are naturally derived bio-active compounds, including live active cultures, that have health-promoting, disease-preventing properties, and that can be delivered in a number of different ways”. In the US, functional foods are developed in one of three ways. The first is by developing a dietary

supplement, which is intended for ingestion in pill, capsule, tablet or liquid form, but is not represented for use as a conventional food or as the sole item of a meal or diet. These products would be clearly labeled as a "Dietary supplements". Examples of this would be botanical supplements, vitamin E capsules, and selenium pills. The second approach is the use of functional foods that are whole foods such as yogurt, whole garlic, and whole carrots. The third method is to fortify regular foods with a nutraceutical component. Examples would include yogurt with added probiotic strains, or milk with extra calcium. Fermented dairy products provide a convenient base for adding a variety of these nutraceutical compounds.

Europe

Based on funds from the EU, 100 European experts worked on a project called FUFOSE (Functional Food Science in Europe). A consensus document defining functional foods was approved at their recent meeting in Madrid. The concepts that were approved are outlined below. Features of a functional food are:

- conventional or everyday food consumed as part of the normal diet;
- composed of naturally occurring components, sometimes in increased concentration or present in foods that would not normally supply them;
- scientifically demonstrated positive effects on target functions beyond basic nutrition;
- provides enhancement of the state of well-being & health to improve the quality of life and/or reduce of the risk of disease; and
- authorized claims.

The European perspective is that functional food science will serve to establish claims based either on enhanced function (type A claim) or reduced disease risk (type B claim) based primarily on changes in relevant and validated markers of exposure, biological functions and disease endpoints.

DEVELOPING FUNCTIONAL FOODS

Nutrition is a relatively new science. For most of the last 100 years, nutritionists were concerned primarily with preventing deficiencies. In the past 20 years the emphasis moved to preventing excess. As nutrition moves into the new millennium, the emphasis changes to enhancing the quality of life. There has been a tremendous improvement in the knowledge of diet and genetics. However, consumers frequently receive conflicting messages; and it is often difficult for them to separate good science from exaggerated claims.

There are 52 nutrient RDIs (Recommended Daily Intakes), which are set, not at an optimal level, but at a level that will prevent deficiency. There are nutrient nutraceuticals, for example the antioxidants, vitamin C, vitamin E, and also beta-carotene that play roles in reducing the risk of cancer and cardiovascular diseases.

There are also thousands of other chemicals coming from various food entities for which no formal nutritional requirement has been established. However, these

nutraceutical or functional ingredients show benefits for risk reduction and enhancement of “structure or function” (see p. 46) of the body. One example is lycopene, a strong antioxidant, which shows benefits for prostate cancer.

Drugs versus foods

Both the US and Europe draw clear distinctions between functional foods and drugs, but in the US the line is often unclear. In contrast, Europeans have firmly stated that functional foods should be part of nutrition and should have nothing to do with pharmacology. The European scientific community has clearly stated that claims for functional foods should not discuss cure of disease or prevention of disease, but rather reduction of the risk of disease.

Typical food companies spend 1.5% of their budgets on research, while pharmaceutical companies spend 15 to 20% on research. A different scientific standard is used to measure the efficacy of functional foods than is used for drugs.

There are several ways to develop a functional food, namely:

- a natural food, such as garlic;
- a food to which a component is added, for example dairy products with probiotic or prebiotic;
- a food from which a component has been removed, like hypoallergenic rice;
- or a food in which bioavailability of a compound has been increased, as in the example of inulin and calcium in a yogurt.

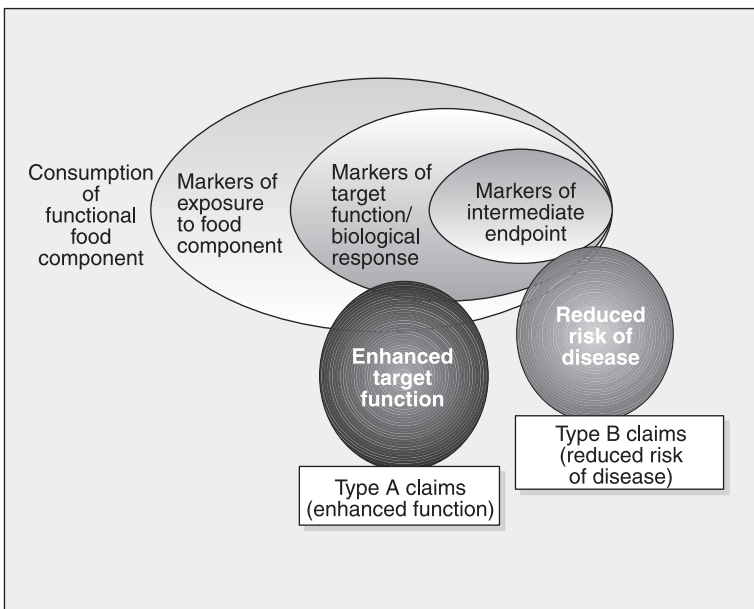


Figure 1. Relationship between functional foods, markers and claims, based on the European perspective.

[From: M.B. Roberfroid et al., *Br J Nutr* 1999; 81 (1): S25.]

The first step in the development of a functional food is the identification, as well as some understanding, of the interaction mechanism between the food component and a body function. On such a basis, a functional effect can then be defined and demonstrated in relevant models including human nutrition studies, which are different from clinical studies. These studies should be hypothesis-driven, and should look for changes in validated and relevant biological markers. The demonstration of these effects must also include safety assessment.

Claims and the marketplace

Following human nutrition studies, claims can be defined. Claims are any representation relating to nutritional properties. They may be a marker of a target biologic response, and have no relationship to disease. For example, an improvement in calcium absorption is a modification of the function without any relation to disease. There may also be an effect on an intermediate marker, such as a reduction in blood pressure, which would lead to the reduction of the risk of the cardiovascular disease.

The ultimate goal of the scientific community and food industry should be to develop functional foods that improve the quality of life. To do this, they must also educate consumers to make some changes in their eating habits and their lifestyles. Academia must also strongly urge the food industry to stay on the scientific side. The food industry needs to be reminded to keep the quest for functional foods a scientific challenge, and not just a marketing challenge.

CHAPTER II

DIGESTIVE HEALTH

PROBIOTICS, PREBIOTICS, AND SYNBIOTICS

The concept of probiotics was first introduced by Ilya Metchnikoff in 1908, who observed the long life of Bulgarian peasants who consumed fermented milk foods. Yogurt and other fermented milks containing probiotics may be considered the first functional foods. In 1989, Füller defined probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”.

Just in the past years, the concept of prebiotics has been introduced. Prebiotics are non-digestible food components that increase the growth of specific microorganisms in the gastrointestinal tract. One example is the inulin-type fructans, for example chicory inulin and its hydrolysate, oligofructose, which are natural food ingredients found in miscellaneous edible plants. They are non-digestible oligosaccharides and thus are dietary fibers.

Synbiotics are a combination of prebiotics and probiotics. Researchers at this symposium presented strong evidence that selection, identification and cultivation of specific strains of probiotic bacteria, in an intestinal environment enhanced by the ingestion of carefully selected and engineered prebiotic foods, can lead to the reduction or displacement of undesirable microorganisms responsible for a variety of gastrointestinal disorders.

MUCOSAL DIGESTIVE BARRIERS

The epithelial lining of the gut lumen, which protects the body from pathogens, is only one cell line thick. Mucosa of the gastrointestinal tract functions as a barrier excluding numerous antigens derived from microorganisms and food. Many foreign antigens are

excluded by stomach acidity, peristaltic movement and intestinal flora. A small percentage is presented to the immune system. Of these, some are absorbed, and some are eliminated by degradation. It is important that some are absorbed so that antigen-specific memory can determine which are good and which are bad, and subsequently exclude pathogens.

There are many factors in the environment that interfere with normal barrier function, like antibiotic therapy, thus allowing pathogens that are present to leak across the gut. In intestinal inflammation, the integrity of the barrier is disrupted, and a greater amount of antigens cross the mucosal barrier and the routes of transport are altered. This may evoke aberrant immune responses and release of pro-inflammatory cytokines with further impairment of the barrier function.

It is important to stop this inflammatory response. Recent demonstration that the gut microflora is an important constituent in the intestine's mucosal barrier has popularized the concept of probiotic therapy. Antigens can be degraded by probiotics. An increase in the number of beneficial bacteria will decrease the number of specific immune responses.

Peyer's patches

Mucosal barrier permeability is one essential step for the action of probiotics. This permeability is controlled by Peyer's patches, which are lymphoid follicles, containing all the products necessary to mount immune responses.

An epithelial cover regulates permeability, and controls immune responses from neonatal through adult life. In this epithelium, are found special cells that make some holes into the monolayer of enterocytes. These lymphoid follicles are separated from the lumen by a specialized epithelium that contains M cells, taking up foreign material and microorganisms and delivering them by transepithelial transport from the external environment to the lymphoid follicles.

Researchers developed an in vitro model to study transport of specific bacteria across the digestive barrier. With an electron microscope, they can demonstrate that bacteria, viruses and immunostimulants are preferentially taken up by M cells and directly delivered to underlying antigen presenting cells (APC) and lymphoid cells.

Modulation of mucins

The intestinal epithelial cells lie in a strategic position between the host immune system and noxious agents of the intestinal lumen. These cells are not passive bystanders, but produce cytokines in response to pathogens. They secrete substances that are directly protective, for example antibacterial peptides and mucins.

Certain pathogens adhere to epithelial cells and cause effacement lesions through localized actin accumulation, for example enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). Studies have shown a quantitative inhibition with increasing mucin. Upregulation of mucin gene expression may be a mechanism whereby probiotics

act. The genes for expression are in the bacteria, not in the cells. In comparing various probiotic strains on the market, there are differences in the ability to upregulate gene expression. Those probiotics that upregulate expression are the same ones that inhibit pathogens.

Studies on various probiotics species revealed the following. Mucins inhibit EPEC epithelial cell adherence, which confirms the importance of innate immunity in host protection. Selected antibiotics can alter the expression of intestinal cell mucin genes. Preincubation or coincubation of selected probiotics can inhibit microbial adherence to epithelial cells.

This may provide the mechanism for the prevention of acute infections and improvement of certain chronic inflammatory bowel diseases, IBD, in which the bacteria seem to be important in the development of the disease. If a bacterium is already established, it seems unlikely that adding probiotics after the fact will have much clinical effect. Expression of both MUC3 (major small intestinal mucin) and MUC2 (major colonic mucin) may explain why probiotics are beneficial against viruses, which cause trouble in the small intestine, and also pathogenic bacteria, which cause trouble in the large intestine. Epithelial cell adherence does not seem to be important for the effects on the epithelial cells, which raises questions about the criteria for probiotics to adhere to the epithelial cells.

DIARRHEA

Acute gastroenteritis rotavirus infection, the most common cause of acute childhood diarrhea, results in partial destruction of the intestinal mucosa. The composition of the intestinal microflora is also affected by the infection and its protective function is reduced.

The current accepted guidelines for the treatment of acute diarrhea are based on correcting dehydration by oral rehydration solutions. Probiotics have been shown to reduce significantly the duration of diarrhea compared to a pasteurized milk product. If the probiotic is administered early in the course of acute diarrhea, the clinical benefit is greater.

Probiotics have been shown to reduce gut permeability caused by rotavirus. Also the gut immune defense is promoted. A significant increase in cells secreting IgA against rotavirus has been detected, which could explain the preventive potential of probiotics in diarrhea. Studies have shown that the use of probiotic therapy can decrease the duration of most cases of rotavirus infection by one day. Probiotics are an efficient way to treat infectious diarrhea in infants and elderly.

ACUTE GASTRO-ENTERITIS

The intestinal microflora is a complex bacterial community, which inhabits the distal digestive tract of animals and humans. The predominant population in this community

are anaerobic species. A scanning micrograph of fecal mass reveals that 50% of it is bacterial cells; and that there is a great mixture of species.

When scientists try to analyze these bacteria they encounter two problems. The first is that, even with the best anaerobic techniques, only 40% of bacteria can be cultivated. The second problem is that each human has a microflora of characteristic composition. This makes it hard to compare composition of microflora between humans. However, it is possible to compare between patients of various inflammatory bowel diseases (IBD) and healthy controls.

Samples were taken from patients with IBD, Crohn's disease (CD), and ulcerative colitis (UC). Also some specimens were taken from patients with ankylosing spondylitis (AS), a chronic inflammatory disease of the sacroiliac joints and spine. Many patients with CD or UC also have AS.

Experimental animal studies, with conventional transgenic rats that have HLA-B27 marker, or gene knockout mice, reveal that these animals develop IBD. However, if they are maintained germ-free they do not show the disease. This indicates that intestinal bacteria have something to do with etiology of conditions; and intestinal microflora may provide fuel that gives chronic inflammation.

The challenge is to analyze the composition of the microflora of humans for this marker. Current methods of analysis include culturing, phylogenetic analysis, and fluorescent in situ hybridization. None of these methods is ideal. What is needed is a good screening method, so scientists can compare bacterial community composition.

1. One week before.

2. After one week
at 5 grams per day.

3. One week later



Figure 2. Sample of a PCR profile comparing fecal bacteria one week after consuming three different prebiotic substances: FOS, GOS, and inulin.

[Source: G.W. Tannock, K. Munro, J. Burton, 2000, unpublished observations.]

An alternate method is to separate species using polymerase chain reaction (PCR)/ denaturing gradient gel electrophoresis. As various 16S DNA fragments or “species” migrate through the gel, each type reaches a point where it is less stable. The point at which their movement stops leaves a map of the bacterial community. Such maps of fecal samples from each human are unique, almost like a thumbprint. The bacteria can be identified by cutting fragments eluted from these gels. By sequencing fragments, and cloning, mucosal populations can be identified.

The evidence indicates that sulfate reducing bacteria, *Bacteroides vulgatus*, and *Klebsiella* species, are found in high concentrations in colitis patients. By detecting sulfite reducers using PCR detection, researchers can determine their etiology in various inflammatory conditions. In addition, PCR detection can be used to identify species of *Helicobacter*, which may be involved in ulcerative colitis.

Currently, probiotics and prebiotics are aimed at healthy populations. It may be possible through the methods described to accurately analyze the microflora associated with particular diseases, and to modify the composition of intestinal microflora through “designer” probiotic and prebiotic substances to manipulate such abnormal microfloras.

CHAPTER III

IMMUNE RESPONSE SYSTEMS

INFANT EXPOSURE TO ALLERGENS

The incidence of allergies in the US has doubled in the past two decades. This same trend is being seen in all industrialized countries. Interestingly, in more polluted areas, there are less allergies. Studies show that often, early dietary factors interfere with microbial exposure, and that allergies develop more frequently in a sterile world. In addition, 25% of allergy sufferers also have atopic conditions such as asthma.

The gut microflora give signals to the newborn system to mature. When a baby is born there is a high production of T helper 2 (Th2) type immune responses. T helper 1 (Th1) is cell-mediated immunity. During pregnancy Th1 is downregulated and Th2 is upregulated. Infants who are exposed to antigens, such as food antigens, become sensitized. They produce antibodies against the antigens they are exposed to as part of their normal adaptation to the world. One hypothesis is that modern society uses more antibiotics and gives less exposure to microbes. Because our food supply is more sterile, there is also less exposure to microbes in the diet. This limited exposure to normal antigens creates a higher incidence of allergies, asthma and eczema.

Formulas or solid food promote Th 2 response, and bring the balance back to normal. This exposure needs to happen early in life, preferably before 6 months of age. Those infants who are 4 months old, and have had nothing orally but breast milk and vitamin D have a high risk of developing asthma later. These infants frequently also have early atopic eczema.

Tests to assess these infants looked at gut barrier actions and measured fecal α -1 anti-trypsin, which is a protein whose presence in the feces demonstrates protein loss in stools. There was a significant response in the gut of babies who have atopic eczema. Babies who had allergies demonstrated an inflammatory response. After taking biopsies

from the intestines of these infants, researchers were able to measure transport across the mucosal barrier, and compare total transport and differentiated transport by macromolecular absorption. With eczema patients, there was much more antigen transport across the barrier compared to controls.

Among the possible mechanisms of probiotic therapy is promotion of the gut defense barrier. In food allergy, an inflammatory response leads to disturbed gut barrier function. There is not just a concern of leaky gut, but also of slower growth. With such infants, extensively hydrolyzed formula is considered the standard treatment. When this formula was supplemented with a *Lactobacillus* strain, in a recent randomized, double-blind, placebo-controlled study, infants showed significant improvement after one month of treatment. The initially high production of TNF- α , an inflammatory response, decreased after one month with a probiotic treatment, but not in the control group which received the standard treatment.

Another group supplemented the breast-feeding mother with probiotic treatment. This group showed the least eczema. These results indicate that probiotics modify the structure of potentially harmful antigens and reduce their immunogenicity. Many of the probiotic effects are mediated via immune regulation, in particular via promoting intestinal IgA responses and controlling the balance of pro-inflammatory and anti-inflammatory cytokines. On the basis of these findings, probiotics are considered as potential innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction and downregulate hypersensitivity reactions.

IMMUNE DECLINE IN ELDERLY

Immune responses decline with the ageing process. These changes include decline in CD3+ mature T cells, a decline in immune function, a change in the Th1 to Th2 ratio, and a decline in lymphocyte proliferation, IL2. These changes all provide an advantage to bacteria in disease situations. Nevertheless, it was recently shown that such decline is not significant in young healthy elderly (70-85 years old) and becomes significant only in the very old healthy elderly (> 85 years old).

In contrast, undernutrition in aged persons is always associated with a profound immune defect, the intensity of the immunodeficiency being correlated to the decreased nutritional status. In addition, the self-sufficient healthy elderly with decreased nutritional status also show decreased immune function if the deficit relates to nutrients active on immune functions: protein, vitamins B₆, B₉ (folic acid), or zinc. Those deficits are quite frequent in aged individuals. In self-sufficient home-living elderly, protein energy malnutrition (PEM) is observed in 2-4%, and micronutrient deficit in 9-26% for vitamin B₆, 2-5% for B₉, and 10-30% for zinc. A decline in folic acid consumption is almost always tied to a decline in immune response.

It has been shown that the decreased immune responses observed in self-sufficient home-living elderly with nutritional deficits are always corrected with appropriate nutritional supplement.

GALT system

From in vitro observations, scientists have determined that probiotics are able to boost the macrophages, or the intestinal immune level. From animal models they see immune boosting effects of probiotics on IgA cells, on the intestinal T cells, and in other responses in the mucosal or the GALT (gut-associated lymphoid tissue) system. This immune system is known to be preserved in aged healthy animals and probably also in aged healthy humans.

Much research is conducted on the importance of some nutrients on intestinal permeability and on GALT activity during the aging process. In fact, they all focus on the activity of different amino acids, proteins or foods on crypt and villosity epithelial heights and functions, and on their consequences or relationships with the GALT activity, and the ensuing activities of the systemic immune responses. The goal of this research is to prevent the decline of the systemic immunity, which is one of the major factors that accelerate the aging process.

RESPONSES TO INFECTIOUS DISEASES

Two different approaches to combating the pathogen, *Haemophilus influenzae*, were presented. The first was based on lactoferrin, a normal constituent of human and bovine milk, and the second was based on a specific fermented milk supplement.

Lactoferrin as a pathogen inhibitor

An unanticipated function of the protein lactoferrin has been discovered. Lactoferrin is a serine protease. It has substrates which are present on bacterial cells that cause human disease. Bacteria make enzymes that cut human IgA in the region where it separates the antigen binding fragments, to inactivate the antibody. Many human pathogens do this.

Haemophilus influenzae is one of the most important pathogens in children, and causes otitis media, or ear infections. Lactoferrin is an iron binding protein that is abundant in human secretions. Lactoferrin has been shown to cleave both the Hap adhesin, which serves to directly bind the microorganism to the host epithelial cell, and the precursor protein of IgA protease, a microbial enzyme that cleaves human secretory IgA.

Lactoferrin shows this activity in its natural form, when it is not acidified. This research shows great promise for developing an inhibitor with very specific biochemical functions, with the power to modify bacterial surfaces, and control childhood pathogens such as *H. influenzae*.

Fermented milk as an immune booster

Another study started with the hypothesis that consumption of milk fermented with *Lactobacillus casei* DN-114001, Actimel[®], could activate both the innate and adaptive immune systems in healthy individuals. In a random test, eighty-eight subjects aged 18-50, consumed 100 ml daily of either Actimel[®] or a diluted milk placebo, for 28 days. Peripheral blood was drawn on day 0 and following consumption at days 9, 18 and 28. The blood was tested for various immunological parameters. These included frequency and distribution of various T cell and B cell subsets, presence of various Th1 and Th2 cytokines, and response to three microbial antigens. These antigens were tetanus, *Candida* and influenza, which represented a bacterial, a yeast, and a viral infection.

The cells were tested *in vitro* for proliferation upon exposure to the antigen. The supernatants were kept and tested for cytokines. Frequency of CD4+, CD3+, CD8+ and CD25+ subsets was measured. Proliferation, as a function of time, was analyzed statistically using the general linear mixed model.

In the case of specific adaptive immunity, there was a positive time trend upregulation of the proliferation response in the Actimel[®] group and the placebo group to all microbial antigens. For *Candida* and tetanus, the changes were not significant. However, for the influenza antigen, there was a statistically significant time trend change that was different in the Actimel[®] group from the placebo group. The trend was consistent with both helper T cells and cytokine cells, suggesting that both populations might have been primed in the Actimel[®] group in contrast to the placebo group. And in the case of innate immunity, there was a significant time frame change in natural killer ability in the Actimel[®] group, however the difference was not significant.

Further study is suggested to see if larger test groups might lead to more statistical differences, or if sub-grouping by response to influenza antigen, might show greater difference in upregulation. Finally, there needs to be an *in vivo* test to determine if Actimel[®] consumption actually leads to a change in the frequency or the length of flu infection in those individuals, and to determine the correlation between the *in vitro* and the *in vivo* response.

CHAPTER IV

FUNCTIONAL DAIRY FOODS AND THE RISK FOR VARIOUS DISEASES

HYPERTENSION

An inverse relationship between intake of dairy products and blood pressure levels was first suggested by several epidemiological surveys in the early 1980's. Subsequent laboratory and clinical investigations provided further evidence of the association between calcium and blood pressure, but the results of these studies were often inconsistent due to variations in study design and methods, study participants, and calcium sources.

In the management of high blood pressure, lifestyle is important. Overweight is the biggest predictor of hypertension; exercise improves blood pressure, and moderate consumption of alcohol is acceptable. The overall adequacy of the diet is very important. Unfortunately, many of the currently recommended solutions to hypertension do not rely on science.

The recently published results of the large and carefully executed "Diet and Blood Pressure in America" study, demonstrated a dramatic blood-pressure lowering effect of diets rich in dairy products, fruits, and vegetables. The study revealed that individuals with low calcium intake ate less cheese, yogurt and ice cream. Results of the NHANES I (National Health and Nutrition Examination Study) study showed that to prevent high blood pressure, the most important food group that needs to be added to the diet is dairy products.

Recent reviews of a very large database, comparing all randomized trials, suggested that for African Americans, the elderly, and those who are salt sensitive, increasing

mineral intake, specifically calcium, will be important for the regulation of blood pressure. Those with low calcium intake responded positively to calcium supplements. This effect is present in both normal and hypertensive subjects, and is more pronounced for high risk individuals.

In another study, the DASH diet (Dietary Approaches to Stop Hypertension), subjects ate 3 servings per day of low-fat dairy products, and 8-10 servings per day of fresh fruits and vegetables. High, normal and mild hypertensives were studied. The study was published in the *New England Journal of Medicine* in 1997. The normal group had a 3 mm drop in systolic pressure on the fruit and vegetable diet, and a 6 mm drop on the combination diet (dairy plus fruit/vegetable). This compares to a 0.6 mm drop achieved by lowering salt intake in another study.

Also in the DASH diet, individuals with mild hypertension achieved an 11.6 mm reduction in blood pressure. The test was heavily weighted to African Americans. No individuals stopped the trial because of lactose intolerance, probably due to the fact that yogurt was emphasized. The authors concluded that blood pressure reduction was rapid; and it was independent of sodium and weight change. Public health implications of this diet as a preventive measure against hypertension could lead to a 27% reduction in stroke and a 15% reduction in coronary heart disease.

The Vanguard studies, a multi-center dietary intervention study carried out at various universities, compared a comprehensive diet containing 100 to 115% of the US recommendation for all macro- and micronutrients, to the Step 1 and Step 2 American Heart Association diets. This test included four trials involving 1300 subjects. In a high-risk group for diabetes, dislipidemia, hypertension and obesity, results showed that a comprehensive diet intervention has a significant effect on blood pressure, weight, lipid profiles, homocysteine, hemoglobin A_{1c}, and insulin. The overall conclusion was that when nutritional adequacy improved, the quality of life improved.

The DASH II diet findings were recently reported at the American Society of Hypertension. In this 12-week study, 3 meals per day were provided. The control diet, which was low in fruits, vegetables, and dairy products, was compared to the DASH diet, at three sodium levels. This was a randomized cross-over test, with 30-day intervention. Subjects were 57% black, 57% women, and 41% hypertensive. The average BMI approached 30, and the group was heavily weighted for sodium sensitivity. Subjects on the DASH diet showed a greater reduction of systolic blood pressure than the control group.

DASH II implications are summarized below:

- Low-fat dairy foods plus fruits and vegetables are far more effective than sodium restriction in changing blood pressure.
- Low-fat dairy foods plus fruits and vegetables virtually eliminate sodium's effects on blood pressure.
- All population segments benefit from the improved diet.
- A national nutrition policy to prevent and manage hypertension must focus on the DASH diet, not on salt.

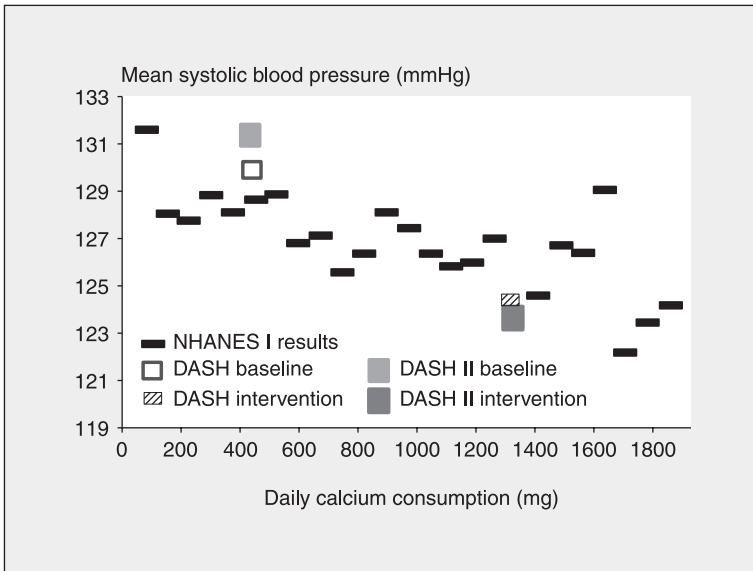


Figure 3. Effects of calcium on systolic blood pressure in NHANES I, DASH and DASH II. [Source: D. McCarron, Oregon Health Sciences University, Portland, USA.]

One lesson from these studies is that single-nutrient interventions do not exist. Foods are the issue, not individual nutrients. Dairy products are critical in achieving results. In addition, especially in the US, lowering weight and getting adequate exercise are both paramount. It is important to prevent any evidence of deficiency, but sodium restriction is a mute point. The greatest cardiovascular benefits are achieved by weight loss. Dietary patterns characterized by fruits, vegetables, whole grains, low-fat dairy products, and lean meats, are associated with a lower risk of mortality.

CARDIOVASCULAR DISEASE

In the 1970's and 1980's the simple lipid hypothesis dominated the scientific community. Emphasis was on the total fat and saturated fatty acids of the diet, and the ratios of LDL (low density lipoproteins) to HDL (high density lipoproteins) cholesterol. This focus led to an increased consumption of skimmed milk, and reduced consumption of cheeses, creams and other higher fat dairy products. This resulted in a lower total fat and saturated fatty acids (SFA) intake, and a higher intake of polyunsaturated fatty acids (PUFA) from margarines and spreads. Little emphasis was placed on the beneficial effects of other fatty acids in the diet, particularly the monounsaturated fatty acids (MUFA), the omega-3 fatty acids, and conjugated linoleic acids.

Current advances in cardiovascular nutrition indicate that high MUFA diets are more cardioprotective than low-fat diets. This is particularly true for those subjects who

show a decline in HDL in response to a low-fat diet. The challenge then is to find effective simple strategies to substitute MUFA in the diet.

After feeding a diet rich in SFA versus a diet high in MUFA, scientists were able to show a significant reduction in expression of adhesion molecules on leukocytes with subjects on the MUFA diet. This reduction is also beneficial in reducing tendency to inflammation. Studies also show that when you give fat acutely to individuals, it leads to activation of factor 7, a predictor of increased clot formation. Individuals on a high MUFA diet show less activation of factor 7 than individuals on a high SFA diet. Thus cholesterol reduction is important, but is not the only factor in determining cardiovascular risk. High MUFA diets are shown to reduce inflammatory and coagulation tendencies.

Currently 87% of dietary PUFA intake is n-6 or omega-6 class, and consumption of omega-3 PUFA is down. The population eats only 1.4 grams of alpha-linolenic acid and less than 0.2 g per day of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are very powerful long chain PUFA helpful in preventing cardiovascular disease.

Omega-3 PUFA are able to reduce triglyceride blood levels, inhibit platelet aggregation, reduce blood clotting, are anti-arrhythmic, reduce blood pressure, and are anti-inflammatory. Purportedly, about 1 gram per day of DHA and EPA can reduce heart disease risk. In subjects who ate 2-3 servings of oily fish per week, there was a significant improvement in survival versus a control diet group which ate a healthy diet.

OSTEOPOROSIS

Osteoporosis is an important public health problem. In the US, 28 million people suffer from osteoporosis; and the majority of these are women. The annual cost of osteoporosis is estimated to be USD 14 billion per year. The disease is a global issue, and it is estimated that in 2020, half of all osteoporosis in the world will be in China.

The role of dairy products in the prevention of this disease is clear to all. In the US, dairy foods are the major contributor of calcium in the diet. Estimates are that 75% of dietary calcium in the US is supplied by dairy foods. Still, the population does not consume enough calcium. For males, median intake is 800 mg per day, significantly less than the RDA of 1000 mg; for females, median intakes are around 600 mg per day. Significant marketing and nutrition education are needed to encourage use of dairy products and other foods to increase the level of calcium in the diet.

There are three main organ systems involved in the metabolism of calcium and phosphorus: the skeleton, the kidney and the intestine. Besides the level of dietary calcium, other body processes that can be manipulated through functional foods are urinary loss of calcium, efficiency of calcium absorption, and bone formation or bone resorption. These could promote optimal bone mineral density, optimal bone structure, and reduce the risk of bone fracture.

Two specific approaches to fortify dairy products with functional foods have been suggested. First, dietary phytoestrogens could be used to reduce bone loss; and second,

dietary oligosaccharides could be used to enhance calcium absorption. There are many sources of dietary estrogens in the diet, as well as various synthetic compounds that have shown estrogenic effects *in vivo*. There are also many naturally occurring phytoestrogens, coming from plant foods, animal foods and fungi.

Current emphasis on phytoestrogens centers on isoflavonoids, particularly isoflavones, and lignins. Two of these isoflavones, daizein and genistein, are found in soy products. However, the type of soy can determine the level of these compounds. For example, tofu and soybeans are not equivalent as vehicles for delivering bioactive compounds. The three main isoflavones found in soybeans, daizein, genistein, and glycitein, have similar structures. These compounds have the potential to interact with the estrogen receptor. Evidence from studies over the course of the last 20 years with synthetic isoflavones, such as ipriflavone, indicates that isoflavones may be beneficial in the area of osteoporosis.

Current research has explored the role of ipriflavone in preventing bone loss. One test utilized ovariectomized (OVX) rats. Ovariectomy creates an estrogen deficiency state over the course of the experiment, which is one month. Studies have compared a soy protein diet to casein, and measured bone mineral density. Actual study results revealed that protective effects on bone come with the isoflavones, and not with the soy protein.

There is very little data from human studies. In one study, on three groups of women, as isoflavone dosage increased, a positive effect on bone mineral density was shown in older women.

In summary, evidence suggests that synthetic and dietary isoflavones can influence bone metabolism in rats and humans. Additional studies are needed on humans in particular, to define the optimal and safe dose, to identify the form that would have the most positive effect on bone, and to demonstrate the long-term efficacy on bone mineral density and fracture incidence.

Enhancing calcium absorption

Several studies have tested whether dietary oligosaccharides can enhance calcium absorption in humans. One recent study evaluated factors that affect absorption in the colon rather than in the small intestine. This was a cross-over study, with 5 grams of oligofructose, in 3 meals per day, for 9 days, versus a control diet. The oligofructose was supplied by orange juice. Study subjects were young male adolescents, age 14-16. These individuals had a high calcium intake of 1267 mg per day. In the last 2 days, calcium absorption was measured. A sophisticated stable calcium isotope test was administered with 200 mg of calcium.

This study waited 36 hours to measure calcium absorption, as opposed to previous studies that had used the standard 24 hours waiting-period and found no effect. Because of the colonic effect, researchers were able to show a positive effect on calcium absorption, or influx of calcium across the gut. There was a 12% increase in fractional calcium absorption in the oligofructose group as compared to the control group with no

oligofructose. These results show a significant potential role for prebiotics in the achievement of peak bone mass.

CANCER

The search for cancer prevention involves lofty goals that are not easy to achieve.

Science is looking for a way to slow the onset of carcinogenesis, and to prevent tumors from progressing. Some food substances such as garlic are beginning to show promise in this area, and there is hope that simple dietary substances may be very relevant in cancer prevention.

In human nutrition, interactions between nutrients are important. Very high or low intakes of certain nutrients may create imbalances with other nutrients. A good example of this is the relationship between zinc and vitamin E. In an animal model, a decrease in dietary zinc will inhibit the attainment of maximum levels of vitamin E. This has also been confirmed in human studies.

Recent studies to compare risks for breast cancer have compared the Western diet to a control diet. Results showed that subjects on the Western diet, which is high in fat, high in phosphate, low in calcium and low in vitamin D, showed a higher rate of proliferation in the small ducts where breast cancer occurs as compared to the control diet. Researchers concluded not only that the western diet has an adverse effect on risk factors for cancer, but also that the combination of nutrients is important.

Both overnutrition and undernutrition may be causative factors in cancer. Overweight relates to a higher incidence of cancers of the breast, colon, prostate, and probably the uterus; while underweight increases the risk of cancers of the stomach, esophagus, and liver. Consumption of fruits, vegetables and grains is protective. Currently researchers are seeking to identify the specific micronutrients involved in risk reduction. There has been a great deal of interest in selenium, omega-3 fatty acids, folic acid, and antioxidant vitamins.

Role of dairy foods

There has been some concern about dairy products and increased risk of prostate cancer, but dairy foods may be protective against breast and colon cancer. Current focus is on finding the mechanisms that account for changes. One possibility is that vitamin D in its active form, 1,25 D, serves to slow down cancer development. Increased calcium may suppress the concentration of 1,25 D. Therefore, it is important to have adequate amounts of vitamin D in the dairy products, or to take additional vitamin D.

Purportedly, calcium in milk may have anti-proliferative effects, and inhibit tumor promotion. It is thought that fermentation and production of probiotics may reduce the progression of preneoplastic lesions. There are many mechanisms through which dairy products might work.

Probiotics may have a role in cancer prevention by influencing microbial flora. This may be due not only to the presence of the probiotic bacteria, but also to the removal of other bacteria by competition in the gut. Probiotics may also improve nutrient bioavailability. In addition, they may have immunologic effects, may stimulate IgA response, and may effect production of cytokines.

Immunity and cancer

The GI tract is the body's first barrier, and the immune system plays a critical role in cancer prevention. Primary immune deficiencies are associated with increased risk for gastric cancer. Immune factors are critical in determining cancer development, and immunodeficiency may be one of the ways in which cancer develops. Again, there is a risk for both overnutrition and undernutrition. Individuals with bone marrow transplants are at an increased risk of cancer. Weight loss has a severe prognosis.

How might probiotic bacteria be helpful specifically in colon cancer prevention? They may enhance post-immune response. They may crowd out organisms that are involved in producing carcinogens, and they may neutralize carcinogens. They may alter the metabolism of intestinal flora, and may produce antitumor factors. In the human colon, there have been studies on several lactic acid bacteria, specially *Bifidobacterium longum*, that show that this organism may reduce tumor ornithine decarboxylase activity, ras p21 expression, and have strong antitumor activity. The biomarkers reflect this. Some safety issues such as the possibility of uncontrolled growth and proinflammatory immune response may warrant further study.

To summarize, nutrition is enormously linked to cancer prevention. This is true for nutrition in general, as well as for specific nutrients and secondary plant products. Further study is needed into the mechanisms of action and validation of these agents. Probiotics may be a vital key to cancer prevention.

Two specific fatty acids outlined below show promise in the reduction of cancer risk. One is of dairy origin, and the other of marine origin, but might be a candidate for inclusion in dairy foods.

Conjugated linoleic acid

Conjugated linoleic acid, CLA, occurs naturally in red meat and dairy products, as a by-product of ruminal hydrogenation. CLA has potent anti-carcinogenic effect, reduces fat deposition, and has anti-atherogenic properties. To date, most of the studies using CLA have been done in small experimental animals, and have been using high-dose levels. A recent paper showed that levels of CLA in human adipose tissue strongly correlated with intake of milkfat. Milkfat is an important dietary source of CLA. The data for CLA and human health benefits is weak, and in animal studies the data is still equivocal. Two studies have shown reductions in cholesterol, but did not show a dose response.

Anti-carcinogenic effects were shown in animals at levels that would be the equivalent of ingesting 3 grams per day of CLA. Anti-atherogenic effects have been found at very high levels, equivalent to human consumption of 400 grams per day, which would not be feasible.

Docosahexaenoic acid

Docosahexaenoic acid (DHA), is a major polyunsaturated fatty acid (PUFA) most often derived from marine sources. DHA can be synthesized from alpha linoleic acid, but this biosynthesis is not very efficient, and a deficiency may lead to impairment of functions such as visual acuity and learning ability. DHA is highly concentrated in the brain, retina and spermatozoa.

Despite some knowledge of the biological functions of DHA, relatively little is known about its metabolic fate. In a recent study, elderly people were given a daily intake of 150 mg DHA, plus 30 mg EPA (eicosapentaenoic acid), for six weeks. Using ¹³C-labeled DHA, researchers were able to show that DHA esterified in lysophosphatidylcholine is the main provider of DHA to erythrocytes and the brain. A daily intake of only 100 mg DHA in triglycerides by elderly people, a population in which an oxidative stress may be evidenced, appears to be able to reverse this oxidative stress. It is concluded that a low intake of DHA might be useful, both for adequate supply to target tissues, especially the brain, and to prevent lipid peroxidation. DHA thus finds a role as an anticarcinogen, and may also have beneficial effects against atherogenesis and arrhythmia.

CHAPTER V

ADDING FUNCTIONAL INGREDIENTS. THE CHALLENGE

FOOD SYSTEMS

Dairy foods are rich sources of protein, calcium and a variety of vitamins, minerals and bioactive compounds. They provide an ideal food medium for delivering probiotics and other functional ingredients. The message was strongly relayed by many of the experts in various fields, that functional foods should be foods and not pills.

Elderly populations often experience PEM (protein energy malnutrition). For these individuals who may be lacking protein, calcium and vitamin D, yogurt provides an excellent source of these and other nutrients. It provides an added advantage for all populations who may have lactase deficiency, because the probiotic bacteria in yogurt produce lactase, which is the enzyme which breaks down lactose. It has been demonstrated in numerous tests that yogurt is well tolerated by individuals with decreased lactase levels.

Probiotics can be delivered through yogurt, fermented milks, cottage cheese and similar dairy products, and also through fortified juice, and infant formulas. Two other vehicles for delivery are pills and nutritional supplements. There is no guarantee that pills will contain the advertised bacteria, or that the probiotics will be viable when they reach the body. Fermented milk products may have a short shelf-life.

One challenge is to incorporate prebiotics into a wider range of food. A novel application was a biscuit formulation. In a recent double-blind, randomized, placebo controlled test with 31 volunteers, a prebiotic was served in a biscuit formulation for a 21-day treatment period. Using fluorescent in situ hybridization, researchers confirmed that there

was a significant increase in Bifidobacterium levels in the active group, thereby confirming that the prebiotic nature of the FOS did hold up in this real food product.

Development of prebiotics should look at enhanced application in food system. These food ingredients should exhibit good storage, varying sweetness for different applications, as well as pathogen binding ability.

ENHANCING FUNCTIONALITY OF PREBIOTICS AND PROBIOTICS

It is estimated that in Europe, 10 million people regularly consume probiotics. Prebiotics are gaining in popularity, and the most commonly used are fructooligosaccharides, lactulose, and trans-galactooligosaccharides. Other oligosaccharides are being evaluated as potential prebiotics.

To enhance the effectiveness of probiotics and prebiotics, it may be helpful to collaborate more closely with nutritionists, to study the mechanisms of host-microbe interactions, and to examine many of the new technologies which have been described in this symposium. Specific methods to enhance functionality may include the following.

- **Targeted activities for the distal colon:** as most large gut disorders are of left-sided origin, the persistence of prebiotics and probiotics towards this area is desirable. Most release occurs in proximal regions, where carbohydrates enter through the ileocecal valve. As food moves through the large intestine towards the distal area, there is more proteolysis, resulting in more phenolic compounds and carcinogens. More sacrolytic balance is needed to alleviate ulcerative colitis and bowel cancer.

- **Encapsulation:** to more fully protect probiotics in the gastrointestinal tract, lyophilized cultures have been encapsulated. This has often included materials such as gelatin, shellac and amylose. However, encapsulation with a prebiotic may offer both a protective capacity, as well as increased levels of growth substrate.

- **Synbiotics:** the combination of probiotics and prebiotics, called synbiotics, may offer the dual advantages of each as well as provide a selective substrate for the live microorganisms in the gut. A good example would be a mixture of bifidobacteria and FOS. Moreover, the use of reverse enzyme technology may allow the probiotics to generate their own substrate. Current research focuses on a mixture of prebiotics that are of varying molecular weights.

- **Anti-adhesive properties:** receptor sites for a variety of gut pathogens involve oligosaccharide sequences. If these could be incorporated into existing prebiotics they may act as “decoy” molecules. That is, the pathogen would bind to the prebiotic at an appropriate site, instead of the gut wall. Thus, the first line of pathogenesis would be compromised.

- **Attenuative properties:** the oligomer cellobiose is able to repress virulence in *Listeria monocytogenes*. When exposed to rotting vegetation, in a natural environment, *Listeria* is not a pathogen. In foods, where there is no cellobiose, the virulence is allowed

to be expressed. This downregulatory process may be a further facet to consider in prebiotic research.

- **Species-level changes:** most existing prebiotics tend to act at the genus level. However, finer control of microflora modulation may be directed towards distinct species. For example, *Bifidobacterium infantis* is a more powerful inhibitor of gut pathogens, such as *E. coli* H0157, than other bifidobacteria. Certain galactooligosaccharides can confer species level changes in bifidobacteria.

- **Activity at low dosage and with no side-effects:** the minimum active dosage varies according to prebiotic type and excessive dosages may result in excessive gas production. As this arises from non-specific metabolism, prebiotics targeted more closely at bifidobacteria and lactobacilli would be highly desirable. In human trials, doses of up to 40 grams of FOS per day, has been reported with little or no adverse side effects. Minimal operative dose of lactulose is about 10 grams per day, for FOS it is 8 grams per day, and in vitro data indicate it may be as low as 4 grams per day. For probiotics, there seems to be no upper dosage limit.

Whenever human trials are done on probiotics, the starting level of bifidobacteria may determine the impact of the prebiotic on probiotic growth. At lower initial microbial levels, the increase in growth may be more significant.

DEVELOPING NOVEL PREBIOTICS

Recent advances in glycototechnology hold much promise for achieving many desired enhancements to current prebiotics and probiotics. There are a number of oligosaccharides that have been recognized as prebiotics, and quite a range of these products are on the market in Japan. Biotechnological approaches to the manufacture of oligosaccharides fall into two general categories: enzymatic synthesis and controlled degradation of polysaccharides. The challenge is to design oligosaccharides that have specific rheological, functional and organoleptic properties.

Pathogen binding

Pathogens will bind to receptors on the cell. Subsequently, bacteria will liberate toxins, and toxins will often bind to a different set of receptors on the cell. The challenge is then to make food ingredients that will intercept toxins and bacteria, and to get these into the colon at sufficiently high concentrations to have a useful inhibitory effect. It is also important to understand selectivity, so that the prebiotic will increase numbers of desirable organisms.

The first challenge is to make the oligosaccharides with receptor activity. Several prebiotics are manufactured by the hydrolysis of the parent polysaccharide. Some work has been done with converting dextran into isomaltooligosaccharides of a controlled molecular weight. These would have varying rheological properties, varying technological properties

such as color formation, and hopefully better persistence through the colon. One advantage of the isomaltooligosaccharides produced to date is low gas production.

Bacteriophages as prebiotics chemists

Bacteriophages are viruses that infect bacterial cells. They possess an enzyme that breaks down the polysaccharide capsule of the bacteria. The phages then have access to the bacterial cell and can affect it. Bacterial extracellular polysaccharides are very complex structures, and bacteria are wonderful carbohydrate chemists. By looking at these polysaccharides, we can increase the structural complexity of novel oligosaccharides. The beauty of the capsular depolymerase enzyme is that cleavage of the polysaccharide always occurs at a defined point. These capsular polymerase enzymes reduce the polysaccharide down to an oligosaccharide so that it can be evaluated for the prebiotic properties. Some will be novel and patentable.

These capsular depolymerase enzymes exhibit great specificity and are easy to obtain by fermentation. Many of these bacterial polysaccharides also have receptor activity, in that pathogen receptor sequences often form part of the repeating sequence of the polysaccharide. This offers the potential for large-scale manufacture of receptor sequences for testing as candidate anti-adhesive, or decoy, oligosaccharides.

Metabolism of fructooligosaccharides

By studying the molecular basis through which lactic acid bacteria, especially probiotic bacteria, metabolize fructooligosaccharides (FOS), scientists are able to identify strains that provide the best fermentation substrates.

To determine which bacteria ferment FOS, scientists first identified 19 FOS-fermenting strains of *Lactobacillus* and *Bifidobacterium* using an agar medium containing pure FOS. HPLC analysis of fermentation broths revealed that FOS-fermenting strains consumed only GF2 and GF3, and none fermented GF4, which are various fractions of FOS. This method was also useful to identify mutant strains.

The enzymes that hydrolyze FOS are located in the cytoplasm, and researchers wanted to identify methods by which fructooligosaccharides are transported by probiotic bacteria. To measure transport, researchers synthesized radio-labeled [^{14}C] FOS, from radio-labeled [^{14}C] sucrose. Studies revealed that fructose, glucose, and sucrose have some affinity for the FOS transport system, but not galactose or lactose.

In conclusion, a convenient, easy method has been developed to identify strains that ferment FOS. Most of the *Bifidobacteria*, and some of the *Lactobacilli* ferment FOS, but bacteria involved in yogurt fermentation do not. Transport is essential for FOS metabolism by probiotic bacteria.

ALTERING DAIRY FOODS

Much of the research presented at the symposium focused on probiotics and prebiotics. However, other approaches to developing functional dairy foods might include adding garlic, bovine colostrum, isoflavones, or a variety of other functional ingredients.

Another approach is to manipulate the fatty acid content of dairy foods to produce a more favorable fatty acid profile. Altering the composition of feed to dairy cattle has shown success. Animals fed canola seed oil, show increased MUFA content, increased PUFA content, and reduced LDL and total cholesterol content.

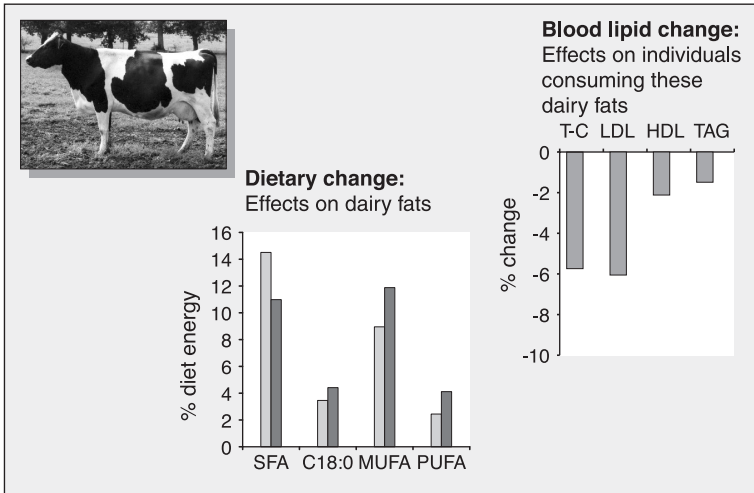


Figure 4. Modifying blood lipids through feed-lot technology; effects on triglycerides and cholesterol levels.

[Source: C. Williams, University of Reading, UK.]

In terms of public health, a modest increase in PUFA is warranted in the population as a whole. There is still debate about optimal levels. One problem is that people do not like the flavor of fish oils. Another problem is that these fats are unstable when added to foods during processing. However, increasing the amounts in animal feed could increase omega-3 PUFA in the food chain. Unfortunately, these foods may be hydrogenated in the rumen and then become ineffective.

Studies in the UK suggest that using fish oils in dairy cattle feed could increase the content of PUFA in milk. However, the content in enriched milk is extremely low. There are questions about the efficacy of this measure as a potent means of increasing supplies of these fatty acids in food. There is also the issue of sustainability of sufficient fish oil production.

There are alternative strategies for increasing omega-3 PUFA. One strategy is to synthesize DHA and EPA from alpha-linolenic acid. Another option is to feed canola oil to dairy cattle, and protect them from hydrogenation in the rumen. This has been tested and produces a milk and a cheese that is pleasant and higher in alpha-linolenic acid content.

MEASUREMENT

IN VITRO MEASURES

In evaluating structure/function relationships, an initial step is to run simple batch culture experiments, and then to measure certain markers such as increasing bifidobacteria, decreasing clostridia, and increasing butyrate production. The next step would be to compare and rank prebiotic oligosaccharides based on these properties. For example, low gas production is a desirable characteristic. Isomalto-oligosaccharides (IMO), with the lowest gas production, received a rank of 1; inulin, with the highest gas production, received a rank of 7.

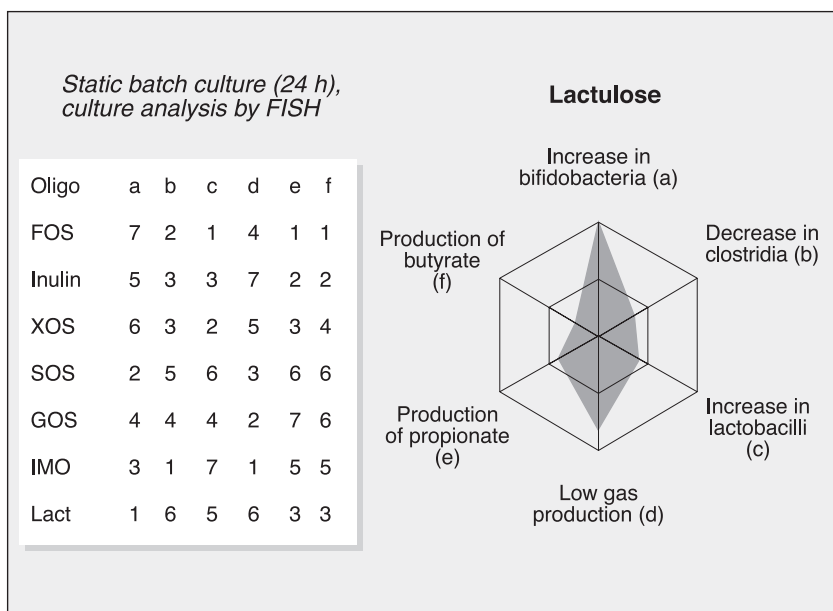


Figure 5. A rank method for evaluating structure-function relationships of prebiotics.
[Source: B. Rastall, University of Reading, UK.]

A more sophisticated system, a “prebiotic index”, is being developed which would use standard test methodology to quantify selectivity markers. The idea is to come up with a number that will increase if Bifidobacteria and Lactobacillus numbers increase, and decrease if E. coli numbers decrease, etc. All of these population changes would be factored to come up with an index number. This model is still under development, but shows much promise.

DESIGNING HUMAN TRIALS

Earlier functional foods were defined from a government and food industry perspective. However, from a consumer perspective, they might be defined as “products that contain extra ingredients that manufacturers ‘claim’ to give an extra benefit”. Claims are a vital part of functional foods, and human intervention studies or “clinical trials” are considered as the ultimate scientific proof for health claims.

Well-designed studies will produce outcomes or biomarkers relevant for substantiating the claim. They should not be misleading, and they should not be medical. The emphasis should be on human volunteer studies, and not clinical trials. In the Netherlands, voluntary codes have been adopted. They are outlined below. Studies should:

- be based on scientific evidence;
- include studies on humans;
- deal with products not ingredients;
- contain reproducible data;
- be relevant for target group; and
- be consistent with nutrition guidelines.

In clinical trials, the “gold standard” is the double-blind, placebo-controlled, parallel-designed trial. For human volunteer studies, the standard will have to be modified in several ways. Everyone agrees that the tests should be “randomized”, as to which group receives the active treatment and which group receives the placebo. They should also be “placebo-controlled”, but with nutrition research, it is often hard to determine an appropriate control. Also, with nutrition studies it is often difficult to make a “double-blind” study; for example if an individual consumes 400 grams per day of brussel sprouts, it is difficult to be blinded to this fact. A “parallel” design will usually be the standard.

In nutrition research, tests may be either a parallel design or a cross-over design. In a parallel design, one group receives the A treatment, and the other group receives the B treatment. In contrast, in a cross-over design, one group receives the A treatment first, followed by a wash-out period, then the B treatment. The other group receives the B treatment first, then a wash-out, and finally the A treatment. A parallel design gives the opportunity for shorter-term experiments. The advantage of cross-over tests is that they can compare more treatments; but the disadvantage is that they take longer, which often limits the test to fewer treatments.

In designing human volunteer studies, it is important to look at safety, and strict diet control. Confirmation of mechanisms must also be considered. A smaller study often allows for stricter control. Researchers should assess voluntary intake before the start of the trial. Compliance is often difficult with a recall diet. Efficacy is often established in larger studies with mixed foods, in larger doses than would normally be consumed. The research must then correlate this to normal dosages and normal use. To keep up compliance in trials, researchers should check on what volunteers eat, keep up motivation, include markers for compliance, and maintain a good atmosphere.

Each study requires careful consideration of the size and characteristics of the study group. For example in a cholesterol test, it is best to choose a slightly hyper-cholesteremic group. In a bone density test, good subject choices would be post-menopausal women or adolescents. In a test of probiotics, a healthy group might not show improved immune function; while a group with lowered immunity might show a greater test effect. The inclusion criteria for a sample population would be an individual with apparently good health, stable, eating the standard Western diet, and with no history of drug use.

Another necessity in volunteer studies is to have good clinical practice. This includes complying with ethical standards, designing tests that are scientifically sound and well described, and having independent audits, and traceable data and results. Such tests would be a standard for regulatory authority, but there is a lot of work to comply with these criteria.

In tests, functional foods will have an important role in maintaining homeostasis. This may not be reflected in mean levels, repeated measurements may be more informative, and alternative statistics may be required.

In conclusion, when testing functional foods, human trials are crucial. Careful consideration of trial design and markers, and study population, will determine success. Some alternatives to double-blind, placebo-controlled trials should be considered.

VALIDATING APPROACHES

Objectively seeking cause and effect relationships for probiotic strains remains a daunting challenge. What is the correct definition of a probiotic? How does one define selection criteria? How can in vitro selection criteria be validated with in vivo studies?

In a recent review of the clinical studies conducted from 1988 to 1998, it was found that *Lactobacillus rhamnosus* strain GG can shorten the course of rotaviral diarrhea by one day. Other health effects of probiotic bacteria have not been validated so well. Scientific rigor must be brought to this field. Since 1998 there has been an explosion of new clinical data suggesting positive outcomes for probiotics. Well-designed studies are definitely needed to confirm probiotic intestinal roles, functional activities, and interactions with other resident microflora. The field of probiotics is exploding with interest for in vivo information.

Traditional selection criteria when considering viable cells have been that they should be able to survive gastric acidity, adhere to the intestinal epithelium and colonize the intestine, at least temporarily. There is some discussion as to whether viable cells are needed, whether adherence is important, and over the whole issue of colonization of the intestine.

There are four key areas of selection criteria for probiotics. These are:

- appropriateness,
- technological suitability,
- competitiveness,
- performance and functionality.

Criteria for **appropriateness** are that strains must be accurately identified: they should be normal inhabitants of the species targeted, and they should be non-toxic and non-pathogenic. Most are considered GRAS (Generally Recognized As Safe), although this cannot always be demonstrated with clinical studies, and is often based on a historical perspective. A desirable antibiogram is important, as is immunocompromised compatibility. Lastly, they should be non-inflammatory.

In evaluating **technological suitability**, one must consider whether the bacteria are amenable to mass production and storage. Also, their viability must be determined, as well as their organoleptic qualities. Finally, scientists must determine if the species is genetically stable and genetically amenable.

Competitiveness measures how well the probiotics survive in the GI tract, and their resistance to acid and bile. Acid tolerance can be evaluated through testing in a gastric juice formulation (pH 2, 0.5% NaCl, and 0.3% pepsin). It has been shown that certain strains fall off from 100% to as low as 30-40% in 60 minutes. *Lactobacillus* can be resistant to bile at levels of 0.15 to 1.5%. Scientists have so far not validated this resistance in vivo, nor have they validated adherence in vivo. Studies show that when feeding stops, some strains are cleared from the GI tract within two to three weeks.

The last measure is **functionality**. Here the issue is what are the clinical benefits. Do the bacteria have a desirable metabolic activity; do they have an impact as antimicrobials; and are they immunostimulatory? Can this activity be demonstrated in vivo? For example, can *L. johnsonii* interfere with *Helicobacter pylori* adhesion? In some areas bacteriocins are well defined, and sometimes there is no validation of the active agent.

The distance between in vitro and in vivo grows larger. However, the gap will close rapidly in the next five years, because of genome sequencing efforts on lactic acid bacteria. The list is over 50 and exploding. There are at least 3 genotyping projects going on around the world, focusing on *Lactobacillus acidophilus*, *johnsonii* and *plantarum*.

With further study, molecular mechanics will become clearer, and strain to strain comparisons will look at some of the active principles that are involved in the modulation of the immune system. Clinical trials can then be used to correlate specific bacterial genotypes with performance and functionality, by a direct comparison of strains to the parent or the wild types. When scientists better understand the mechanistic basis of probiotic

functionality, they will be in a better position to understand survival/tolerance, metabolic activities, antimicrobial activity and immunostimulation.

In the end, to validate in vitro selection criteria, one must have the in vivo validation of specific active principles that are responsible for probiotic effects. This is a scientific platform that the industry is obligated to pursue. The question remains, what role will be left to industry, to government, and to science? This research is expensive, and there may be a need to move into government and medical funding.

CHAPTER VII

REGULATORY CHALLENGES

In the regulatory arena, probiotics have perhaps a distinct advantage over other functional foods because of their cultural acceptance. In France, probiotics have had a wider latitude in terms of regulatory acceptance, and in the EU a more comfortable place in both the PARNUTS (foods for particular nutritional uses) and novel foods area.

Industry has led science in the area of marketing functional foods. In addition, the Internet has created a new marketplace for functional foods that operates below the regulatory framework. As a result, the definition of science has changed, and the level of science necessary to correlate to acceptable products in the marketplace has also changed. In Europe, most products are regulated by what they are. Many herbs can be used as drugs. In contrast, in the US, what is said on the label determines whether the product is a drug, which must go through pre-market approval, or a food or dietary supplement, which does not.

US REGULATIONS

The chart below outlines the United States claims continuum:

A **drug claim** states that a product treats or cures a disease.

A **health claim** describes a long-term reduction of disease risk. An example would be that fiber helps reduce the risk of colon cancer. In the US, there is a petition to establish a health claim for *Lactobacillus*.

“Medical Foods” used to be primarily enteral nutrition products delivered through the alimentary canal. Now the industry is a multi-billion community delivering nutrition through candy bars and focusing on the geriatric population. It is difficult to define “Foods for Special Dietary Use”. However, these are intended for individuals with diabetes,

dysphagia, phenylketonuria, and similar conditions. There is a huge market for these products that needs scientific direction.

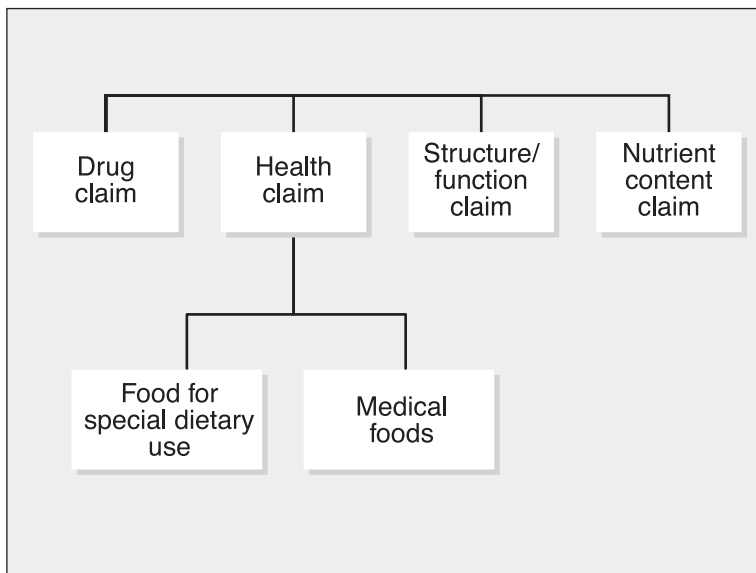


Figure 6. In the United States, the “intended use” determines the product category. [Source: S. Bass, Sidley and Austin, Washington DC.]

A **nutrient content claim** states the presence or absence of certain nutrients, such as “Good Source of Calcium,” or “Low Fat”.

A **structure/function claim** generally applies to functional foods. In 1994, the US Congress passed the Dietary Supplement Health Education Act (DSHEA). This law opened up a new category of claims. Unfortunately, the definitions for this new category of claims are very unclear. There is no clear standard for proving an immune claim. For example, it is uncertain how many in vitro markers really mimic the complexities of the immune system. In the US, a recent court decision, the Pearson decision, ruled that a food company must be allowed to give more information to the public, as long as it is disclaimed. Even though the FDA held meetings, on this issue, the policy is unclear.

Congress moved structure/function claims to a separate section for dietary supplements. Structure/function claims are outlined in the Code of Federal Regulations, §403(r) 6, and are defined as:

- something that affects the structure/function of the body – but not disease;
- there must be a documented mechanism;
- may contribute to a state of well-being.

For the structure/function claim that probiotic bacteria help build the immune system, an example of the documented mechanism would be the way in which probiotics work on the various cells in the immune system, such as T cell proliferation, or macrophage

effect. The criteria for contributing to a state of well-being is meant to address products like St. John's wart that have a non-specific systemic effect.

ESTABLISHING CLAIMS

On January 6, 2000, FDA issued a structure/function regulation for dietary supplements that said that the breadth of the claims would be the same for supplements as for foods. Below are several FDA examples of structure/function claims, which would be acceptable for functional foods, as opposed to disease claims, which could not be used for functional foods.

1. General pain relief, (acceptable) – alleviate arthritic pain (not acceptable).
2. Sleep defects (acceptable) – used as a sleeping pill (not acceptable).
3. Laxative effect (acceptable) – chronic constipation (not acceptable).

For conventional of functional foods, claims can only be made for nutrients; however for dietary supplements, claims may be made for non-nutrients. There must be no unreasonable risk of illness or injury. The difficult part is to establish efficacy, as there are no standards under FDA law. One must first establish a protocol.

To establish a scientific protocol, one must establish endpoints that are targeted to the type of claim, usually after consultation with academia, private industry, or a trade association advisory group. The FDA has declined, to date, to establish a standard for such protocols. It is up to scientists and industry groups to self-regulate. Companies may also use independent panels, either official or unofficial GRAS panels, for determining efficacy as well as safety.

A dietary supplement is not just a pill that has a functional ingredient such as *L. acidophilus* in it. It is generally a drink, a bar, or some other conventional food. However, you can not sell a conventional food as a dietary supplement, for example potato chips with St. John's wart. In the US, Actimel® is considered a dietary supplement.

In enforcing advertising of food supplements, the Federal Trade Commission (FTC), and not the FDA, has major responsibility. The FTC does not have a group of scientists. FTC has two documents on their web-site which explain the advertising policy for dietary supplements. The address is [www.FTC.org/dietary supplement](http://www.FTC.org/dietary%20supplement). The FTC standards include "reasonable basis" and reliance upon scientific experts and published studies. The preferred evidence to support a claim is clinical studies; the second option is good quality animal studies, and the third alternative is epidemiological evidence and historical use. The last option falls far short of the first two in credibility.

WORLDWIDE REGULATIONS

In Europe, the situation is different. The development of regulations for functional foods is moving rapidly at national level, but not at EU level, due to the PARNUTS

directive. The EU has established a draft list of functional foods, and it contains only RDA and RDI nutrients. However, probiotics will get favorable treatment because of cultural acceptance. The EU commission's recent draft paper on dietary supplements will invoke the precautionary principle, ensuring that this area moves forward slowly in Europe as compared to US, Japan, Australia and Canada.

In defining endpoints, industry has forged ahead of government. Europe has set up some precautionary principle documents, but politics have often complicated the regulations. EU had to sue two countries to have them approve ingredients. Scientists will ultimately direct the functional food world, but now industry rules. Japan has FOSHU; and Canada has put millions of dollars into a new functional food and nutraceutical division. The EU is establishing a dietary supplement policy and has expanded its PARNUTS products with a novel foods directive. Companies are penetrating national markets in the EU, and are moving forward to get consumer acceptance. The Internet is carrying a lot of the market below the national regulatory framework.

One major difference between the EU and the US lies in the substantiation of scientific protocols. Safety remains the top issue in EU, but the US situation is very much driven by market forces.

A new group, the Research Based Dietary Ingredients Association is working with ILSI, the International Life Sciences Institute and a number of larger companies to establish scientific parameters for efficacy and safety. They are establishing panels of academic scientists to review new ingredient claims for safety and efficacy, which will hopefully be the self-regulatory model. They will be establishing an immune category that would include probiotics.

The goal is to ensure that science, not sales, will be the driving force in the functional food industry. Hopefully, when functional foods get into the hands of lower level marketing companies, the science will be good, the products will be safe, and there will be some margin incentive.

Functional Dairy Products

For nearly a century, scientists have been aware of the benefits of probiotics. These live micro-organisms, when they are ingested in sufficient quantities, have a positive impact on health, which goes beyond conventional nutritional effects. It is known that some probiotics create the best possible conditions for the innate and adaptive immune systems to work together.

Research has now reached a new stage with the establishment of the “functional foods” concept, which covers a broader range of products, including “prebiotics” and “synbiotics”.

The aim of this book is to supply a clear definition of “functional foods” and to explain how they act on the body to enhance physiological function and reduce the risk of disease. It will thus be seen that these foods have a significant role to play in reducing the risk of digestive and cardiovascular diseases and cancer, as well as in promoting the immune system.

This book bears witness to the impact of scientific research on the way we eat, and the innovative image it gives to the human diet as foodstuffs become active ingredients that will soon offer a guarantee of enhanced health. A book to be read attentively, both for its scientific interest and for the prospects it opens up.



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