SPECIAL GUEST EDITOR SECTION

Need for Accurate and Standardized Determination of Amino Acids and Bioactive Peptides for Evaluating Protein Quality and Potential Health Effects of Foods and Dietary Supplements

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Accurate standardized methods for the determination of amino acid in foods are required to assess the nutritional safety and compositional adequacy of sole source foods such as infant formulas and enteral nutritionals, and protein and amino acid supplements and their hydrolysates, and to assess protein claims of foods. Protein digestibility-corrected amino acid score (PDCAAS), which requires information on amino acid composition, is the official method for assessing protein claims of foods and supplements sold in the United States. PDCAAS has also been adopted internationally as the most suitable method for routine evaluation of protein quality of foods by the Food and Agriculture Organization/World Health Organization. Standardized methods for analysis of amino acids by ion-exchange chromatography have been developed. However, there is a need to develop validated methods of amino acid analysis in foods using liquid chromatographic techniques, which have replaced ion-exchange methods for quantifying amino acids in most laboratories. Bioactive peptides from animal and plant proteins have been found to potentially impact human health. A wide range of physiological effects, including blood pressure-lowering effects, cholesterol-lowering ability, antithrombotic effects, enhancement of mineral absorption, and immunomodulatory effects have been described for bioactive peptides. There is considerable commercial interest in developing functional foods containing bioactive peptides. There is also a need to develop accurate standardized methods for the characterization (amino acid sequencing) and quantification of bioactive peptides and to carry out dose-response studies in animal models and clinical trials to assess safety, potential allergenicity, potential intolerance, and efficacy of bioactive peptides. Information from these studies is needed for determining the upper safe levels of bioactive peptides and as the basis for developing potential health claims for bioactive peptides. This information is, in turn, needed by regulatory agencies for developing appropriate policy and regulations on adding these substances to foods and for determining if health claims are scientifically substantiated.

Accurate standardized methods for measuring amino acid levels are required for evaluating protein quality of foods, including foods with protein content claims, and sole source foods, such as infant formulas and enteral nutritionals, as well as protein and amino acid supplements and protein hydrolysates; for determining amino acid composition for food composition tables and surveys; and for assessing intakes relative to requirements. Monitoring of fermentation and correlating flavor trends in food development and assessing the need for amino acid fortification levels also make use of amino acid determination. Moreover, the detection of D-amino acids, acetylated amino acids, and nonprotein amino acids in processed and genetically modified food proteins, and low levels of antinutritional amino acid residues such as lysinoalanine, formed during processing is important for nutritional adequacy and food safety considerations.

There is also a need to develop accurate standardized methods for the characterization and quantification of bioactive peptides derived from animal and plant food sources. Various potential health effects have been attributed to food-derived bioactive peptides. These include antimicrobial properties, blood-pressure lowering [angiotensin converting enzyme (ACE) inhibitory] effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption and/or bioavailability, cyto- or immunomodulatory effects, and opioid activity (affecting appetite, behavior, and gastrointestinal motility; 1). As a result of these findings, the food and supplement industries are interested in

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commercializing products containing bioactive peptides, either as "functional foods" or as "nutraceuticals" in supplement form.

Several bioactive peptides are already on the market in Japan, Europe, and the United States or under commercial development in these countries. Responsible manufacturers of the commercially available bioactive peptides have likely followed a careful protocol for assessment of safety and efficacy, but the extent of premarket assessment of these products by governments may be limited, especially with respect to claims assessment. Certainly, the safety, allergenic potential, and adequacy, including bioavailability of the new bioactive peptides, should be thoroughly assessed before they are made widely available to consumers (2). Analytical methodology for determining the levels of some of the commercially available bioactive peptides in foods has been developed by the industry. However, this methodology has not been standardized or validated. Suitable analytical methods for determining the levels of specific bioactive peptides in foods and biological samples such as blood are needed for assessing the safety of new bioactive peptides, and to monitor the post-market safety surveillance of the commercially available products. The safety assessment may require compositional analysis, structure/toxicity analysis, bioavailability, evaluation of historical and intended exposure, clinical/epidemiological studies, and evaluation of special considerations such as potential for adverse food or drug interactions (3). Moreover, studies in humans would be needed to assess potential allergenicity, potential intolerance, and efficacy (the latter for verification of claimed health benefit) of bioactive peptides. Data from these studies would be required for determining the upper safe levels of bioactive peptides and as the basis for developing potential health claims for these substances. This information is, in turn, needed by regulatory agencies for developing appropriate policy and regulations on adding these substances to foods and for scientific substantiation of health claims.

This paper provides an update on the currently available analytical methods for the determination of amino acids and bioactive peptides in foods, and highlights the need for the standardization or validation of these methods for assessing protein adequacy and potential health effects. It also provides information on the commercially available bioactive peptides and on regulatory frameworks used in various countries to assess the safety and efficacy of these products.

Protein Quality

The quality of a dietary protein is determined by the pattern and concentration of indispensable or essential amino acids, the protein digestibility, and the bioavailability of its amino acids. To satisfy the protein and amino acid requirements of humans, the diet must supply enough indispensable amino acids (IAA) in required proportions and enough total amino nitrogen for synthesis of dispensable or nonessential amino acids. These are required to support the synthesis of body protein and the production of other nitrogen-containing compounds such as hormones and neurotransmitters involved in a range of physiological functions.

An IAA is defined as an amino acid that cannot be synthesized in the body, or at least not in adequate amounts. Nine amino acids, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, are not synthesized in adequate amounts by mammals and are, therefore, IAA for humans. Dispensable amino acids including alanine, arginine, aspartic acid, cyst(e)ine, glutamic acid, glycine, hydroxyproline, proline, serine, and tyrosine are not essential in the diet, as they can be synthesized from IAA and amino nitrogen. Because cystine can replace part of the requirement for methionine, and tyrosine a part of the requirement for phenylalanine, cystine and tyrosine are also included when considering IAA contents of diets, and these pairs are then expressed as total sulfur amino acids (methionine + cystine) and total aromatic amino acids (phenylalanine + tyrosine). Most dietary proteins contain a mixture of all 20 common amino acids in varying proportions.

Protein Digestibility-Corrected Amino Acid Score for Predicting Quality of Proteins

The only true measurement of protein quality of foods is an assessment, in humans, of growth, nitrogen balance, metabolic balance, or some other appropriate test, preferably carried out with suitable subjects from the target population of interest. Such assessments directly reflect how well the food meets human needs, and this is a function of the proportion and content of IAA, the digestibility of protein, and the bioavailability of amino acids in the food or food product.

Human studies for protein quality assessment, which are the gold standard, cannot be carried out on a routine basis for reasons of cost and ethics. Therefore, in vitro and animal assay techniques have been developed that correlate well with data from human studies for each food product. Criteria for a valid and useful assay for routine protein quality assessment include accuracy, precision, reproducibility, proportionality to protein quality, and low cost.

A method based on a comparison of amino acid content of food with human requirements is considered internationally to be the most suitable approach for routine evaluation of protein quality of foods (4). The amino acid score should be corrected for incomplete digestibility of protein and for the unavailability of individual amino acids, especially those that are susceptible to damage by processing treatments.

The Food and Agriculture Organization/World Health Organization (FAO/WHO; 4) has recommended the protein digestibility-corrected amino acid score (PDCAAS) as the preferred method for routine assessment of protein quality of properly processed and highly digestible food products for human nutrition. In other words, the foods should contain minimal amounts of residual antinutritional factors, and the digestibility of the protein should be a good approximation of the bioavailability of individual amino acids.

Analyses required for the determination of PDCAAS include proximate composition (levels of moisture, nitrogen and resultant crude protein, fat, and ash), amino acid profile,

calculation of amino acid score (IAA in test protein/IAA in requirement pattern) and protein digestibility as determined by the standardized rat balance method (4).

Since the adoption of the PDCAAS in 1991 by FAO/WHO (4), a number of important technical issues relating to this method were noted in 2007 by WHO/FAO/United Nations University (UNU; 5). These include the use of the revised human amino acid requirement values, methods for assessing protein digestibility (fecal vs ileal), and the reduced bioavailability of some amino acid residues (such as lysine) in proteins which have been chemically transformed during the manufacturing of processed foods and may not be detected in the protein digestibility assessment. In addition to these concerns is the controversial issue of truncating the amino acid score (and the resultant PDCAAS value), i.e., expressing the maximum value for individual proteins as no greater than 1.0 or 100%, when actual calculated PDCAAS values are higher than this (5). It was also recognized by WHO/FAO/UNU (5) that there is no official standardized method for amino acid determination in foods.

Determination of Amino Acids in Foods and Dietary Supplements

Determination of the total amino acid content of foods and supplements requires protein hydrolysis by various means that must take into account variations in stability of individual amino acids and resistance of different peptide bonds to the hydrolysis procedures. Modern methods for separating and quantifying free amino acids either before or after protein hydrolysis include ion-exchange chromatography (IEC), high-performance liquid chromatography (LC), gas chromatography, and capillary electrophoresis (CE). Chemical derivatization of amino acids may be required for chromatographic separation to improve detection.

The hydrolysis conditions for the preparation of protein hydrolyzates and the chromatographic determination of amino acid in foods have been reviewed (6). More recently, advancements in the application of CE to the analysis of amino acids, biogenic amines, peptides, and other food components have been reviewed (7). IAA contents in food and feed components may also be precisely analyzed by near infrared reflectance spectroscopy (NIRS; 8).

Standardized methods for hydrolysis and analysis of amino acids by IEC have been developed (9–11). Hydrolysis with 6 M HCl at 110°C for 24 h is the most commonly used method for the release of most amino acids except sulfur amino acids and tryptophan (9). A pre-oxidation with performic acid followed by 6 M HCl hydrolysis is widely used for accurate determination of cyst(e)ine and methionine (which are partially destroyed after acid hydrolysis) as cysteic acid and methionine sulfone, respectively (9, 10). Similarly, an alkaline (4.2 M NaOH) hydrolysis is commonly used for accurate release of tryptophan (9–11). The 3 hydrolysis procedures were compared in a collaborative study by Satterlee et al. (9). Amino acids in the protein hydrolyzates were determined by IEC, and the interlaboratory variability for the determination of the nutritionally important amino acids was found to be about 10% in most cases (9–11).

Since the publication of these reports on standardization of hydrolysis procedures and determination of amino acids by IEC, it has become apparent that there is a need to develop correction factors for certain amino acids based on different time duration, usually 6 M HCl hydrolysis at 24, 48, and 72 h because of the different effects of hydrolysis time on different amino acids (12). Moreover, LC has replaced IEC as the analytical technique for quantifying amino acids in most laboratories, especially those in North America. LC, usually employing reversed-phase C8 or C18 silica-based columns, uses a precolumn derivatization method following hydrolysis for analyzing amino acids (6) The LC methods are simpler, faster, have greater sensitivity, and use less expensive LC systems that operate at higher pressures than dedicated ion-exchange-based amino acid analyzers. Phenylisothiocyanate (PITC), benzylisothiocyanate, *o*-phthalaldehyde (OPA), 5-dimethylamino-1-naphthalene sulfonyl chloride (dansyl chloride), 9-fluorenylmethyl chloroformate, and 6-aminoquinoyl-*N*-hydroxysuccinimidyl carbamate are among the compounds that have been used for precolumn derivatization of amino acids in foods.

PITC reacts quantitatively with both primary and secondary amino acids to form relatively stable phenylthiocarbamyl derivatives, and has been most frequently used for precolumn derivatization of amino acids in foods and biological samples. Cohen et al. (13) described the Waters (Millipore, Bedford, MA) PicoTag system, based on PITC derivatization, followed by LC separation with UV detection at 254 nm for determination of free amino acids in plasma, urine, spinal fluid, and tissue samples. Sarwar et al. (14) adapted this methodology using a Waters PicoTag Amino Acid Analysis Column for accurate determination of all amino acids except tryptophan in hydrolyzates of foods and feces. Separation of all amino acids was completed in 12 min, with column washing and equilibration bringing the total run time to 20 min. Performic acid oxidation of samples was required prior to PITC derivatization for analysis of methionine as methionine sulfone and cysteine–cystine as cysteic acid. Because tryptophan exhibits strong UV absorbance, it was detected without PITC derivatization in the same food and feces samples following a separate 4.2 M NaOH hydrolysis by a simple isocratic LC method using a Waters C18 μBondapak column. The intralaboratory variation of the LC method was found to be similar to that of IEC. When similar hydrolytic conditions were used in preparing protein hydrolyzates, amino acid data obtained with the PITC method were generally in close agreement with those obtained by IEC. However, the interlaboratory variability of the PITC derivatization method and other LC methods has not been determined. Therefore, there is a need to develop accurate, standardized, and/or validated methods of amino acid determination in foods and dietary supplements using LC techniques.

Determination of Bioactive Peptides in Foods and Dietary Supplements

Chemical and Enzymatic Hydrolyses

Protein digestion generates many peptides in the gut lumen. Some of these peptides possess biological effects. These bioactive peptides are inactive within the original protein but once released can function as regulatory compounds with hormone-like activity which is based on the inherent amino acid composition and sequence (15). Food-derived bioactive peptides commonly contain 2–9 amino acids (16). This range may, however, be extended to 20 or more amino acid units such as the peptide lunasin, a food-derived bioactive peptide with anticancer bioactivity in a skin cancer mouse model, contains 43 amino acids with a molecular weight of 5400 Da (17). To exert a potential physiological effect such as an antihypertensive effect after oral administration, ACE-inhibitory peptides have to reach the cardiovascular system in an active form (15). Therefore, they need to remain active during digestion by human proteases and be transported through the intestinal wall into the blood. It is known that dipeptides and tripeptides can be absorbed intact from the gastrointestinal tract. Studies in rats have shown that larger peptides (10–51 amino acids) generated by food protein digestion can also be absorbed intact through the intestinal tract and produce biological effects (18). But the potency of the bioactivity after absorption is inversely correlated to chain length (15).

Enzymatic and acid hydrolyses are the 2 main methods to generate peptides from proteins. Although the acid hydrolysis method is relatively simple and less expensive, it is more difficult to control, and amino acid damage may occur. On the other hand, enzymatic methods are easier to control and do not cause amino acid damage as they use mild conditions. Therefore, enzymatic hydrolysis is the most frequently used method to produce bioactive peptides from food proteins (16). Proteinases (endopeptidases) such as trypsin, subtilisin, chymotrypsin, thermolysin, pepsin, proteinase K, papain, and plasmin are most commonly used for the production of peptides from food proteins (19). Desirable results can be obtained with combined enzymatic hydrolysis and acid hydrolysis. Fermentation is also known to be an efficient way to produce some bioactive peptides (20). Bioactive peptides can be released by the microbial activity of the fermented food or through enzymes derived from the microorganism.

Isolation, Purification, Characterization, and Quantification

The isolation, purification, characterization, and quantification of bioactive peptides have been reviewed (16). Salting out and solvent extraction are often used before further purification stages. LC is the most frequently used technique to isolate and purify bioactive peptides. Commercially available reversed-phase columns have been used for rapid separation and detection of peptides from a protein hydrolysate, while normal-phase LC has been preferentially used for the separation of hydrophilic peptides. IEC, CE, and capillary isoelectric focusing are used to separate peptides based on their charge properties. Size-exclusion chromatography (SEC) in aqueous separation systems and gel-permeation chromatography in nonaqueous separation systems is a separation technique entirely based on molecular size. Ultrafiltration, crystallization, counter-current distribution, partition chromatography, and low-pressure hydrophobic interaction chromatography have also been used for protein fractionation and purification of peptides (16).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can be used to determine the molecular weight and the purity of bioactive peptides (16). This methodology is helpful for relatively large peptides, but the resolution of SDS gel is usually low for small peptides. Size-exclusion LC can give an indication of particle size. By using appropriate columns and conditions, LC may provide useful information for peptide characterization. Because LC and size-based analyses cannot give direct amino acid sequence information, amino acid analyzers and protein sequencers are frequently used to determine amino acid composition and sequence of unknown peptides (16).

Advances in the application of mass spectrometry for the characterization and quantification of bioactive peptides have recently been reviewed (21).

Commercial Functional Foods Containing Bioactive Peptides

Internationally, several products are commercially available or under development by companies that are intended to exploit the hypotensive potential of peptides derived from milk proteins (22). These functional foods are either in the form of fermented milk drinks or as a milk protein hydrolysate preparation containing ACE-inhibitory tripeptides, IPP and VPP. These products are sold in Japan, the United States, and Europe. Casein hydrolysates containing the dodecapeptide, FFVAPFPEVFGK, have been commercially produced by Japanese and Dutch companies. A whey protein hydrolysate preparation containing hypotensive peptides has been commercially developed in the United States (23). A Danish product has been launched commercially that uses a specific strain of lactic acid bacteria in the production of yogurt that may reduce blood pressure (24). Numerous fermented milk drinks which contain ACE-inhibitory peptides (VPP, TTMPLW, and RY) have become commercially available in Spain (25).

Regulatory Considerations

Japan

Japan was the first country to adopt a regulatory framework for allowing claims on functional foods. In 1991, Japan introduced the Food for Specific Health Use (FOSHU) licensing system, according to which health claims must be substantiated through scientific evidence before FOSHU approval is granted (26). In February 2005, a new FOSHU regulation was implemented by the Japanese Ministry of

Health, Labour and Welfare (MHLW). The new FOSHU was designed to increase the number of approved functional foods with health claims (27). The Japanese MHLW has adopted 3 new categories into its FOSHU regulation. One of the categories is Standardized FOSHU, meaning that if a product meets FOSHU standards set out by the MHLW and includes an MHLW-approved ingredient, it could go through a faster approval process (27). In 2005, there were 537 FOSHU approved products with an estimated retail value of U.S. $6.3 billion (28). At present, there are 7 different ACE-inhibitory peptide products marketed with FOSHU approval. These include peptides derived from milk, fish, fungal, and seaweed protein sources (22).

United States

In the United States, health-related claims for food products or their components are regulated under the Food, Drug and Cosmetic Act (FDCA), 1938, the Nutrition Labeling and Education Act (NLEA), 1990; the Dietary Supplement Health and Education Act (DSHEA), 1994; and the Food and Drug Administration Modernization Act (FDAMA), 1997 (28). Structure/function (S/F) claims for conventional foods have been permitted for many years under the FDCA, but the effects considered acceptable for claims on these products derive from nutritive value rather than non-nutritive effects. The enactment of NLEA in 1990 permitted several health claims for relating consumption of certain foods, food components, or diets with reduced risk of osteoporosis, hypertension, heart disease, or some types of cancer. The U.S. congress passed the DSHEA in 1994 to broaden the availability of all dietary supplements by authorizing them to carry claims for specific effects on "structure or function" of the body or on "well-being" from consumption of a nutrient or dietary ingredient but not therapeutic or specific disease prevention claims. The DSHEA defines a dietary supplement as "a product (other than tobacco) that is intended to supplement the diet and that bears or contains one or more of the following dietary ingredients: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total daily intake, or a concentrate, metabolite, constituent, extract or combinations of these ingredients" (29). Moreover, although legally classified as food rather than drug, a dietary supplement is intended for ingestion in pill, capsule, tablet, or liquid form; is not represented for use as a conventional food or as the sole item of a meal or diet; and is labeled as a dietary supplement. The U.S. Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition is charged with the responsibility for the regulation of dietary supplements. These are not premarket reviewed and the labels must contain the disclaimer, "This statement has not been evaluated by the FDA. This product is not intended to diagnose, treat, cure, or prevent any disease." To use these claims, manufacturers must have substantiation that the statements are truthful and not misleading, and they must notify the agency no later than 30 days after a product that bears the claim is first marketed. Dietary supplements that were produced before 1994 are assumed safe, whereas the safety of those marketed after 1994 is the responsibility of the manufacturer. The role of FDA in regulating dietary supplements can be found in a guidance document which has been posted on the FDA website (30).

European Union

In the European Union (EU) Food Laws, there is no single regulatory framework for functional foods or nutraceuticals. There are numerous regulations which depend on the nature of the foodstuff (31). The general food law regulations are applicable to all foods. Moreover, legislation on dietetic foods, on food supplements or on novel foods may also be applicable to functional foods depending on the nature of the products and their intended use. Two proposals on nutrition and health claims and on the addition of vitamins and minerals and other substances to foods, which are currently in the legislative process, would have a major impact on future marketing of functional foods or nutraceuticals in Europe (31). The cornerstone of EU legislation on food products, including functional foods, nutraceuticals, and dietary supplements, is safety. Decisions on the safety basis of legislation are based on risk analysis, in which scientific risk assessment is carried out by the European Food Safety Authority and risk management is conducted by the European Commission, the Member States, and in the case of legislation, together with the European Parliament (31). The Process for the Assessment of Scientific Support for Claims on Foods (PASSCLAIM) does exist within the EU (30, 31). This program provides a generic guidance for the evaluation of scientific support for health-related claims for foods and food components (32, 33). According to PASSCLAIM, low-density lipoprotein (LDL) cholesterol and blood pressure are well-established markers generally accepted as related to changes in risk of cardiovascular diseases. Based on PASSCLAIM assessment, the evidence appears to be sufficient to support claims related to diet and cardiovascular disease, including the following: "May lower SBP, may lower DBP, may reduce left ventricular hypertrophy, may lower the risk of stroke, may lower the risk of heart failure, may lower risk of cardiovascular disease/coronary heart disease" (34).

Canada

In Canada, bioactive substances intended to have health benefits could be considered as a functional food or nutraceutical. In 1998, the Canadian Department of Health proposed conceptual definitions for functional food and nutraceutical to aid in discussion and in categorizing these products (35). These definitions are in general use among manufacturers, product developers, and others interested in this area in Canada but are not found in any regulation. They are noted below.

A *functional food* is similar in appearance to, or may be a conventional food, is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond nutritional functions.

A *nutraceutical* is a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease.

According to these definitions, nutraceuticals are mostly derived from foods and sold in dosage forms like capsules and pills, in which form they would most likely be sold as Natural Health Products, a new subcategory of drugs. Becuase, by definition, nutraceuticals are derived from foods, there is a tendency to assume that they are safe, unlike the case for drugs or synthetic substances. However, the safety of bioactive substances, especially at intakes significantly greater than normally encountered in the diet, needs to be assessed. A nutraceutical could also be used to "fortify" a food and make it a functional food.

Therefore, bioactive peptides could be sold as natural health products or as an ingredient of functional food. In Canada, natural health products fall under the Natural Health Products (NHPs) Regulations of the Food and Drugs Act which came into effect on January 4, 2004, following extensive consultations with stakeholders and the Canadian public regarding an appropriate regulatory framework. NHPs are usually sold in capsule, pill, tablet, liquid, or bulk form and certain other forms, such as gum or bars. Several documents have been developed by Health Canada to provide industry with clear guidance on how to comply with the regulations. The regulations, policies, and guidance documents for industry regarding natural health products can be found on the Internet (36, 37).

The safety of addition to food of bioactive peptides derived from, extracted, or concentrated from foods would be addressed through the regulations governing novel foods, as these would be considered ingredients with no history of safe use as food in their isolated form. The regulations for novel foods are in Division 28 of the *Food and Drug Regulations* which were promulgated in October 1999. Manufacturers of foods with added bioactive substances intended to have health benefits are also interested in making health claims for their foods. The overarching provision in the Food and Drugs Act is that such claims must not be false, misleading, or deceptive.

Nutrient content claims (the statements that characterize the amount of a nutrient) are regulated in Canada, and only those that are permitted by the regulation can be made. A few examples are "good source of protein" or "low in saturated fat." For non-nutrients, only simple declarations of the amounts of bioactive substances contained in a food are permitted because there is typically no recommended dietary intake that can serve as the basis for judging the relative importance of a given amount of the substance. Since January 1, 2003, the food industry has had the option to use any of 5 health claims that show how certain dietary choices may help to reduce the risk of certain major chronic diseases in Canada. These were claims that FDA had previously permitted under the Nutrition Education and Labeling Act of 1990. Following assessment of the additional evidence that had accumulated in the 10 years since the FDA reviews, these 5 claims were revalidated and set out in the *Food and Drug Regulations*. They deal with relationships between sodium and potassium and hypertension; calcium, vitamin D, physical activity, and osteoporosis; saturated and *trans* fat and heart disease; fruit and vegetables and some cancers; and fermentable carbohydrate and tooth decay. Health Canada has also similarly assessed the scientific evidence of 5 additional health claims since they were permitted by FDA in 1990. The scientific evidence was found to uphold 2 of those 5 diet–disease relationships, one relating to a diet high in vegetables, fruit, and whole grains with reduced risk of heart disease, and one relating a diet high in folic acid with reduced risk of neural tube birth defects. In the case of a third claim for types of soluble fiber, Health Canada is considering these on the basis of industry submissions for each fiber. Another type of claim may be made for energy or nutrients in food products. These are referred to as "biological role claims" and are permitted under a regulation that permits statements to the effect that energy or a nutrient is generally recognized as a factor in maintaining the functions of the body necessary to the maintenance of good health and normal growth and development. Health Canada is reviewing the management of health claims in Canada, including seeking the best approach for structure–function claims for non-nutrients, a type of claim that might best suit food products with added bioactive peptides.

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