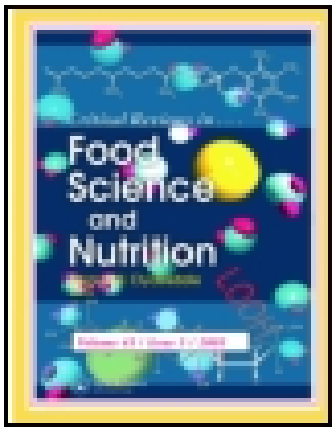

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Dietary Bioactive Peptides: Human Studies

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Dietary Bioactive Peptides: Human Studies

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Abstract:

Current opinion strongly links nutrition and health. Among nutrients, proteins and peptides which are encrypted in their sequences and released during digestion could play a key role in improving health. These peptides have been claimed to be active on a wide spectrum of biological functions or diseases, including blood pressure and metabolic risk factors (coagulation, obesity, lipoprotein metabolism, peroxidation), gut and neurological functions, immunity, cancer, dental health and mineral metabolism. A majority of studies involved dairy peptides, but the properties of vegetal, animal, and sea products were also assessed. However these allegations are mainly based on *in vitro* and experimental studies which are seldom

confirmed in humans. This review focused on molecules which were tested in humans, and on the mechanisms explaining discrepancies between experimental and human studies.

INTRODUCTION

Bioactive substances are defined as ôfood components that can affect biological processes or substrates and, hence, have an impact on body function or condition and ultimately healthõ (Möller et al., 2008). Dietary proteins contain several hundreds of encrypted sequences of amino acids which potentially agree with this definition.

The sequence and activity of these bioactive peptides (BioPep) were extensively reviewed (Teschemacher, 2003; Korhonen & Pihlanto, 2006; Meisel, 2005; Mine, 2007; Möller et al., 2008; Tidona et al., 2009; Agyei & Danquah, 2012; Udenigwe & Aluko, 2012), but only a few reports assessed their activity in human.

To be active, Biopep must be released from food protein precursor through two main ways: 1. *In vivo* hydrolysis by digestive enzymes, or by GRAS non digestive enzymes, or by microbial enzymes in large bowel and/or 2. Release during food processing or cheese ripening by bacteria or their enzymes. Some of bioactive peptides, such as caseinomacropeptide (CMP) released from ə-casein in the stomach of the newborn, bind digestive membrane receptors (e.g. opiod receptors) and are active in the gut. Other must be absorbed before reaching their target organs, via:

- Paracellular route: diffusion through tight junctions (large water-soluble peptides)
- Passive transcellular diffusion (hydrophobic peptides)
- Transporter located at the basolateral membrane (hydrolysis-resistant small peptides)
- Endocytosis: binding of peptides to the cells and transport through the cell via vesiculization (usually large polar peptides)
- Lymphatic system (large lipophilic peptides).

Small peptides (di- and tri-peptides) are absorbed more efficiently than larger ones, which are prone to be hydrolysed by enterocyte peptidases; however several BioPep have been shown to be absorbed by intestinal cell-cultures (Caco-2) and could be detected in blood stream or target organs, in newborn or adults (Chabance et al., 1995; Chabance et al., 1998; Dia et al., 2009; Iwai et al., 2005; Foltz, et al, 2007). Nevertheless, only a small fraction is transported intact by human intestinal enterocytes toward their target receptors (Vermeirssen et al., 2004).

The present review focuses on peptides whose biological activity was tested in humans and suggests some clues to explain the discrepancies between human and experimental studies. Lastly, some safety issues on these ñnovel foodsò were given. Since some BioPep are multifunctionnal acting on functions of several organs or systems (Meisel, 2005; Korhonen & Pihlanto, 2006; Udenigwe & Aluko, 2012), they have been reviewed according to their functions and not to their origin.

BLOOD PRESSURE

Reports from large cohorts suggest that increased protein intake is associated with a reduced risk of increased blood pressure (BP) and coronary heart diseases. One of the main mechanisms involved in BP regulation is the angiotensin converting enzyme (ACE); ACE catalyses the conversion from angiotensin I to angiotensin II, a hormone which results in vasoconstriction, and subsequently in an increase of BP. In addition, ACE degrades bradykinin which has vasodilatatory properties. Thus ACE-inhibitors lower hypertension. ACE inhibitory peptides are generally short sequences. Small amounts of peptides containing up to six amino acids are able

to pass intact a monolayer of Caco-2 cells but the absorption rate decreases with the length of their chain; peptide length and sequence determine the affinity for the receptor and peptideôs activity: the active site of ACE cannot bind large molecules, and binding to ACE is strongly influenced by the C-terminal of peptides; hydrophobic amino acids (Trp, Tyr, Phe, Pro) are preferred as C-terminal residues. Moreover, the positive charge from Arg and/or Lys residues may increase the inhibitory activity (Foltz et al, 2007).

In vivo BP regulation is a highly regulated homeostasis which involves other mechanisms than ACE, via vasoactive molecules, which could also be found as by-products in fermented foods (Teschemacher, 2003; Aihara et al., 2009; Tidona, et al., 2009; Ballard, et al,. 2012).

Biopep from Milk: Recent human studies report the protective effect of dairy products on hypertension and metabolic syndrome of obesity, so attention was early drawn to the effects of milk-derived peptides, in conjunction with other potentially active dairy components, such as calcium or lipid molecules such as CLA. Many ACE-inhibiting peptides derived from caseins of different mammalian species have been reported (Korhonen & Pihlanto, 2006). A specific peptide mixture, called C12 peptide, from casein induces a significant mean reduction of 6.0 mm Hg of diastolic BP (DBP) in prehypertensive subjects (Cadée et al., 2007). Two peptides produced in fermented sour milk are actually on the market under the registered label of Calpis®; they are VPP (Val-Pro-Pro), found in the primary structure of bovine ß-casein (84ï 86)(74ï 76), and IPP (Ile-Pro-Pro), from ə-casein (108ï 110). Studies used these purified peptides, or milk enriched with them by fermentation by Lactobacillus helveticus, at doses ranging from 1.8 mg to 50 mg/d for up to 8 weeks. Conflicting results were analyzed in meta-

analysis. The effects of casein peptides (e.g. VPP or IPP) on BP are evident in Asian patients and seem to be independent of age and dose; in these populations observed average decreases in systolic BP (SBP) and DBP are around 5 mm Hg and 2 mm Hg, respectively and have no health impact (Cicero et al., 2010); so, the European Food Safety Authority (EFSA) rejected a health claim on the effect of IPP and VPP on BP (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2012). Concerning the use of fermented mik-containing BioPep, yet there is limited but consistent evidence that their consumption improves arterial stiffness and prevents adhesion of inflammatory molecules to endothelium surface (Aihara et al., 2009; Pase et al., 2011). A recent Cochrane review concluded that they have a modest effect on SBP, and no evident effect on DBP. These results do not support an effect of fermented milk or their BioPep on blood pressure (Usinger et al., 2012).

In a clinical trial performed in healthy humans, 5g/d of a whey peptide (NO peptide 47) launched on the market as NOP-47®, increased the arterial reactive hyperhemia following occlusion (Ballard et al., 2012). In another human trial, no effect on BP was detected in patients with mild hypertension after ingestion of milk containing ACE-inhibiting whey peptides (Lee et al., 2007).

Biopep from other sources:

In a short 3-week crossover human intervention trial (7 volunteers), pea protein hydrolysate led to a significant but low reduction in SBP (5-6 mmHg) (Li et al., 2011).

The activity of Val-Tyr peptide from marine source shows a low-intensity ACE inhibiting activity *in vitro* and in human; in a study carried out on mildly hypertensive volunteers, daily ingestion of 3 mg of Val-Tyr led to a significant BP reduction of about 9 and 5 mm Hg for SBP

and DBP, respectively (Kawasaki et al., 2000). An oligopeptide produced by hydrolysis of dry bonito muscle lowered BP in SSR model and hypertensive human subjects, at a dose of 1.5g/d (Fujita et al., 2001). Five g/d of an octapeptide from chicken collagen lowers BP by an average of 11.5 mm Hg in SSR and mildly hypertensive patients (Saiga-Egusa et al., 2009).

COAGULATION

Increased blood coagulation is involved in the development of metabolic diseases and precocious mortality, in addition to increased endothelial reactivity, obesity, dysregulation of glucose metabolism and lipoproteins. The similarity between the clotting process of several mammalian milks, in newborn stomach or during cheese making, through release of ə-casein derived CMP, and blood clotting is well known: CMP presents a high structural homology with human fibrinogen. An antithrombotic peptide from ə-casein was characterized in newborn plasma after milk ingestion (Chabance et al., 1995). No specific related bioactivity was reported in human.

PEPTIDES ACTIVE ON NEUROLOGICAL FUNCTIONS

Full whey protein (Ŭ-lactalbumin) improves memory in man, and induces an anxiolytic effect; these activities could be linked to its high tryptophan content and passage through the blood-brain-barrier which enhances serotonin synthesis (Booij et al., 2006). Other mechanisms are involved in the action of proteins or peptides on neurological functions: dietary peptides can bind opioid receptors which are found in the central and in the peripheral nervous systems, in the immune system and in the endocrine system of mammals.

Biopep from human and bovine milk: Exorphins are opioid peptides, produced mainly from Ŭs1- and ɓ- caseins. ɓ-casomorphins (BCM) consisting of 5 to 8 amino acids peptides (Tyr,Pro,Phe,Pro,Gly,Pro,Ile,Pro) produced by the cleavage of ɓ-casein (region 57-70) are the best known sequences (Teschemacher, 2003; Tidona et al., 2009).

In human, BCM are partially hydrolysed during their absorption. Their actions could be mediated by ε receptors. Since they induce apnea and irregular breathing, modulate sleep patterns, a possible link between BCM intake and sudden infant death syndrome and other severe human diseases (autism, cardiovascular diseases and type I diabetes) has been suggested (Wasilewska et al., 2011). BCM-7 and beta-casokinin-10 (β-Ck-10), issued from the C-terminal part of ɓ-casein (sequence 193-202), display effects on immunity, gut motility (decrease in transit time) and brain functions (Teschemacher, 2003).

Owing to their potential adverse effects on brain function, the metabolism and biological effects of BCM were extensively reviewed (DATEX Working Group on ɓ-casomorphins, 2009); this report shows that BCMs are free from side effects, yet they actually are bioactive.

Ŭ-casozepine is an anxiolytic peptide produced by INGREDIAÊ , corresponding to the sequence 91-100 of the bovine Ŭ(s_1)-casein; in experimental and human studies it reduces symptoms related to stress, particularly digestion, cardiovascular, intellectual, emotional and social problems (Kim et al., 2007).

Biopep from other sources

Hydrolysate from gluten proteins increases gastrointestinal transit time in healthy human volunteers. This effect is blocked by the opioid inhibitor naloxone. Gluten hydrolysate also

produce a naloxone-reversible increase in plasma somatostatin activity, which may be responsible for a slower gut transit (Teschemacher, 2003).

A recent study reports on the effect of a soybean peptide on immune function, brain function, and neurochemistry in healthy volunteers. The peptide decreases adrenalin level in plasma but increases dopamine level, and increases the amplitude of several brain rhythms (Yimit et al., 2012). However, the exact amino-acid sequence of the peptide was not identified and the clinical relevance of these observations remains to be precised.

A fish hydrolysate obtained by fermentation displays anxiolytic properties maybe by lowering the stress-induced response by pituitary-adrenal axis, sympathoadrenal activity and brain gamma amino butyric acid (GABA) content (Bernet et al., 2000).

GUT AND METABOLISM

In preterm infants, the reduction of gastrointestinal transit time upon oral administration of a protein hydrolysate formula could be caused by release of opioid receptor ligands. In adult humans, oral administration of BCM slowed gastrointestinal transit. When humans are given an intra-gastric administration of one of four solutions containing either caseins, whey proteins or their hydrolysates, hydrolysates elicit about 50% more gastric secretion than the whole protein solutions; this effect is accompanied by higher glucose dependent insulinotropic polypeptide (GIP) plasma. Similar glucagon-like peptide-1 (GLP-1) and peptide YY plasma responses were elicited by the four solutions (Calbet & Holst, 2004).

DIGESTIVE DISEASES

Lactoferrin (Lf) from milk displays similar activities in *in vitro*, experimental and human studies. Oral recombinant human Lf supplementation limits the increase in small intestinal permeability in non-steroidal anti-inflammatory drugs (NSAID)-induced gastroenteropathy in healthy volunteers (Troost et al., 2003). The exact mechanism of action of such interesting activity is not known. TGF-ϐ is a growth factor present in high concentrations in the milk of several species, including human, and it is also produced in small amounts in the intestine of newborns. TGF-ϐ has pleiotropic functions, including the regulation of the immune function, cellular growth and differentiation. Following oral administration, TGF-ϐ is quickly absorbed in newborn and is detected in the plasma of healthy human volunteers (de Medina et al., 2010). Owing to its biological activities, TGF-ϐ could contribute to the treatment of inflammatory bowel diseases (IBD): in a short study, 7 children with IBD (Crohn disease (CD)) were given a casein- and lactose-free, TGF-ϐ enriched formula for 8 weeks; they showed a significant improvement of the disease activity, confirmed by ileal biopsies in 6 of them, and of their nutritional status. In a following study conducted during 8 weeks in 29 children with CD a 79% remission rate was achieved, with complete healing in ten cases (Fell, 2005).

FEEDING (APPETITE, SATIETY) AND OBESITY

Proteins have a satiating effect, which potentiates the slimming effects of low-energy diets in overweight treatment. Several causes may be involved in that effect, including the dose and physical characteristics of the proteins, their form (solid vs. liquid) and amino acid composition,

and the release of BioPep. These direct effects are additive to those of opiod peptides which slow transit time.

Whey proteins, whether native or hydrolysed (+50% activity) stimulate the release of gastric inhibitory peptide (GIP), also known as glucose-dependent insulinotropic peptide, and GLP-1. Whey proteins also have direct insulinotropic activity, maybe due to their amino acid composition, and stimulate the satiety hormone Cholecystokinin (CCK) secretion (Jakubowicz & Froy, 2012). The potential modulatory effect of Lf was tested in overweight subjects, given 300 mg/day of bovine Lf for 8 weeks: it allowed a significant reduction in visceral fat, body weight and BMI (Ono et al., 2010).

The ə-casein derived glycomaropeptide (GMP) was extensively studied: it exists as a mixture of different glycoforms due to the binding of carbohydrates, including sialic acid (N-acetylneuraminic acid, NeuNAc). GMP triggers stimuli from intestinal receptors without being absorbed. The signal would be then transmitted to organs through regulatory digestive peptides. The extent of glycosylation which influences GMP binding to the receptors could be a key factor of its biological activity. GMP has a pronounced gastric inhibitory activity and is able to stimulate the release of CCK. Initial experimental studies suggested a stimulating activity of a slightly glycosylated form of GMP (Yvon et al., 1994; Froetschel et al., 2001). Subsequent human studies gave contrasting results: for Burton-Freeman (Burton-Freeman, 2008), pre-meal satiety was greater after a whey protein preload compared to control and GMP preload in women, but no difference was evident in men. CCK concentration followed a pattern that predicted the subjective satiety in women, but not in men. Test meal intake was not different by

preload: GMP alone was not considered by the authors as critical in pre-meal whey-induced satiety; however, it may have a role in compensatory intake regulation. Other authors found no significant difference in CCK levels, neither subjective measures of satiety nor in food intake between treatments at the given preload level, whatever the degree of glycosylation of the GMP (Keogh et al., 2010).

In fact whey proteins, which are insulinotropic in their native form, more than their content in GMP, could explain the satiating effects observed in some studies (Veldhorst et al., 2009).

Oral ingestion of a hydrolyzed gelatin meal in human subjects with normal weight and in obese patients increased post-prandial circulating levels of several gut peptides, including GLP-1, underlying a potential slimming effect (Rubio et al., 2008). A blend of black soy oligopeptides, some of them being identified, given at a dose of 5.2g/meal has an anti-obesity effect, associated with a rise in HDL cholesterol levels and a decrease in triglycerides levels (Kim et al. 2013).

METABOLISM

Inflammation, insulin resistance and imbalance between beneficial HDL cholesterol and LDL cholesterol, and increased serum triglycerides favor the accumulation of cholesterol by vascular endothelium, the development of atherosclerosis and the occurrence of cardiovascular disease. A specific metabolic risk is associated to the so-called metabolic syndrome, which clusters obesity, hypertension, dyslipoproteinemia and resistance to insulin or overt diabetes. So increasing uptake by liver (by LDL-receptors) or esterification of cholesterol (by lecithin-cholesterol acyltransferase) is of health interest.

The hydrophobicity of peptides, including the presence of a proline residue, is required for their biological activity. The optimum length of peptides appears to be four amino acids (Foltz, et al., 2007; Agyei & Danquah, 2012).

Some ecological studies have linked the intake of ϐ-casomorphin BCM7 with insulin-dependent-diabetes (IDDM). However, no cause-effect association was established (DATEX Working Group on ϐ-casomorphins, 2009).

Bovine serum immunoglobulin display a cholesterol-lowering effect in human subjects with mild hypercholesterolemia (Earnest et al., 2005). These results agree with the effects observed after bovine colostrum administration; immunoglobulins reduce fat accumulation and facilitate the movement of glucose to the muscle, which could explain their positive effect on physical performance in sportsmen. Whey fractions and peptides have beneficial effects in type 2 diabetic patients: bovine colostrum decreases blood glucose, cholesterol, and triglyceride levels (Kim et al., 2009). Postprandial triglyceride response to a fatty meal ingested with different whey proteins or whey hydrolysate does not differ; however insulin response is significantly enhanced by whey hydrolysate (Mortensen et al., 2012).

Lunasin is a 43 amino acid-containing polypeptide found in soybeans, barley, rye, and wheat which inhibits the production of 3HMG-CoA reductase, and increases the production of LDL receptors (Hernández-Ledesma et al., 2009). Human studies confirm the bioavailability of lunasin: lunasin-derived peptide sequences are observed in plasma after ingestion of lunasin-containing protein (Dia et al., 2009a). They also confirm the experimental effects of soy peptides on blood cholesterol and triglycerides (Hori et al., 2001; Kohno et al., 2006). Beneficial effects of collagen peptides from

salmon skin are observed in rat models and diabetes type 2 patients, which show biological improvement: decrease of levels of free fatty acid, and of inflammatory markers, rise of adiponectin and bradykinin (Zhu et al., 2010). Ingestion of a gelatin hydrolysate (20g) induces insulin secretion in normal and obese subjects; according to the authors, this observation could be used to enhance satiety in obese patients (Rubio et al., 2008).

Yet biological properties of these proteins can be related to the peptides encrypted in their sequences, it remains unclear if peptides released by their hydrolysis are more potent than the whole proteins. However, precise mechanisms involved in the potential protective effects of proteins or their hydrolysates and the mechanisms involved remain to be confirmed in large human studies (Udenigwe & Aluko, 2012).

CARIES

Tooth enamel is a polymeric substance consisting of crystalline calcium phosphate embedded in a protein matrix. Dental caries develop by acidic demineralization (calcium and phosphorus solubilization) of tooth enamel. Anticariogenic effects of phosphorylated peptides from caseins (caseinophosphopeptides: CPPs) have been reported; a human *in situ* caries model has been developed to study the ability of CPP, in association with amorphous calcium phosphate, to prevent enamel demineralization. However, more work should be done *in vivo* on the effects of BioPep on dental caries in human (Gurunathan et al., 2012).

IMMUNITY

Several BioPep have been reported to act on immune functions and/or as antimicrobial agents; they are usually short (3-20 amino acid residues), hydrophobic and cationic. The action of these immunomodulatory peptides is relatively nonspecific and the exact mechanism of their actions as well as their *in vivo* metabolism is largely unknown. Most of the studies on the effects of proteins and peptides on immunomodulation have been conducted *in vitro* with cells of the specific and unspecific immune system. Also, the relationship between structure characteristics of food peptides and their antimicrobial activity has not yet been clearly stated.

Concerning HIV, it should be kept in mind that peptides described as HIV protease inhibitors have usually a restricted therapeutic value because of their limited ability to penetrate cell membranes. So, further research on pharmacokinetics and therapeutic value of these peptides using *in vivo* and clinical studies is needed to firmly establish their therapeutic potency (Agyei & Danquah, 2012).

Biopep from milk proteins: Lf and its derived peptide lactoferricin exert antimicrobial action either by chelation of iron or by destabilization of bacterial membranes, by binding to their lipopolysaccharides (LPS). Lf also modulates the inflammatory process, and inhibits virus replication. It exhibits antiviral activities in human: against hepatitis C, human papillomavirus, herpes simplex virus, adenovirus, HIV, human CMV (Ebringer et al., 2008). The interest of Lf was further confirmed in several clinical trials. Infection and its severity in neutropenic patients caused by chemotherapy are reduced. In a controlled study in elderly subjects, Lf enhanced the activity of white blood cells (Trümpler et al., 1989). Administration of 200 mg of Lf to healthy adult males increases T-cells (Mulder et al., 2008). Bovine Lf supplementation was also

examined in human infants. Newborns (<4 months of life) fed a formula supplemented with Lf (850 mg/L) had significantly fewer lower respiratory tract illnesses and significantly higher hematocrit levels at 9 months (King et al.,2007).

Very-low-birth-weight infants (VLBW) were assessed for development of sepsis. They were randomly assigned to receive orally administered bovine Lf (BLF) (100 mg/d) alone, BLF plus Lactobacillus rhamnosus GG (LGG) or placebo from birth until day 30-45 of life. Compared with placebo, BLF supplementation alone or in combination with LGG reduced the incidence of late-onset sepsis in VLBW (Manzoni et al., 2012). In another study in children, supplementation with BLF did neither improve the incidence nor the prevalence rate of diarrhea, but led to a lower prevalence of colonization with Giardia species and of incidence of invasive fungal infection (Ochoa et al., 2008). Colostrum is a rich source of immunoglobulins which increases salivary IgA; subjects consuming the fairly high dose of 60g/day of concentrated bovine colostrum reported fewer symptoms of upper respiratory tract infections than those taking whey proteins (Brinkworth & Buckley, 2003). Colostrum prevents the depression of innate immunity induced by an acute prolonged physical stress, such as exercise (Davison & Diment, 2010).

Biopep from other sources: Oral intake of 3 g/d of wheat protein hydrolysate increased *ex vivo* natural killer activity (Horiguchi et al., 2005). Peptides used in these studies were not well characterized; their activity was assessed in partial models which are not representative of *in vivo* immunity.

Apart from its use as a natural food preservative, egg white lysozyme displays immunomodulatory effects in human: it exhibits antiviral activity, mainly on skin infections,

such as herpes simplex, and restores immune responses in immune-depressed patients undergoing anti-cancer treatments. When associated with immunotherapy, it improves chronic sinusitis, and normalizes humoral and cellular responses in patients with chronic respiratory tract infection (Asakura et al., 1990). Some small marine peptides stimulate *ex vivo* resistance of human immunocytes to HIV infection (Kim et al., 2011).

INFLAMMATION / PEROXIDATION

An important component of immunity is the inflammatory response. Food-derived BioPep have been mostly reported to modulate *ex vivo* the production of proinflammatory mediators in macrophages, by decreasing the production of free radicals, pro-inflammatory interleukins and prostaglandins. (Dia et al., 2009b). Ingestion of 2 g of a milk protein concentrate decreases the activity score of osteoarthritis (Zenk et al., 2002). Casein increases *ex vivo* the stimulated production of IL-2 by human T helper-1 (Th-1) lymphocytes (Phelan et al., 2009). However in these studies, the active component(s) is/are not identified.

Peroxidation reactions are involved in several pathological processes: they contribute to the development of dyslipoproteinemia and cardiovascular diseases, aging, and cancer. Dietary proteins and peptides may prevent peroxidation either by their content in amino acid which could stop the peroxidation chain, or by their ability to scavenge inducers of peroxidation, such as iron. Bovine Lf administered to healthy adult increases blood antioxidant capacity (Mulder et al., 2008). Most of the available studies were conducted *in vitro*.

CANCER

Care must be taken to dissociate toxic effects for cells from true anticancer properties. Results from *in vitro* studies using large molecules must be handled with care, since they do not take into account their *in vivo* metabolism before they reach their target cells (Udenigwe & Aluko, 2012). Only a few peptides were tested as drugs in human.

In a double-bind study the topical application of the complex lactalbumin-oleic acid reduces skin papillomas resistant to conventional treatment (Gustafsson et al., 2004). A recent clinical study involving 73 healthy volunteers demonstrated that a commercial milk peptide mixture that induces apoptosis in colon cancer cells *in vitro* is safe to consume orally. All clinical blood markers remained within normal levels without clinically significant side effects. The milk peptide mixture improves insulin sensitivity, and white cells blood count (Kreider et al., 2011). However, this preliminary study involves healthy individuals and additional work is needed to confirm its beneficial effect in cancer patients.

Owing to the limited environmental resource of native molecules from sea products, BioPep used in trials are obtained by laboratory synthesis (Mayer et al. 2010). Several well characterized microtubule interfering agents are currently tested in human by pharmaceutical laboratories: Cemadotin from sea slug, Plitidepsin (Aplidin®) from tunicate, Elisidepsin (Irvalec®), and Tasidotin, produced by bacteria, are in phase II trials. Hemiasterlin is a tripeptide of sponge origin in phase I trials. So far, very few marine compounds have reached the market, may be due to difficulties in accessing their source, in isolation and purification procedures as well as to ecological and safety considerations (Suarez-Jimenez et al., 2012).

MINERAL METABOLISM

Mineral and trace metals status are strongly related to health and development; e.g. calcium and bone development, iron and nervous development, iron, zinc and immunity; their deficiency is highly prevalent around the world, from infant to old people. These deficiencies are either secondary to insufficient dietary intakes or to a poor absorption rate. Diet composition has strong influences on this absorption: proteins have different effects depending on their origin: muscle proteins and hemoglobin enhance trace element absorption, while vegetal, milk and egg protein have rather an inhibiting effect.

Caseins have a strong affinity for divalent cations, by their clusters of phosphorylated serins, which keep these minerals in a soluble state; however, most of these cations are released at the acidic pH of stomach, an become insoluble and unavailable for abosrption; iron whose strength of binding is about 100 times higher than calcium and others cations remains in a bound form, but is released too far in the gut to be efficiently absorbed. Enzymatic hydrolysis of caseins improves iron absorption (Hurrell et al., 1989). Therefore, attempts have been made to hydrolyze caseins, to purify and concentrate the phosphorylated sequences, of caseinophopshopeptides (CPPs) which bind minerals.

The metabolism of ɓ-CN (1-25)-Fe labelled with ^{59}Fe was studied in young women; although its absorption was not significantly higher than ferrous sulfate, Fe administered as ɓ-CN(1-25)-Fe displayed an enhanced tissue uptake (Aît-Oukhatar et al., 2002). In adults, CPPs increase the absorption of zinc supplied in oat or whole grain baby food (Hansen et al., 1997). Fortification of the diet of postmenopausal women with CPPs increases calcium absorption and bone mineral density (Heaney et al., 1994).

Whey proteins, mainly Ŭ-lactalbumin, can bind zinc or calcium (Hurrell et al., 1989). In spite of this specific binding site, lactalbumin does not affect iron absorption in formula-fed babies, neither does GMP (Szymlek-Gay et al., 2012). Daily supplementation of healthy adults with 40-300 mg of a whey protein fraction named milk basic protein stimulates bone formation (increase in osteocalcin), and reduces bone resorption (Uenishi et al., 2007).

CONCLUDING REMARKS

Comparison between in vitro and human studies

A very large number of BioPep issued from enzymatic hydrolysis of dietary proteins were described, whether enzymes were of digestive origin or not. Using liquid chromatography-mass spectrometer/mass spectrometer (LCï MS/MS) analysis coupled to Data bases, one can identify even more potential peptides encrypted in proteins, without any certainty on their release, metabolism, and activity *in vivo*. Allegations on health properties of BioPep usually rely on *in vitro* experiments, which were sometimes completed by experimental studies. Human data are scarce; when compared to *in vitro* data, their results are somewhat disappointing.

Some assumptions can be raised to explain these discrepancies:

1. *In vitro* models neglect the industrial process of production as well as the digestive and metabolic steps which occur before peptides can reach their target;

a) Process

- Production at an industrial scale of sufficient amounts of peptides for a human trial includes several steps of purification and concentration, which can alter the structure or the interactions of peptides with surrounding matrix; these changes are liable to modify the bioavailability and activity of peptides (Udenigwe & Aluko, 2012).
- Process used to produce proteins influences the release and metabolism of their peptides: Maillard reaction, which makes lysine residues unavailable for hydrolysis, and more generally food processing decreases the susceptibility of proteins to hydrolysis (Seiquer et al., 2006; Bouzerzour et al., 2012).
- Care should be taken to the presence of unknown compounds in products used for experiments, besides peptides, which could contribute to the activity attributed to the peptides, and to their potential side effects.
- Storage can alter the bioavailability of free, concentrated peptides (Rao et al., 2012), and shorten their shelf-life.

b) Metabolism during gut transit

Owing to the extensive hydrolysis throughout the gastrointestinal tract, from stomach to jejunum, and the activity of brush border peptidases, most of peptides do not reach the absorption step. In addition, the metabolism of peptides depends on several physiological and biochemical factors:

- Luminal events can strongly change the biological effect of peptides: when ingested in a free form their metabolism in the gut can differ from peptides ingested in a protected, encrypted form in whole proteins (Meisel, 2005; Ricci-Cabello et al., 2012). In addition, sequential hydrolysis of protein during digestion can release BioPep with different or opposite and

antagonist activities (Yamauchi et al., 2003). However in some cases, shortened peptides showed an enhanced activity (Terashima et al., 2011). Providing peptides in a fed or fasting state also influences their metabolism (Foltz et al., 2007).

- The clusters of phosphorylated serine residues of caseinophosphopeptides (CPPs) (Ser(P)-Ser(P)-Ser(P)-Glu-Glu) prevent their hydrolysis by digestive proteases, protect their mineral-binding capacity, and give them a better chance of being absorbed (Boutrou et al., 2008).
- Glycosylation of peptides active on endoluminal receptors, such as caseinomacropeptide (CMP), can improve their binding efficiency and their activity. (Bouzerzour et al., 2012).

- Absorption pathways: use of the paracellular pathway or transcytosis pathways, instead of enterocyte uptake, could protect absorbed BioPep from endogenous hydrolysis (Satake et al., 2002) such as suggested for CPPs (Pérès et al., 1999).

2. Doses are usually higher in *in vitro* than in human studies; future *in vitro* studies investigating potential biological activities of peptides should use physiologically relevant concentrations and experimental time (Foltz et al., 2010).
3. Compared to *in vitro* models, human physiology is highly regulated by interactions between several biological mechanisms, which could lessen the *in vitro* activity observed in simple *in vitro* models.

Safety issues

Attention must be paid to safety aspects of BioPep:

- It should be shown that they are not toxic for humans. Specifically, peptides active on cancer cells or viruses should not induce death of healthy cells.
- Conditions of production must be safe: protein source, enzymes used and process must have an accepted food grade (GRAS). Peptides or hydrolysates produced could or not belong to the category of Novel Foods, according to European legislation and similar ones from other states, depending of the native protein and of the process used.

Claims on health effects must rely on strong, randomized controlled trials (RCT), submitted to the European Food Safety Authority (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2012; Schaafsma, 2009).

CONCLUSIONS

Since available *in vitro* models are unable to predict the bioactivity of diverse food derived peptides (BioPep) (Foltz, van der Pijl, & Duchateau, 2010), the development of research in the promising field of BioPep, with well-designed studies in humain, must be supported to give scientific strength to their allegations.

Therefore, health allegations concerning bioactive peptides cannot be only drawn from *in vitro* and animal models. To be valid a research approach should associate the demonstration of stability of peptides during digestion, and the confirmation in human of results obtained in *in vitro* or experimental models.

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