

Bioactive diet components and gastrointestinal tract health

Rafał Filip¹, Radosław Piotr Radzki², Marek Bieńko², Iwona Sierakowska-Łuszczewska³, Stefan Grzegorz Pierzynowski⁴

¹ Department of Endoscopy, Institute of Agricultural Medicine, Lublin, Poland

² Department of Biochemistry and Animal Physiology, Veterinary Medicine Faculty, University of Life Sciences, Lublin, Poland

³ Department of Animal Anatomy, Veterinary Medicine Faculty, University of Life Sciences, Lublin, Poland

⁴ Department of Cell and Organism Biology, Lund University, Lund, Sweden

Abstract: Some dietary components that may not be required for human existence may markedly influence the quality of life by modifying physiologic processes. These compounds can influence, in combination or alone, numerous biological functions by serving as antioxidants, immunoregulators, regulators of gene expression, modulators of several cellular processes, including growth and apoptosis. Many diseases are associated with impaired cell proliferation or differentiation and, what is most important, also with deregulations in programmed cell death, which, in the end, can lead to the promotion of the diseases such as cancer. Biologically-active molecules are capable of modifying the biochemical pathways and/or influencing specific proteins regulating apoptosis in gastrointestinal cells, which can be used for both prevention and treatment. Dietary fibres, cruciferous vegetables, flax and curcumin showed positive effect in the protection of colon cancer. Similarly, a strong inverse relationship between stomach as well as colon cancer risk and allium vegetables intake has been proved. Limonoids from citrus fruits are also considered as promising molecules with anticancer activity, such as perrillyl alcohol, which was tested in patients with advanced malignant tumours. Although the relationship between soy intake and cancer risk has not so far been clearly elucidated, it is known that isoflavones from soy may reduce the risk of estrogen-dependent breast cancer. Although functional foods of animal origin are not that well recognized, some of them, however, seem to be worthy of particular interest. Although interactions among nutrients have been inadequately examined, a few examples of negative and positive interactions exist, e.g. some components may provide beneficial effects by influencing the structure and functions of the wall as well as environment of the gastrointestinal tract, including phytohaemagglutinin (PHA) and other components, such as alpha-ketoglutaric acid (AKG).

Key words: functional foods, intestine, apoptosis, AKG, PHA

Abbreviations: AKG – Alpha-ketoglutaric acid, BW – Body weight, CCK – Cholecystokinin, EGF – epidermal growth factor, FELASA – Federation of the European Laboratory Animal Science Association, GI – gastrointestinal, Gln – Glutamate, N – Nitrogen, NPY – Neuropeptide Y, PHA – Phytohaemagglutinin, TGF- β – transforming growth factor-beta

INTRODUCTION

Functional foods – a definition

Some dietary components that may not be required for human existence may markedly influence the quality of life by modifying physiologic processes. These compounds can influence, in combination or alone, numerous biological functions by serving as antioxidants, immunoregulators, regulators of gene expression, modulators of several cellular processes, including growth and apoptosis. Although all foods may be called “functional”, as they provide micro- and macroelements, vitamins and proteins, or positive sensations like taste and a pleasant aroma. However, the term *functional food* applies to food providing an additional physiological benefit beyond that of meeting basic nutritional needs.

Recently, a large number of biologically-active molecules have been isolated from foods and their potential, together

with the mode of action, thoroughly described; however, the primary plant and animal foods that have been linked with physiological benefits have been known for a long time.

GI tract cell proliferation, differentiation and apoptosis

The homeostasis of GI tract epithelial cells is maintained by the balance between cell proliferation and apoptosis. Growth of the intestine, as well as the regenerative process, are complex and involve epithelial cell migration and proliferation, changes in cellular function, adaptation of subepithelial tissues, and contraction of the injured area. This requires interaction of both different cell types and signaling molecules, e.g. epidermal growth factor (EGF) and transforming growth factor-beta (TGF- β) [1]. Apart from mesenchymal-epithelial interactions, recent studies suggest a role for different gene products. It has also become obvious that immune cells and cytokines are important factors in intestine mucosa regeneration. [2] On the other hand, the importance of the genetically programmed form of cell death – apoptosis in growth regulation and maintaining the tissue size, has recently been emphasized [3]. Apoptotic cells are present along the whole length of the villi, and in the crypts

Corresponding author: Rafał Filip MD, PhD, Department of Endoscopy of IAM, Jaczewskiego 2, 20-090 Lublin, Poland.
E-mail: r.s.filip@wp.pl

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and apoptosis plays an important role in the exchange of the enterocyte population, facilitating maturation of the mucosa [3]. Among the diseases of the GI-tract, inflammatory bowel disease (IBS), celiac disease, as well as gastritis may be attributed to the appearance of pro-apoptotic factors. On the other hand, any factor inhibiting apoptotic process may promote cancer cell development [4].

However, the regulation of intestinal cell death by luminal (nutritional) factors has so far not been clearly elucidated; recent data indicate that the cessation of feeding may promote apoptosis in apical enterocytes [5]. It has also been shown in rat model that in the gut mucosa of fasted animals, progress of the apoptotic process may be inhibited by indigestible nutrients [6]. On the other hand, biologically-active molecules capable of modifying the biochemical pathways and/or influencing specific proteins regulating apoptosis gave a remarkable opportunity for manipulating the life and death decisions of the gastrointestinal cells, and led to the development a new therapeutic strategies.

Mechanism involved in regulation of apoptosis by dietary bioactive agents

The alteration of cell death contributes to most of the known illnesses. In consequence, the identification of the key components in cellular regulation of apoptosis became targets of therapeutic strategies such as Bcl-2 proteins as integral regulators of the mitochondrial apoptotic pathway, caspases as the executioner enzymes, endogenous caspase inhibitors, as well as death receptors that trigger apoptosis from the surface of the cell [7, 8]. Apoptosis, which is in fact a programmed cell death, is also considered a main pathological mechanism involved in, e.g. autoimmunity, allograft rejection or inflammation [9].

Apoptosis may be induced in both an extra- or intracellular manner, including triggering the cell cycle disruption, detachment of cells from their surrounding tissue, loss of trophic signaling or DNA damage [10]. Many pathways may be targeted by biologically active food molecules and in that way both stimulate and inhibit apoptotic processes [11, 12]. In the living cell, apoptosis may be induced *via* two effector mechanisms, intrinsic, or mitochondrial-mediated, and extrinsic, or death receptor-mediated [13].

Cell intrinsic pathway. The intrinsic pathway of apoptosis relies primarily on the permeabilization of mitochondrial membranes, with associated release of apoptogenic mitochondrial proteins, leading to activation of caspase 9 and downstream cleavage of caspases 3, 6, or 7 [14]. Activation of cytochrome C triggers the above-mentioned caspase cascade activation and, as a final consequence, induction of apoptosis [8, 10]. The intrinsic mitochondrial pathway also involves the Bcl-2 family proteins [9]. The basic role of Bcl-2 family proteins is to act as molecular integrators of both simultaneous cellular pro-death and prosurvival signals, therefore, these proteins, mostly Bcl-2 and Bcl-xL, are considered a promising approach in chemoprevention [15]. Caspase 3 links intrinsic pathways with extrinsic pathway mechanism.

Extrinsic Death Receptor Pathway. The integral part of the extrinsic death receptor pathway are the members of the tumor necrosis factor (TNF) receptor family, which among 20 different cytokine receptors include TNF-related apoptosis inducing ligand (TRAIL), TNFR1 as well as Fas receptors [16, 17]. Intracellular interactions are very complex and involve

death-inducing signaling complex (DISC), which is essential in the extrinsic pathway because of its capability to activate the initiator caspases 8 and 10 [18].

Gene expression. The crucial point for the development of molecules targeting apoptosis was elucidation of the molecular mechanisms of the apoptotic process, followed by recognition of genes and its products that are involved in the regulation of apoptosis. Recent data indicate that a number of dietary components have the potential to influence selected physiological processes, such as apoptosis, cell signaling, and cell cycle, probably by the modification of transcription and translation [19]. On the other hand, variations in individual responses to the exposition on bioactive dietary compounds, followed by activation or inactivation of apoptotic processes, may be explained by the genetic polymorphism [8, 20].

Cellular Signaling. Apoptosis may be also initiated by the reduction of growth factor-induced proliferative signaling. What is of importance here is that the interaction of growth factor receptors and their ligands, including insulin-like growth factor (IGF), EGF, and vascular epithelial growth factor (VEGF), may induce a signal for the neoplastic transformation of cells. Therefore, the capability to interrupt the above-mentioned signaling by decreasing proliferative and proapoptotic signaling would be a great benefit for the supplementation of biologically active dietary components [8].

THE INFLUENCE OF NUTRITIONAL FACTORS ON GI-TRACT CELL PROLIFERATION, DIFFERENTIATION AND APAPTOSIS

Based on the large number of animal, epidemiological, as well as clinical studies, it is recognized that a plant-based diet can modify GI-tract cell proliferation, differentiation and apoptosis, and in this manner, reduce the risk of several diseases, of which cancer seems to be the most studied.

Dietary fibres. Studies with dietary fibres (mainly wheat bran and cellulose) have shown their positive effect in the protection against colon cancer. However, there is also some evidence which shows that the supplementation of dietary fibres may also support tumour development. The protective effect may be attributed to both the dilution and absorption of carcinogens within the gut lumen, followed by acceleration of the luminal content transit time [21, 22]. On the other hand, the negative effect may be a result of the stimulating effect of epithelial cell proliferation and migration, which may promote carcinogenesis [21, 22]. Butyrate, and other short chain fatty acids produced in the colon lumen during the fermentation process of dietary fibres, may also increase epithelial cell proliferation by modification of the metabolic acidity of colonic microflora [23].

Curcumin. Curcumin (*Curcuma longa* L.), a principal curcuminoid of the Indian curry spice turmeric, is capable of inhibiting skin cell proliferation and tumour formation. The ability of curcumin to induce apoptosis in a variety of cancer cell lines in culture and its low toxicity have led to scientific interest in its potential for cancer therapy, as well as cancer prevention [24]. However, its effect on GI tract cells differentiation, proliferation, and apoptosis, has not been adequately studied; in animal model studies, curcumin was shown to increase the rate of proliferation of colon epithelial cells and pool size, and in this way, prevent colon cancer [25].

Garlic (*Allium sativum* L.). Based on several epidemiological studies, allium vegetables, including garlic, are capable of protecting against cancer of the GI tract [26, 27]. Although its action on carcinogenesis has not yet been clearly elucidated, a strong inverse relationship between stomach [28] as well as colon [29] cancer risk and allium vegetables (mostly garlic) intake has been proved. A number of biologically active garlic compounds, mostly oil- and water-soluble (e.g. allicin), sulfur-containing elements, have been identified to date, of which several have been shown to inhibit tumorigenesis [30].

Cruciferous vegetables (*Cruciferae* Juss.). The most extensively studied molecule with the chemopreventive potential is Indole-3 carbinol (I3C), a product of glucosinolates hydrolysis by myrosinase. Both the substrate and enzyme can be found in cell vacuoles of cruciferous vegetables, such as broccoli, cauliflower, brussels sprouts and cabbage. It was shown in rats exposed to the colon carcinogen 1,2-dimethylhydrazine, that consumption of cruciferous vegetables significantly decreased the numbers of aberrant crypt foci (ACF) and mucin-depleted foci (MDF), which in fact means the reduction of colon cancer risk in rats [31]. Moreover, I3C alone can also reduce the risk of breast cancer by interfering with the estrogen metabolism. Hydroxylation of estrogen hormones in the C-16 and C-2 positions, involves competing cytochrome P-450-dependent pathways, each sharing a common estrogen substrate pool. The increased formation of 2-hydroxylated (catechol) estrogen metabolites relative to 16-hydroxylated forms, may protect against cancer, as catechol estrogens can act as antiestrogens in cell culture [32, 33]. On the other hand, there are also *in vivo* data showing that I3C can also enhance carcinogenesis [34].

Citrus fruits. Among phytochemicals, limonoids are one of the most promising molecules with anticancer activity. Limonene, which can be found in lemons, grapefruits or oranges, was shown to be effective in the chemoprevention of both spontaneous and chemically-induced neoplasm [33-36]. Limonene metabolite-perrillyl alcohol, was also tested during clinical trials in patients with advanced malignant tumours [37].

Soybean (*Glycine max* L.). Since the early 1990s, soy has been recognized as a high quality source of protein; however, since some anticarcinogens have been identified in soybeans, including phytoestrogens, saponins, phenolic and phytic acids, isoflavones and protease inhibitors, soy became of particular interest as a potential anticancer-drug source. The use of soybean in human and animal nutrition is limited because of previously observed damage of the intestinal mucosa structure in animals fed soybean supplemented diets. However, soaking and subsequent boiling may reduce the negative effects of soybean on gut mucosa [38]. Although the relationship between soy intake and cancer risk are not yet clearly elucidated, it is known that the population of Southeast Asia (high daily soy intake) have reduced risk of estrogen-dependent breast cancer [39]. This effect is attributed to isoflavones, which are heterocyclic phenols structurally similar to the estrogenic steroids, and may act as antiestrogens by competing with the more potent, naturally-occurring endogenous estrogens (e.g., 17 β -estradiol) for binding to the estrogen receptors α and β [33-39].

Flax (*Linum* L.). Flaxseed was proved to act as a factor to decrease tumours of the colon and mammary gland, as well as of the lung [40, 41], which was probably the effect of fibre-associated compounds – lignans (enterodiol and its oxidation

product, enterolactone). Lignans are formed in the GI tract by bacterial action on plant lignan precursors [42].

Tomatos (*Lycopersicon esculentum* Mill.). The beneficial effect regarding the reduction of cancer risk is attributed to the carotenoid lycopene [43]. Instead of prostate cancer, other cancers whose risk have been inversely associated with serum or tissue levels of lycopene, include the digestive tract, cervix, breast, bladder and skin [44, 45]. Since lycopene is the most efficient quencher of singlet oxygen in biological systems, its antioxidant capacity seems to be responsible for its possible potential in cancer risk reduction [46].

Functional foods of animal origin are not that well recognized; however, some of them, including animal fat, seem warrant particular interest. Beef fat contains 3.1 to 8.5 mg conjugated linoleic acid (CLA)/g fat, which has been shown to be effective in suppressing forestomach tumours in mice, aberrant colonic crypt foci in rats, and mammary carcinogenesis in rats [47]. CLA is also an effective anticarcinogen in mammary the tumour model. These results are not due to displacement of CLA in cells, which suggests that there may be a unique mechanism by which CLA modulates tumour development [48].

Bacteria. From over 400 species of bacteria inhabiting the human GI-tract, *Bifidobacterium* and *Lactobacillus* are considered beneficial for health. The role of probiotics in the reduction of colon cancer risk is of considerable interest since the fact was recognized that lactic acid cultures can alter the activity of faecal enzymes (e.g., β -glucuronidase, azoreductase, nitroreductase) that are thought to play a role in the development of colon cancer [49].

The interaction of components of the diet is far from being elucidated. However, there is evidence showing that supplementation with the combination of dietary bioactive components, in the case where each single molecule targets the multiple mechanism or exhibit synergy or potentiating, may be more beneficial in comparison to the provision of a single dietary component.

EFFECT OF COMBINED ORAL SUPPLEMENTATION OF PHA AND AKG ON GI TRACT MORPHOLOGY, GROWTH AND NITROGEN BALANCE IN RATS

Although interactions among nutrients have been inadequately examined, a few examples of negative and positive interactions exist, e.g. some components may provide beneficial effects by influencing the wall and environment of the gastrointestinal (GI) tract, including phytohaemagglutinin (PHA), and other components such as alpha-ketoglutaric acid (AKG).

Phytohaemagglutinin, PHA

The interaction of red kidney bean (*Phaseolus vulgaris* L.) lectin, phytohaemagglutinin (PHA), with the GI-tract is well documented [50, 51]; indeed, it has been shown that PHA accelerates the turnover of GI-tract cells. Such rapid cell proliferation might be expected to result in both increased gut growth and functional maturation and, as a further consequence, an increase in the use of dietary amino acids for protein synthesis by enterocytes [51, 52]. Animal studies have shown that over a 3-day period oral administration of PHA to young suckling rats results in gut maturation in terms of epithelial development, decreased lactase, but increased

maltase and sucrase activities, as well as a reduction in macromolecular absorptive capacity, sometimes referred to as gut closure [53].

Alpha-ketoglutarate, (AKG). Glutamine and its derivative – AKG, are considered one of the crucial molecules in transmembrane amino acid transport, protein metabolism, and both gene and cellular redox regulation [54]. Animal, and indeed human studies alike, have shown that 95% of luminal glutamate and 70% of glutamine, but only 40% of AKG, is metabolized (first pass) to CO₂ by the intestinal mucosa [55, 56]. Deamination of amino acids releasing amino nitrogen for growth results in the production of ammonia, which is generally protonated to ammonium at physiological pH. There is, however, some evidence that the ammonia ion itself may be an essential part of the appropriate environment for growth of enterocytes [57]. Indeed, ammonia, which is toxic and therefore maintained at a very low level in the blood, has been shown to be converted from extracellular or intramitochondrially generated ammonia to urea by enterocytes in post-weaned pigs [57]. Alternatively, AKG, which serves as a natural scavenger of ammonium, facilitates its conversion to amino acids and protein [58], and by reducing levels of ammonium in the body it has a beneficial effect on nitrogen metabolism. In this way, one might also regard AKG as having a role as a protective agent for kidney function [59], with a knock-on benefit for bone metabolism [60, 61].

The rationale for the combined oral administration of PHA and AKG was based on the speculation, that an accelerated cellular turnover in enterocytes upon addition of PHA, resulting in an increase in ammonia production, in the presence of AKG, will rather promote its incorporation into amino acids and reduce the levels of urea produced, enhancing amino nitrogen levels for growth.

Effect of PHA and AKG on GI tract weights

The results showed that oral PHA administration increases total GI tract weight. After the addition of AKG, the observed changes were more pronounced in both the small intestine and large intestine [62, 63].

Effect of PHA and AKG on GI-tract morphology and CCK/NPY/Gln expression

The morphometric analysis of duodenum taken from younger animals treated with PHA in combination with AKG showed a significant difference compared to that of the Control group with regard to crypt depth (138% increase). However, no difference in the thickness of tunica mucosa, nor height and basal width of enterocytes, was found. The results of immunohistochemical examination of the duodenum showed no difference in the abundance of CCK positive cells in the tunica mucosa in AKG+PHA treated animals. However, expression of NPY in granules of neuronal cells of the submucosal parasympathetic ganglia, without any expression in goblet cells, was noticeable [62]. Morphometric analysis of the proximal part of the GI tract taken from older animals treated with AKG+PHA, revealed a significant difference increase in both crypt depth and tunica mucosa thickness [63].

Effect of PHA and AKG on N excretion

Oral PHA administration combined with AKG led to a significant reduction in N excretion in urine. Calculation of the relative level of N in faeces to the total N intake (Faecal

N/intake N %) showed that in AKG+PHA treated animals the percentage of N excreted in faeces increased, compared to the control group, in favour of N excretion *via* the faeces [62].

Underlying mechanisms and possible practical application

Plant lectins are known to bind avidly to the mucosal surface of the GI tract, inducing dose- time-dependent and fully reversible hyperplastic and hypertrophic growth of the small intestine [50-53]. Earlier studies suggest that an increase in the growth of the small intestine following PHA treatment is achieved through an elevated rate of cellular production [50-53]. It is very likely that PHA acts as an extraneous growth factor in the gut, binding to cell surface receptors of the brush border membrane in a similar fashion to some other hormones and peptide growth factors. It has certainly been shown that PHA induces extensive proliferation and changes in the metabolism of epithelial cells *via* activation of secondary messenger pathways, such changes being the result of activated by PHA of brush border epithelial receptors [50-53]. In the presented experiments, crypt depth and the thickness of the tunica mucosa in the proximal part of the small intestine was significantly increased in the PHA treated rats comparing to that of the controls. Moreover, the effect was far more pronounced in the animals administered with a high dose of PHA. These data not only support but also augment previous findings in which authors have attributed an increase in the weight of the small intestine to an increase in the rate of cell production, as indicated by a greater crypt depth [64, 65]. It has also been shown that PHA significantly stimulates small intestinal growth by a CCK independent mechanism, and pancreatic hypertrophy by a CCK dependent mechanism [66, 67]. Moreover, PHA *in vitro*, directly and dose dependently releases CCK from isolated intestinal mucosa cells [68]. The exact mechanism of lectin-induced CCK release from CCK cells in the intestine has yet to be elucidated, but it is hypothesised that lectins influence CCK cell activity by binding to the glycosyl side chains of voltage-gated channels [69]. The results also indicate that oral PHA+AKG treatment may lead to an alteration of NPY expression in the duodenum of rats. The physiological effect of this alteration may be an increase in duodenal intra-luminal pressure, together with involvement in the regulation of inter-digestive motility of the small intestine [62, 63]. The presented experiments also support evidence [56, 57] (IHC analysis) that AKG treatment has a beneficial impact on the level of glutamate metabolized by enterocytes in the proximal small intestine. Thus, it might be speculated that the increased level of glutamate found in AKG treated rats, may be seen as indicative of an increased part of the ingested feed nitrogen bound within the gastrointestinal tract made available for further metabolic processing to, for example, glutamine and proline formation following such treatment. Another interesting outcome from AKG+PHA supplementation is that such treatment alters the proportion of N excreted in urine *cf* that of faeces, so that faecal N excretion appears to be favoured. Moreover, it is important to note that the addition of AKG alone to the diet did not exert any influence on the amount of N excreted in the urine or faeces [62, 63]. Although the presented results do not elucidate underlying mechanisms, one might speculate that above-described AKG+PHA synergy is achieved either through inhibition of the senescent down-regulation of the proliferative and metabolic genes, identified by Lee *et al.* [69], or a synergetic stimulation of cellular growth and provision

of metabolic reserves necessary to support protein synthesis. The presented study also leads to the conclusion that elderly rats appear to have a reduced ability to utilize nitrogen for growth or other metabolic processes, which is in agreement with the findings of animal and clinical reports [62, 63, 70, 71]. Although it is difficult to anticipate the practical application, these findings reveal new perspectives for the treatment of injured intestinal mucosa, as well as some potential in prolonging the inter-dialysis period in patients with chronic renal failure.

CONCLUSIONS

In the recent decade, bioactive components derived from foods have become an object of considerable interest for nutritionists and other health professionals due to their great potential as health promoters. Therefore, a better understanding of how diet influences an individual's genetic potential, overall performance, and susceptibility to disease, can have enormous implications for society. Many diseases have been associated with impaired cell proliferation or differentiation and, what is most important, also with aberrantly regulated apoptotic cell death, ultimately leading to inhibition of apoptosis and propagation of diseases such as cancer. Better knowledge of the mechanisms will provide the opportunity for dietary intervention to prevent different diseases, including cancer, through, e.g. induction of apoptosis, since diet is a significant environmental factor in the overall cell neoplastic conversion and can exacerbate or interfere with carcinogenesis. Therefore, both mitochondrial-mediated and the death receptor-mediated effector mechanisms of apoptosis seem to be one of the most studied therapeutic targets of bioactive diet components.

REFERENCES

- Zabielski R, Le Huërou-Luron I, Guilloteau P: Development of gastrointestinal and pancreatic functions in mammals (mainly bovine and porcine species): influence of age and ingested food. *Reprod Nutr Dev* 1999, **39**(1), 5-26.
- Thompson JS, Saxena SK, Sharp JG: Regulation of intestinal regeneration: new insights. *Microsc Res Tech* 2000, **51**(2), 129-137.
- Godlewski MM, Shupecka M, Woliński J, Skrzypek T, Skrzypek H, Motyl T, Zabielski R: Into the unknown – the death pathways in the neonatal gut epithelium. *J Physiol Pharmacol* 2005, **56** (Suppl 3), 7-24.
- Ciccocioppo R, Di Sabatino A, Gasbarrini G, Corazza GR: Apoptosis and gastrointestinal tract. *Ital J Gastroenterol Hepatol* 1999, **31**(2), 162-172.
- Iwakiri R, Gotoh Y, Noda T, Sugihara H, Fujimoto K, Fuseler J, Aw TY: Programmed cell death in rat intestine: effect of feeding and fasting. *Scand J Gastroenterol* 2001, **36**(1), 39-47.
- Kakimoto T, Fujise T, Shiraiishi R, Kuroki T, Park JM, Ootani A, Sakata Y, Tsunada S, Iwakiri R, Fujimoto K: Indigestible material attenuated changes in apoptosis in the fasted rat jejunal mucosa. *Exp Biol Med (Maywood)* 2008, **233**(3), 310-316.
- Fischer U, Schulze-Osthoff K: Apoptosis-based therapies and drug targets. *Cell Death Differentiation* 2005, **12**, 942-961.
- Martin RM: Targeting Apoptosis with Dietary Bioactive Agents. *Exp Biol Med* 2006, **231**, 117-129.
- Reed J: Apoptosis-regulating proteins as targets for drug discovery. *Trends Mol Med* 2001, **7**, 314-319.
- Sun S, Hail N, Lotan R: Apoptosis as a novel target for cancer chemoprevention. *J Natl Cancer Inst* 2004, **96**, 662-672.
- Danial N, Korsmeyer S: Cell death: critical control points. *Cell* 2004, **116**, 205-219.
- Lockshin R, Zakeri Z: Apoptosis, autophagy, and more. *Int J Biochem Cell Biol* 2004, **36**, 2405-2419.
- Reed J: Apoptosis mechanisms: implications for cancer drug discovery. *Oncology* 2004, **18**, 11-20.
- Guimaraes C, Linden R: Programmed cell death: apoptosis and alternative death styles. *Eur J Biochem* 2004, **271**, 1638-1650.
- Hail N: Mechanisms of vanilloid-induced apoptosis. *Apoptosis* 2003, **8**, 251-262.
- Bouralexis S, Findlay D, Evdokiou A: Death to the bad guys: targeting cancer via Apo2L/TRAIL. *Apoptosis* 2005, **10**, 35-51.
- Reed J: Dysregulation of apoptosis in cancer. *J Clin Oncol* 1999, **17**, 2941-2953.
- Perik P, de Vries E, Gietema J, van der Graaf W, Sleijfer D, Suurmeijer A, Van Veldhuisen D: The dilemma of the strive for apoptosis in oncology. *Crit Rev Oncol Hematol* 2005, **53**, 101-113.
- Davis C, Milner J: Frontiers in nutrigenomics, proteomics, metabolomics and cancer prevention. *Mutat Res* 2004, **551**, 51-64.
- Milner J: Molecular targets for bioactive food components. *J Nutr* 2004, **134**, 2492-2498.
- Jacobs LR: Effect of dietary fiber on colonic cell proliferation and its relationship to colon carcinogenesis. *Prev Med* 1987, **16**(4), 566-571.
- Jacobs LR: Relationship between dietary fiber and cancer: metabolic, physiologic, and cellular mechanisms. *Proc Soc Exp Biol Med* 1986, **183**(3), 299-310.
- Jacobs LR: Modification of experimental colon carcinogenesis by dietary fibers. *Adv Exp Med Biol* 1986, **206**, 105-118.
- Karunagaran D, Rashmi R, Kumar TR: Induction of apoptosis by curcumin and its implications for cancer therapy. *Curr Cancer Drug Targets* 2005, **5**(2), 117-129.
- Kim SJ, Hellerstein MK: Pharmacological doses of dietary curcumin increase colon epithelial cell proliferation in vivo in rats. *Phytother Res* 2007, **21**(10), 995-998.
- Block G, Patterson B, Subar A: Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr Cancer* 1992, **18**, 1-29.
- Ernst E: Can allium vegetables prevent cancer? *Phytomed* 1997, **4**, 79-83.
- You WC, Blot WJ, Chang YS, Ershow AG, Yang ZT, An Q, Henderson B, Xu GW, Fraumeni JF, Wang TG: Diet and high risk of stomach cancer in Shandong, China. *Cancer Res* 1988, **48**, 3518-3523.
- Steinmetz KA, Potter JD: Vegetables, fruit and cancer II. Mechanisms. *Cancer Causes Control* 1991, **2**, 427-442.
- Reuter HD, Koch HP, Lawson LD: Therapeutic effects and applications of garlic and its preparations. In: *Garlic. The Science and Therapeutic Application of Allium sativum L. and Related Species*, 2nd Ed., Koch HP, Lawson LD (eds.), Williams & Wilkins, Baltimore 1996.
- Arikawa AY, Gallaher DD: Cruciferous vegetables reduce morphological markers of colon cancer risk in dimethylhydrazine-treated rats. *J Nutr* 2008, **138**(3), 526-32.
- Michnovicz JJ, Bradlow HL: Altered estrogen metabolism and excretion in humans following consumption of indole carbinol. *Nutr Cancer* 1991, **16**, 59-66.
- Hasler CM: Functional Foods: Their Role in Disease Prevention and Health Promotion. A Publication of the Institute of Food Technologists. Expert Panel on Food Safety and Nutrition. www.NutriWatch.com
- Dashwood RH: Indol-3-carbinol Anticarcinogen or tumor promoter in Brassica vegetables. *Chem Biol Interactions* 1998, **1**(10), 1-5.
- Hasegawa S, Miyake M: Biochemistry and biological functions of citrus limonoids. *Food Rev Intl* 1996, **12**, 413-435.
- Gould MN: Cancer chemoprevention and therapy by monoterpenes. *Environ Health Perspec* 1997, **105**, 977-979.
- Ripple GH, Gould MN, Stewart JA, Tutsch KD, Arzooonian RZ, Alberti D, Eierabend C, Pomplun M, Wilding G, Bailey HH: Phase I clinical trial of peillyl alcohol administered daily. *Clin Cancer Res* 1988, **4**, 1159-1164.
- Godlewski MM, Ślęzak P, Zabielski R, Piastowska A, Gralak MA: Quantitative study of soybean-induced changes in proliferation and programmed cell death in the intestinal mucosa of young rats. *J Physiol Pharmacol* 2006, **57** (Suppl 7), 125-33.
- Messina M, Barnes S, Setchell KDR: Phytoestrogens and breast cancer. *Lancet* 1997, **350**, 71-972.
- Thompson LU: Flaxseed, lignans, and cancer. In: *Flaxseed in Human Nutrition*, (ed) Cunnane S, Thompson LU: AOCS Press, Champaign, IL, 1995, 219-236.
- Yan L, Yee JA, Li D, McGuire MH, Thompson LU: Dietary flaxseed supplementation and experimental metastasis of melanoma cells in mice. *Cancer Lett* 1998, **124**, 181-186.

42. Setchell KDR, Lawson AM, Borriello SP, Harkness R, Gordon H, Morgan DML, Kirk DN, Adlercreutz H, Anderson LC, Axelson M: Lignan formation in man – microbial involvement and possible roles in relation to cancer. *The Lancet* 1981, **1**, 4-7.
43. Gerster H: The potential role of lycopene for human health: *J Am Coll Nutr* 1997, **16**, 109-126.
44. Clinton SK: Lycopene: Chemistry, biology, and implications for human health and disease. *Nutr Rev* 1998, **56**, 35-51.
45. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman JW: Cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol Biomarkers Prev* 1996, **5**, 823-833.
46. Di Mascio P, Kaiser S: Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 1989, **274**, 532-538.
47. Ip C, Scimeca JA: Conjugated linoleic acid and linoleic acid are distinctive modulators of mammary carcinogenesis. *Nutr Cancer* 1997, **27**, 131-135.
48. Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouard PY, Van Amburgh ME, Bauman DE: Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J Nutr* 1998, **128**, 881-885.
49. Mital BK, Garg SK: Anticarcinogenic, hypocholesterolemic, and antagonistic activities of *Lactobacillus acidophilus*. *Crit Rev Micro* 1995, **21**, 175-214.
50. Pusztai A, Ewen SW, Grant G, Peumans WJ, van Damme EJ, Rubio L, Bardocz S: Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. *Digestion* 1990, **46**, 308-316.
51. Bardocz S: Effect of phytohaemagglutinin on intestinal cell proliferation. Role of polyamines. *Arch Lationam Nutr* 1996, **44** (4 suppl 1), 16-20.
52. Linderth A, Prykhod'ko O, Ahren B, Fak F, Pierzynowski SG, Westrom BR: Binding and the effect of the red kidney bean lectin, phytohaemagglutinin, in the gastrointestinal tract of suckling rats. *Br J Nutr* 2006, **95**, 105-15.
53. Linderth A, Prykhod'ko O, Pierzynowski SG, Westrom BR: Enterally but not parenterally administered *Phaseolus vulgaris* lectin induces growth and precocious maturation of the gut in suckling rats. *Biol Neonate* 2006, **89**, 60-68.
54. Wernerman J, Hammarqvist F: Modulation of endogenous glutathione availability. *Curr Opin Clin Nutr Metab Care* 1999, **2**, 487-492.
55. Junghans P, Derno M, Pierzynowski S, Hennig U, Souffrant WB: Utilization of α -ketoglutarate (AKG) in young growing pigs after intravenous and intra-duodenal administration. The EAAP Symposium (European Association for Animal Production) on Energy and Protein Metabolism and Nutrition, Rostock-Warnemünde, Germany, 2003, 521-523.
56. Lambert BD, Filip R, Stoll B, Junghans P, Derno M, Hennig U, Souffrant WB, Pierzynowski S, Burrin DG: First-pass metabolism limits the intestinal absorption of enteral α -ketoglutarate in young pigs. *J Nutr* 2006, **136**(11), 2779-2784.
57. Wu G: Urea synthesis in enterocytes of developing pigs. *Biochem J* 1995, **312**, 717-723.
58. Wiren M, Permert J, Larsson J: α -ketoglutarate – supplemented enteral nutrition: effects on postoperative nitrogen balance and muscle catabolism. *Nutrition* 2002, **8**, 725-728.
59. Welborn JR., Shpun S, Dantzler WH, Wright SH: Effect of α -ketoglutarate on organic anion transport in single rabbit renal proximal tubules. *Am J Physiol* 1998, **274**, 165-174.
60. Filip RS, Pierzynowski SG, Lindegard B, Wernerman J, Haratym-Maj A, Podgurniak M: α -ketoglutarate Decreases Serum Levels of C-terminal cross-linking telopeptide of type I collagen (CTX) and Preserves Bone Mass in Postmenopausal Women with Osteopenia: Six-Month Study. *Int J Vit Nutr Res* 2007, **77**, 89-97.
61. Filip R, Pierzynowski SG: The absorption, tissue distribution and excretion of enterally administered α -ketoglutarate in rats. *J Anim Physiol Anim Nutr* 2008, **92**(2), 182-189.
62. Filip R, Harrison AP, Bienko M, Radzki RP, Pierzynowski SG: Dietary supplementation with phytohemagglutinin in combination with α -ketoglutarate limits the excretion of nitrogen via the urinary tract in rats (*in press*).
63. Filip R, Harrison A, Pierzynowski SG: A Combined Phytohemagglutinin and α -Ketoglutarate Pharmacology Study of Gut Morphology and Growth in Older Adult Rats. *Am J Pharm Toxicol* 2008, **2**, 170-177.
64. Radberg K, Biernat M, Linderth A, Zabielski SG, Pierzynowski SG, Westrom BR: Exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. *J Anim Sci* 2001, **79**, 2669-2678.
65. Banwell JG, Howard R, Kabir I, Arian TE, Diamond RH, Abramowsky C: Small intestinal growth caused by feeding red kidney bean phytohemagglutinin lectin to rats. *Gastroenterology* 1993, **4**, 1669-1677.
66. Herzig KH, Bardocz S, Grant G, Nustede R, Fölsch UR, Pusztai A: Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. *Gut* 1997, **41**, 333-338.
67. Schmidt WE, Roy Choudhury A, Siegel EG, Löser C, Conlon JM, Fölsch UR, Creutzfeldt W: CCK-antagonist L-364,718: Influence on rat pancreatic growth induced by caerulein and bombesin-like peptides. *Reg Pep* 1989, **24**, 67-79.
68. Bouras EP, Misukonis MA, Liddle RA: Role of calcium in monitor peptide-stimulated cholecystokinin release from perfused intestinal cells. *Am J Physiol* 1992; 262: 791-79.
69. Santer R, Leung YK, Alliet P, Lebenthal E, Lee PC: The role of carbohydrate moieties of cholecystokinin receptors in cholecystokinin octapeptide binding: alteration of binding data by specific lectins. *Biochim Biophys Acta* 1990, **1051**, 78-83.
70. Lee HM, Greeley GH, Englander EW: Age-associated changes in gene expression patterns in the duodenum and colon of rats. *Mech Ageing Dev* 2001, **122**, 355-371.
71. Woudstra T, Thomson AB: Nutrient absorption and intestinal adaptation with aging. *Best Pract. Res. Clin Gastroenterol* 2002, **16**, 1-15.