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ARTICLE

Negative element balance according to a survey for consumption of some essential elements in cases of patients with inflammatory bowel diseases

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ABSTRACT Malnutrition is a characteristic feature of inflammatory bowel diseases (IBD), often due to unhealthy nutritional habits. Therefore, nutritional habits (intake of vegetables and fruits) and element content in erythrocytes have been investigated. 50 IBD patients (25 male, 25 female) and 50 healthy volunteers (35 male, 15 female) were asked to complete a questionnaire. In addition to routine laboratory parameters, Ca, Cu, Fe, Mg, Mn, P, S and Zn content in erythrocytes were determined with ICP-OES. Decreased level of Ca (0.975 ± 0.440 μg/g), Mg (1.02 ± 0.24 μg/g) and Zn (0.776 ± 0.482 μg/g) was observed in IBD patients at $P<0.05$ level compared to the control (2.90 ± 2.25 μg/g, 18.28 ± 9.66 μg/g and 1.05 ± 0.48 μg/g). IBD patients consume similar foodstuffs to healthy people although in lesser amount. The intake of nutritional antioxidants was almost the same in both groups, whereas element intake differed because of diverse nutritional habits. According to the survey, in Hungary healthy people consume about 18-66% of essential element requirements. In the case of IBD patients the situation is worse (15-60%) because of lesser intake and malabsorption. Lowest element intakes were observed for Ca and Zn. The mineral element imbalance in IBD patients probably contributes to their deficiency. Since IBD patients and the controls are on similar diet, latent element deficiency may develop in healthy volunteers which may enhance the risk of metabolic diseases.
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KEY WORDS

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Mg-
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Fe

Malnutrition is a serious problem in inflammatory bowel diseases (IBD) e.g. ulcerative colitis and Crohn's disease. Since intestinal permeability is altered, the absorption and bioavailability of different nutritional components e.g vitamins and metal elements (Fe, Zn, Ca, Mg, K) are also changed (Goldschmid and Graham 1989; Bhaskar et al. 1995; Blázovics et al. 2000; Bruwer et al. 2001; et al.; Gasche et al. 2004; Blázovics et al. 2006). As a result, multiple deficiency state may develop and IBD patients may need adequate substitution of different essential agents (Sturniolo et al. 1998; Blázovics et al. 1999a). In deficiency state, the therapy is completed with mineral supplementation but in spite of this, metal element homeostasis often remains modified. In some cases significant lower Fe and Se concentration was measured in the plasma of IBD patients. In other cases, however, no significant difference was found in the Se content (Sturniolo et al. 1998). Since the element balance of IBD patients is also dependent on the severity of the disease, comparison of the data is very difficult.

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As is known, redox homeostasis is altered in IBD patients (Blázovics et al. 1999a, 2004a). Metal elements play an essential role in the human body and in redox homeostasis. The quality and quantity of participating elements alter the redox homeostasis of tissue, cell and subcellular particle of specifically. Both oxidative and antioxidative processes may catalyzed by some elements (Cu, Fe, Mn, Zn). The role of P, S and Se is also very important in the antioxidant function. NF-κB, JNK and p53 signaling proteins play an important role in apoptotic death and free radicals and metals are significant mediators in the apoptotic process as well (Kudrin 2000; Sheikh and Fornace 2000). Transition metal ions are ubiquitous in biological systems. The presence and absence of some transition metal (Cu, Fe, Mn, Zn) and non-metal elements (P, S, Se) significantly modify the signal transduction processes, therefore, optimal tissue concentration is, no doubt, highly important. The most favorable results were attained by substitution of essential trace elements from natural sources (fruits, vegetables, herbal teas etc.; Lugasi et al. 1998; Máday et al. 2000; Szentmihályi et al. 2000; Kocsis et al. 2004; Stefanovits-Bányai et al. 2005).

The main natural sources of antioxidant compounds, such as vitamins, polyphenols (flavonoids, anthocyanidins), as

Table 1. Element content (μg/g) in erythrocyte of healthy controls and sick patients*.

Elements	Control (N=50)	IBD patients (N=50)	Significance (P < 0.05)
Al	0.364 ± 0.313	0.443 ± 0.189	Not sign.
Ca	2.90 ± 2.25	0.975 ± 0.440	Sign.
Cu	0.029 ± 0.024	0.019 ± 0.005	Not sign.
Fe	24.48 ± 4.48	21.63 ± 2.24	Not sign.
Mg	18.28 ± 9.66	1.02 ± 0.24	Sign.
Mn	0.148 ± 0.234	0.024 ± 0.069	Not sign.
P	13.50 ± 4.14	10.86 ± 1.64	Not sign.
S	67.55 ± 15.90	56.11 ± 7.88	Not sign.
Zn	1.05 ± 0.480	0.776 ± 0.482	Sign.

*Data are presented as mean ± SD.

well as essential trace elements, are foodstuffs. Since fresh fruits and vegetables are known to contain these components in largest amount, in this study the nutritional habits of IBD patients and healthy controls were studied. For this purpose questionnaires were completed and blood samples were taken from patients and controls for evaluation and comparision.

Materials and Methods

Hydrogen peroxide was obtained from Sigma (St. Luis, MO, USA), nitric acid of 65% was purchased from Carlo Erba (Milano, Italy). Standard solutions for calibration were prepared from solutions of Spectrascan (Kolbotn, Norway) and High-Purity Standards (Charleston, SC, USA).

Survey

Randomly chosen 50 healthy Caucasian volunteers (Controls; 35 male and 15 female; ages between 25 and 47 years) and 50 IBD patients (25 male, 25 female; ages between 35 and 67 years) filled out questionnaires related to their nutritional habits in the consumption of foodstuffs. From the data obtained was that we could estimate the consumption of fresh fruits and vegetables and the nutritional components. The questions referred to both favored and non-favored fruits and vegetables, the frequency and amount of consumption, as well as the consumption of juice, wine, medicinal plant teas, teas, bread and kinds of bread. The questions were composed so that minimum one positive answer could be obtained. The basic principle of elaboration of the survey was published by Dörnyei et al. (2006).

Before filling out the questionnaires, blood samples were taken from the patients.

Preparation and evaluation of the questionnaire were made by permission of TUKEB 153/2000. The amount of elements consumed from foodstuffs was calculated based on the Table of Nutrients (Bíró and Lindner 1989). Since in our evaluation average values were used, therefore weigthed average was calculated for element intake/requirement from RDA and DRI values of male and female subjects (RDA 1989; DRI 2002). According to the calculation, and considering the ratio of male and female subjects (the two groups showed some differences: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group), the following calculated average requirements (AR) were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.

Methods

The erythrocytes were separated and washed three times with isotonic NaCl solution. After washing and centrifugation procedures (10 min at 3000 rpm), the haemoglobin content of red blood cells was determined by the Haemisol Reagent Kit (HUMAN Vaccine Producing and Research Institute, Gödöllő, Hungary). The haemoglobin content was uniformly adjusted to 1 g/v%.

Inductively coupled plasma atomic emission spectrometry (ICP-AES, AtomScan 25, Thermo Jarrell Ash Co., Franklin, USA) was applied for the determination of Al, Ca, Cu, Fe, Mg, Mn, P, S, Zn concentrations in erythrocytes. Samples (1-2 ml) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (2 ml) in teflon vessels. After digestion, the samples were diluted to 10 ml with deionised water (Blázovics et al. 1999b). Since the washing of erythrocytes was performed with NaCl Na was not measured from the samples. In addition, since the intake of Na in the form of NaCl is generally higher than desired, the consumption of Na was not evaluated.

Routine laboratory parameters were controlled with the Roche/Hitachi Modular equipment followed by Ultra Sound investigations. The data obtained were published by Blázovics et al. (2006).

Means and standard deviations were calculated from the results. T-test was used for comparison of the results between the groups. Significance level was determined at $P < 0.05$.

Subjects

Blood samples of the above randomly chosen volunteers and IBD patients were evaluated. The patients were treated with 5-aminosalycilic acid (5-ASA, WHO proposed therapy). Patients with inactive UC and CD were treated with 5-ASA and 5-ASA plus the immunosuppressor azathioprine, respectively. Patients with moderate UC and CD were given 5-ASA plus a local steroid, and 5-ASA plus azathioprine plus the local antibiotic metronidazole, respectively. Patients with severe UC were treated with 5-ASA plus a local steroid and/or systemic steroid. Patients with severe CD received combined therapy with (metronidazole or ciprofloxacin) plus elementary

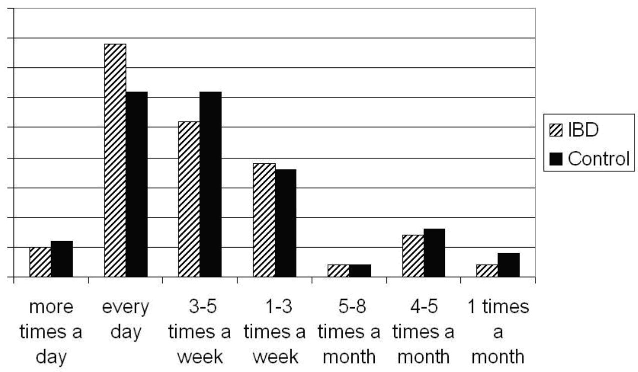

Figure 1. Frequency of fruit consumption.

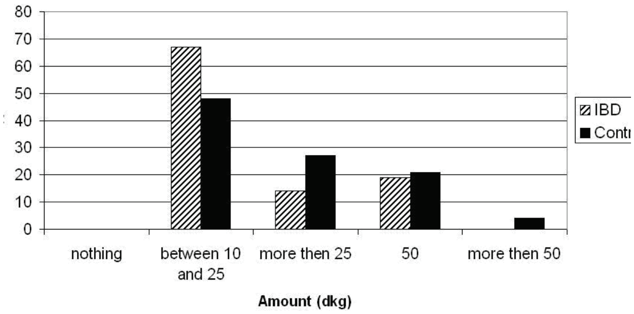

Figure 2. Amount of fruit consumed.

diet and steroid (local and/or systemic) plus antibiotics. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University. Permission number: TUKEB 24/1996, renewed in 2000.

Although our earlier investigations showed that in both males and females the redox parameters of plasma and erythrocytes change according to the severity of the disease independently of the antioxidant therapy (Blázovics et al. 2000; Szentmihályi et al. 2000), in this study the severity of the disease was not examined. This survey examined the nutritional habits of patients compared to the controls independently of the momentary state of the patient. All appreciable data of patients were elaborated from randomly chosen volunteers.

Results

Element homeostasis depends on many factors e.g. the amount of food, nutritional habits, the form of element consumed, the element state of the body. Therefore, the differences in the element content of erythrocytes of healthy controls and IBD patients were monitored first. The element content of erythrocytes is summarized in Table 1. The high value of standard deviations in the control group confirms that the group was chosen randomly from healthy people and some of these "healthy controls" may suffer from other metabolic diseases or latent deficiency state. In spite of this the concentration of Ca, Mg and Zn in the group of IBD patients was significantly lowered at $P<0.05$ calculated by the t-test.

Consumption of fruits

The frequency of fruit consumption and the amount of fruit consumed by the IBD patients slightly differ from the data of controls (Figs. 1 and 2). Although patients generally eat fruit more frequently than healthy people, they eat smaller amounts at a time. The fruits most favoured by IBD patients are peaches (93%), apples (80%), cherries (78%), water-melons (78%), bananas (76%), and apricots (62%). In spite of recommendations, some fruits, with small seeds, which are not favorable for patients, are consumed relatively frequently: strawberries (78%), raspberries (72%), white grapes (62%). Healthy people also like peaches (80%), apples (76%), bananas (60%), cherries (60%), strawberries (60%), white grapes (58%), apricots (58%), water-melons (54%), raspberries (54%), although other fruits are also under consumption: oranges (60%) and pears (54%). The daily amount of fresh fruit was found to be 0.15 kg for IBD patients and 0.17 kg for controls.

Almost totally neglected fruits by the two groups are avocado, blackberries, blueberries, cornel, currant species, gooseberries, maracuja, naseberries, quince apples, quince pears, rose-hips, wild plums. Some of the fruits, which are grown in Hungary, e.g. currant species, naseberries, quince apples, quince pears, rose-hips, wild plums are consumed almost only by small garden owners.

Cherries, walnuts, strawberries, raspberries, pears and plums cause complaints in about one-fifth of IBD patients, while bananas, walnuts, white grapes, apples and plums cause complaints in the controls.

Table 2 shows the daily element intake of fruits for controls and IBD patients calculated from the data of questionnaires. As can be seen, there is only a small difference between the element intake of the two groups in absolute value and in percentage related to AR calculated by RDA and DRI values (1989; 2002). Element consumption ranges between wide intervals depending on nutritional habits, e.g. IBD patients consume K between 368 and 1840 mg. Minimum consumption for an individual seems to be the same in both groups, although the maximum value shifts towards higher values in the case of controls, owing to nutritional habits. In all cases average element intake was found to be below 50% in IBD patients, while it was between 14 and 55% for the control group.

Consumption of vegetables

The frequency of vegetable consumption by controls and IBD patients shows only a slight difference (Fig. 3). IBD

Table 2. Element intake a day from fresh fruits according to the nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Minimum intake for an individual (mg)	368	75	59	1.2	80	0.1	0.7	0.4
Maximum intake for an individual (mg)	2940	597	469	9.4	642	1.1	5.3	3.1
Weighted mean for the group (mg)	1136	231	181	3.6	248	0.44	2.1	1.2
Minimum intake refer to AR (%)	7.8	7.4	15.0	9.5	10.0	15.8	4.4	17.7
Maximum intake refer to AR (%)	62.5	59.7	120	76.1	80.2	127	35.5	142
Average intake refer to AR (%)	24.2	23.1	46.5	29.4	31.0	48.9	13.7	54.8
IBD patients (n=42)								
Minimum intake for an individual (mg)	368	75	59	1.2	80	0.1	0.7	0.4
Maximum intake for an individual (mg)	1840	373	293	5.9	401	0.7	3.3	2.0
Weighted mean for the group (mg)	948	192	151	3.1	207	0.37	1.7	1.0
Minimum intake refer to AR (%)	7.8	7.5	16.3	8.4	10.0	15.8	4.4	18.6
Maximum intake refer to AR (%)	39.0	37.3	81.5	42.1	50.2	79.2	22.2	92.9
Average intake refer to AR (%)	20.2	19.3	42.0	21.7	25.9	40.8	11.4	47.9

AR average requirement (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

patients eat vegetables more rarely than healthy people. The most favoured vegetables of IBD patients are potatoes (95%), tomatoes (90%), cucumbers (81%), carrots (80%), green peppers (76%), lettuce (76%), onions (76%), cauliflowers (71%), green pease (71%), beetroots (71%), kale (67%) and garlic (62%). Of these vegetables garlic and kale may cause complaints sometimes for IBD patients and therefore, some of these patients never eat kale. Although cauliflowers, cucumbers, green peppers and beetroots are not recommended they are much favoured and consumed by IBD patients. Healthy controls also like potatoes (94%), cauliflowers (94%), tomatoes (78%), lettuce (76%), carrots (72%), green peppers (72%) and maize (80%). The total amount of vegetables to be consumed a day is 0.25 kg for IBD patients and 0.30 kg for controls.

Some vegetable almost never consumed s are those rarely available in the market or originating from abroad: brussels sprouts, chard, parsnips, asparagus, rheum and pumpkins. Vegetables cause complaints more frequently in IBD patients than in controls, although the vegatables causing problems are the same in both groups: kale, cabbage, kohlrabies and maize.

Table 3 shows the daily element intake of vegetable for IBD patients and controls calculated on the basis of questionnaires. Similarly to the element intake from fruits, only a slight difference could be observed in the element intake between patients and controls in absolute value and in percentage related to AR. Minimum and maximum consumption seems to be the same in both groups. Average element intake ranges between 4.0 and 17.7% in IBD patients, while it changes between 4.2 and 18.3% for the controls.

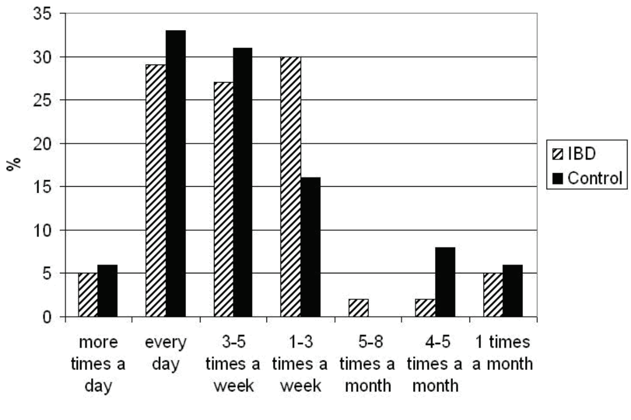

Figure 3. Frequency of vegetable consumption.

Total consumption of fruits and vegetables

Based on the evaluation of the survey, element intake by vegetables and fruits covers only a part of the daily requirements (Table 4). This value ranges between 15-60% for IBD patients and 18-66% in the controls. Although plants are known to be good sources of K and Mn, the intake of K does not reach 30% in IBD group. The low Zn intake (15% of the requirement in general) and Ca (24% of the requirement in general) in the case of IBD patients is a serious problem. The main sources of Ca are dairy products and of Zn are meats, although patients hardly eat any dairy products and the consumption of meat is also reduced mainly because of intolerance.

Discussion

Hungarian people consumes essential elements in relatively small amount as verified by the significantly low element concentration of Ca, Mg and Zn in the erytrocytes of IBD

Table 3. Element intake a day from vegetables according to the nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Minimum intake for an individual (mg)	19	2.21	2.13	0.05	3.84	0.01	0.03	0.01
Maximum intake for an individual (mg)	1665	189	183	3.87	329	0.62	2.34	0.97
Weighted mean for the group (mg)	444	51	49	1.0	88	0.165	0.624	0.26
Minimum intake refer to AR (%)	0.4	0.2	0.5	0.4	0.5	0.8	0.2	0.5
Maximum intake refer to AR (%)	35.4	18.9	46.9	31.2	41.1	68.7	15.6	44.2
Average intake refer to AR (%)	9.4	5.1	12.5	8.3	10.9	18.3	4.2	11.8
IBD patients (n=42)								
Minimum intake for an individual (mg)	19	2.21	2.13	0.05	3.84	0.01	0.03	0.01
Maximum intake for an individual (mg)	1665	189	183	3.87	329	0.62	2.34	0.97
Weighted mean for the group (mg)	429	49	47	0.99	85	0.159	0.60	0.25
Minimum intake refer to AR (%)	0.4	0.2	0.6	0.3	0.5	0.8	0.2	0.4
Maximum intake refer to AR (%)	35.4	18.9	50.8	27.6	41.1	68.7	15.6	46.3
Average intake refer to AR (%)	9.1	4.9	13.1	7.1	10.6	17.7	4.0	11.9

AR average requirement AR (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

Table 4. Element intake a day from fresh vegetables and fruits according to the survey of nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Average intake (mg)	1580	282	230	4.6	336	0.6	2.72	1.46
Average intake refer to AR (%)	34	28	59	37	42	67	18	66
IBD patients (n=42)								
Average intake (mg)	1376	241	198	4	292	0.53	2.3	1.25
Average intake refer to AR (%)	29	24	55	29	36	59	15	60

AR average requirement (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

patients compared to the controls and according to the survey by the low element intake of Zn, Ca, K and Fe from fruits and vegetables. Similar results were published by Geerling and co-authors, namely lowered Ca and P intake was observed in IBD patients (Geerling et al. 2000). Non-favorable nutritional habits may contribute to the development of IBD (Tragnone et al. 1995; Mahmud and Weir 2001) and in IBD patients macro- and microelement deficiency may frequently occur (20-90% of the patients) (Gasche et al. 2004; Fehér and Kovács 2007). Decreased oral element intake, malabsorption and increased intestinal bleeding are the major causes of deficiency. According to the literature, mainly Ca, Mg, Fe, Se and Zn deficiency occurs in IBD patients, although the rate of deficiency depends on the severity of the disease as well (Galland 1988; Rennem et al. 1998; Schoon et al. 1999; Gasche et al. 2004; Vijverman et al. 2006). Probably the most serious problem is the Zn deficiency, since the human body does not store Zn and a high amount of Zn is lost with the faeces, urine and through the skin by sweat (Tapiero and Tew 2003). Zn deficiency may cause diarrhea (Saxena et al. 1993), although the deficiency may be treated by supplementation of elements. High doses of inorganic metal compounds, e.g. Fe supplementation with inorganic $FeSO_4$ may be dangerous because of the increased oxidative stress and cancer risk (Seril et al. 2006). Toxic or large amount of metal elements e.g. Mn, Fe, Al may induce the signal transduction process via activation of mitogen-activated protein kinases (Kaneki et al. 2004; Valko et al. 2006). Administration of high amount of Zn may alter Fe metabolism (Blázovics et al. 2004b). Mg deficiency may cause transient increase in intracellular Ca level, which induces the production of pro-oxidant cytokines (interleukin-1, -6 and -8, tumor necrosis factor-α and -β), different growth factors (EGF-α, TGF-β, NFGF, FGF, PDGF), interferon-α and –γ. The increase in intracellular Mg level inhibits the production of pro-oxidant cytokines via the activation of protein phosphatases (Caddell 2000). Therefore the achievement of optimal metal element level is essential for the proper function of cells and body.

There is no doubt, element supplementation is very important for IBD patients. Since the long-term supplementary diet may cause several unfavorable effects, medical control is essential throughout the treatment. The consumption of fresh fruits and vegetables should be recommended and patients must be informed of plant foodstuffs most favorable from nutritional aspects. For IBD patients most recommended fruits are apples, peaches, apricots, bananas, nectarines and water-melons. According to the survey the patients do eat these fruits but in relatively small amount. At the same time some valuable fruits rich in K, Ca, Mg and Zn, e.g. rose-hips, gooseberries, raspberries, white grapes, currants, walnuts, hazel nuts and almonds are contra-indicated for patients. The situation is similar for vegetables, since the vegetables most rich in elements, e.g. spinach, wood sorrel, mushrooms, garlic are not recommended (Kocsis et al. 2004; Fehér and Kovács 2007). Malnutrition is aggravated by low consumption of milk and meat products.

Plant foodstuffs contain essential mineral elements in the form of organic compounds as well as antioxidant polyphenols and vitamins. In Hungary, as well as in other countries throughout the world, some diseases (e.g. cardiac, vascular diseases and cancer) associated with nutritional habits and life style are responsible for the high rate of mortality (75%). Therefore, several surveys have been made to estimate or determine the consumption of vegetables and fruits or the intake of vitamins and mineral elements. Based on commercial data in Hungary, the yearly ingestion/person of fruits and vegetables was 211.4 kg in 2004, from which the rate of vegetables was 117.7 kg (with 68 kg potatoes). According to the Hungarian Central Statistical Office, the consumption of vegetables and fruits was 195 kg in 2004 (110 kg vegetables and 85 kg fruits) (Hungarian Central Statistical Office 2005). Our survey shows that the consumption of plant foodstuffs (fruits and vegetables) amount to 146 kg/year (0.4 kg/day) for IBD patients and 171.6 kg/year (0.47 kg/day) for controls. The difference may be due to the amount of potatoes eaten as chips or fried potatoes (which were omitted by the participants of survey).

Conclusion

The mineral element imbalance was found to be higher in IBD patients, which probably contributes to the deficiency states. Since both groups, patients and controls are on a similar diet, the low element intake may cause latent element deficiency in healthy people as well, which may enhance the risk of metabolic disorders.

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