Original Article

Tissue accumulation and urinary excretion of chromium in rats fed diets containing graded levels of chromium chloride or chromium picolinate

Munehiro Yoshida^{1,2}, Erika Hatakeyama¹, Ryota Hosomi¹, Seiji Kanda³,
Toshimasa Nishiyama³ and Kenji Fukunaga^{1,2}

¹Laboratory of Food and Nutritional Sciences, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Yamate 3-3-35, Suita, Osaka 564-8680, Japan

²Organization for Research and Development of Innovative Science and Technology, Kansai University, Yamate 3-3-35, Suita, Osaka 564-8680, Japan

³Department of Public Health, Kansai Medical University, Fumizono 10-15, Moriguchi, Osaka 570-8506, Japan

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ABSTRACT — To attempt a risk assessment of the excess intake of trivalent chromium (Cr), tissue Cr accumulation and urinary Cr excretion were examined in weanling rats fed experimental diets containing graded levels of Cr chloride (CrCl₃) or Cr picolinate (CrPic). Thirty-six male weanling 4-weeks-old Wistar rats were divided into six groups and fed a casein-based semi-purified diet (Cr content: < 0.02 µg/g) supplemented with 1, 10, or 100 µg Cr/g as CrCl₃ or CrPic for 28 days. Among the experimental groups, no significant difference was observed in body weight; however, supplementation of 100 µg Cr/g to the diets caused a significant low liver weight irrespective of the chemical species of Cr. Activities of serum aspartate aminotransferase and alanine aminotransferase were significantly elevated in rats given CrPic at 100 µg Cr/g. In the liver, kidney and femur, Cr accumulation increased with elevation of the dietary Cr level. No influence of the difference in the chemical species of supplemented Cr was observed in the liver and kidney, but CrCl₃ caused significantly higher Cr accumulation than CrPic in the femur of rats given 100 µg Cr/g. Daily urinary Cr excretion elevated with the increase of the dietary Cr level. Rats given CrPic showed significantly higher daily urinary Cr excretion than those given CrCl₃, particularly at a dietary Cr level of 100 µg/g. The rate of urinary Cr excretion in rats given CrPic was constant, irrespective of the dietary Cr level, but that of rats given CrCl₃ fell with the increase of the dietary Cr level. These results indicate that the lowest adverse effect level of dietary Cr is less than 100 µg/g, irrespective of the chemical species of Cr.

Key words: Trivalent chromium, Chromium chloride, Chromium picolinate, Excess intake, The lowest observed adverse effect level (LOAEL)

INTRODUCTION

Trivalent chromium (Cr) is an essential trace element for human and animal nutrition (Mertz, 1993) and enhances insulin function in the form of a low molecular weight Cr binding substance (LMWCr), called chromodulin (Vincent, 2004). Severe Cr deficiency induces impaired glucose tolerance and is believed to increase the risk for diabetes (Mertz, 1969). Since a high dose (200 to 1,000 µg/day) of Cr improved glycemia among patients with diabetes (Lamson and Plaza, 2002; Rabinovitz,

2004), some healthy people take a Cr supplement, expecting an improvement of lipid or glucose metabolism. Cr chloride (CrCl₃), Cr nicotinate, and Cr picolinate (CrPic) are used as formulations of trivalent Cr. Among these, CrPic is designed to improve absorption (Evans and Pouchnik, 1993) and is the most common Cr supplement. In the U.S., manufacturers and sellers of Cr supplements ardently promote the benefits of Cr in the prevention and treatment of type 2 diabetes, dyslipidemia, and cardiovascular disease. As a result, estimated sales of Cr supplements to consumers were about \$150 million in

Correspondence: Munehiro Yoshida (E-mail: hanmyou4@kansai-u.ac.jp)

2008, exceeding those of calcium supplements (Nutrition Business Journal, 2009).

In spite of the general public's acceptance of Cr supplements, the scientific basis of the protective efficacy of Cr against disease, including diabetes, is weak. In a systematic review of the effect of Cr supplementation at 200 to 1,000 µg/day on glucose metabolism and lipid levels, it was concluded that no significant effect of Cr on lipid or glucose metabolism was found in people without diabetes (Balk *et al.*, 2007). In contrast, excess intake of trivalent Cr can possibly have adverse effects on humans and animals. In fact, several case reports pointed out the occurrence of adverse effects, including anemia, liver and kidney dysfunctions, or muscle cramps due to the ingestion of CrPic at 200 to 1,000 µg Cr/day (Wasser, 1997; Martin and Fuller, 1998; Wani *et al.*, 2006). However, the possibility that other supplements or drugs ingested concomitantly are associated with these symptoms cannot be excluded in these cases and there are no available data giving clear information on the dose-response relationship between Cr and its adverse effects. Due to limited human data on the toxicity of trivalent Cr, many developed countries have not established a tolerable upper limit (UL) of Cr (Food and Nutrition Board of U.S., 2001; Scientific Committee on Food of European Commission, 2003; Ministry of Health, Labour and Welfare of Japan, 2009).

The Cr intake of Japanese adults was estimated to be about 120 µg/day (Otsuka *et al.*, 2000). Since this estimated value far exceeds 25 to 40 µg/day, which is the recommended dietary allowance of Cr for Japanese adults (Ministry of Health, Labour and Welfare of Japan, 2009), the possibility of dietary Cr deficiency in the general Japanese is extremely low. Thus, it is thought that the use of Cr supplements does not have benefits but rather adverse effects on healthy Japanese. In the present study, we examined urinary Cr excretion and Cr accumulation in the organs of weanling rats fed experimental diets containing graded levels of CrCl₃ or CrPic and attempted a risk assessment of the excess intake of trivalent Cr.

MATERIALS AND METHODS

Reagents

CrCl₃ (Cr (III) chloride hexahydrate) was purchased from Nacalai Tesque (Kyoto, Japan). CrPic (Cr (III) picolinate) was purchased from Tokyo Chemical Industry (Tokyo, Japan). The ingredients of animal diets were purchased from Oriental Yeast Co. (Tokyo, Japan). Cr standard solution (1,000 µg Cr/ml as K₂Cr₂O₇ in 0.1 M HNO₃), rhodium (Rh) standard (1,000 µg Rh/ml of as Rh(NO₃)₂ in

2 M HNO₃), distilled water (HPLC grade), and metal-free grade HNO₃ were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animal experiments

The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the "Guide for the Care and Use of Experimental Animals" of the Prime Minister's Office of Japan. Experimental animals were fed in a room under a controlled 12 hr light (8:00 to 20:00) and dark cycle at a temperature of 23 to 24°C and humidity of 60%. The animals were given experimental diets and deionized water *ad libitum* during the entire experimental period.

Thirty-six male weanling 4-weeks-old Wistar rats were divided into six groups and fed a casein-based semi-purified basal diet (Cr content: < 0.02 µg/g) supplemented with 1, 10, or 100 µg/g of Cr as CrCl₃ or CrPic. The basal diet excluded CrCl₃ from the AIN93G diet (Reeves *et al.*, 1993). On the 23rd to 26th day after the start of feeding, urine samples were collected from all rats. After feeding for 28 days, the rats were anesthetized with sodium pentobarbital, blood was collected from the abdominal aorta, and the liver, kidney and femur were excised, washed with saline, blotted and weighed.

A part of each blood sample was transferred to a heparinized tube and subjected to hemoglobin determination. Serum was separated from the remainder of the blood and subjected to serum biochemical tests (total protein, albumin, triglyceride, total cholesterol, glucose, urea, iron, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT)). The hemoglobin measurement and serum biochemical tests were performed by a commercial service system (Japan Medical Laboratory, Osaka, Japan).

Analysis of Cr

Cr in the organ and urine samples was determined by inductively coupled plasma mass spectrometry (ICPMS) using Rh as an internal standard. Up to 1 g of the liver, kidney or femur was heated with 5 ml of metal-free HNO₃ in a boiling water bath until the disappearance of insoluble components. The volume of the digest was made up to 25 ml with distilled water. One milliliters of urine was diluted with 9 ml of 0.1 M HNO₃. Cr in the diluted digest and urine was directly nebulized to ICPMS. Details of the ICPMS operating condition for Cr determination were described previously (Yoshida *et al.*, 2008).

Histopathology

Liver samples were sectioned and the blocks obtained

were fixed in 4% formaldehyde. Tissues were embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin. A pathologist without knowledge of the animal treatment evaluated the sections.

Statistics

Experimental data were assessed by two-way analysis of variance (ANOVA). When the *F* value was significant ($p < 0.05$) in the Cr level, the Scheffe multiple range test was performed to determine which pairs of the Cr levels were significantly different ($p < 0.05$). In addition, when the *F* value was significant ($p < 0.05$) in the interaction between the Cr level and Cr species, the Scheffe multiple range test was performed to determine which pairs of the means were significantly different ($p < 0.05$). These statistical tests were performed using a personal computer (eMac; Apple Computer, Cupertino, CA, USA) with operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 (Abacus Concept, Berkeley, CA, USA).

RESULTS

Rat growth and organ weights

Table 1 shows the body, liver and kidney weights of rats fed experimental diets containing 1 to 100 µg/g of Cr as CrCl₃ or CrPic for 28 days. Among the experimental groups, no significant difference was observed in the body weight; however, supplementation of 100 µg Cr/g to the diets caused a significantly lower liver weight. In the kidney weight, rats fed diet containing 100 µg Cr/g as CrPic tended to show lower values compared to other groups of rats.

Serum biochemical tests and histopathology of liver

Table 2 shows serum AST and ALT activities in rats fed experimental diets. Dietary supplementation with CrPic at a level of 100 µg Cr/g significantly elevated these serum transaminase activities. In spite of the elevation of AST and ALT activities, no hepatic histopathological changes were observed even in rats fed a diet containing CrPic at 100 µg Cr/g.

No significant difference was observed among experimental groups in the hemoglobin level and serum biochemical tests, except for transaminase activities.

Cr in organs

Table 3 summarizes Cr concentration in the liver, kidney and femur. In all organs examined, Cr accumulation increased dependently on the dietary Cr level. No influ-

ence of differences in the chemical form of supplemented Cr was observed in the liver and kidney, but was manifested in the femur; CrCl₃ caused significantly higher Cr accumulation than CrPic in the femur of rats fed diets containing 100 µg Cr/g.

Urinary Cr excretion

Table 4 shows the urinary Cr excretion of rats fed the experimental diets. Daily urinary Cr excretion elevated with the increase of the dietary Cr level. Differences in the chemical form of dietary Cr markedly influenced urinary Cr excretion. Rats given CrPic showed significantly higher daily urinary Cr excretion than those given CrCl₃, particularly at a dietary Cr level of 100 µg/g. In rats given CrPic, the rate of urinary Cr excretion was constant (1.36 to 1.52%) irrespective of the dietary Cr level. On the other hand, the rate of urinary excretion fell with increased dietary Cr in rats given CrCl₃.

DISCUSSION

In the present study, dietary Cr supplementation decreased liver weight at 100 µg/g, irrespective of the chemical species. Since a difference between CrCl₃- and CrPic-loading was not observed in the hepatic Cr accumulation, the decrease must be associated with the accumulated Cr. Because no histopathological changes were detected, the decrease may not be a disease change. Nevertheless, it can be said that trivalent Cr inhibits liver growth.

In addition to the lowered liver weight, rats fed diets supplemented with CrPic at 100 µg Cr/g showed elevated serum transaminase activities. It has been reported that CrPic causes liver dysfunction in human and animals (Cerulli *et al.*, 1998; Bagchi *et al.*, 2002), and picolinate induces apoptosis in several types of cultured cells (Ogata *et al.*, 2000). Thus, it can be concluded that CrPic impairs liver cells by a mechanism different from CrCl₃.

Cr accumulation in the organs and urinary Cr excretion rose with increased dietary Cr and no difference with the chemical form of supplemented Cr was observed in hepatic and renal Cr accumulation. However, administration of CrPic caused lower femur Cr accumulation and higher urinary excretion than that of CrCl₃ at a dietary Cr level of 100 µg/g. On the other hand, while the rate of urinary Cr excretion of rats given CrPic was constant irrespective of the dietary Cr level, that of rats given CrCl₃ decreased with the increase of the dietary Cr level. In several organs, LMWCr is estimated to exist as the apo form (Yamamoto *et al.*, 1987). After the intake of a large dose of Cr, apoLMWCr probably binds to Cr and carries it into

Table 1. Effect of dietary Cr administration on body, liver and kidney weights of growing rats

Dietary Cr level ($\mu\text{g/g}$)	Body weight (g)		Liver weight (g/100 g body weight)		Kidney weight (g/100 g body weight)	
	CrCl ₃	CrPic	CrCl ₃	CrPic	CrCl ₃	CrPic
1	246 $\pm$ 5	243 $\pm$ 5	4.63 $\pm$ 0.16	4.71 $\pm$ 0.15	0.79 $\pm$ 0.01	0.78 $\pm$ 0.03
10	252 $\pm$ 8	242 $\pm$ 5	4.46 $\pm$ 0.10	4.59 $\pm$ 0.20	0.81 $\pm$ 0.03	0.80 $\pm$ 0.02
100	246 $\pm$ 3	244 $\pm$ 4	4.14 $\pm$ 0.09	4.01 $\pm$ 0.08	0.78 $\pm$ 0.04	0.71 $\pm$ 0.02

Two-way ANOVA followed by the Scheffe multiple range test

CrCl ₃ vs. CrPic	NS	NS	NS
Cr level	NS	$p < 0.001$	NS
1 $\mu\text{g/g}$ vs. 10 $\mu\text{g/g}$	-	NS	-
1 $\mu\text{g/g}$ vs. 100 $\mu\text{g/g}$	-	$p < 0.001$	-
10 $\mu\text{g/g}$ vs. 100 $\mu\text{g/g}$	-	$p = 0.009$	-
Interaction	NS	NS	NS

Means $\pm$ S.E.M. (n = 6).**Table 2.** Effect of dietary Cr administration on serum AST and ALT activities in growing rats

Dietary Cr level ($\mu\text{g/g}$)	AST (IU/ml)		ALT (IU/ml)	
	CrCl ₃	CrPic	CrCl ₃	CrPic
1	102 $\pm$ 3 ^a	107 $\pm$ 2 ^a	43 $\pm$ 4 ^a	39 $\pm$ 3 ^a
10	83 $\pm$ 6 ^a	102 $\pm$ 4 ^a	42 $\pm$ 3 ^a	43 $\pm$ 3 ^a
100	95 $\pm$ 3 ^a	179 $\pm$ 19 ^b	35 $\pm$ 3 ^a	63 $\pm$ 4 ^b

Two-way ANOVA

CrCl ₃ vs. CrPic	$p < 0.001$	$p = 0.004$
Cr level	$p < 0.001$	$p = 0.030$
Interaction	$p < 0.001$	$p < 0.001$

Means $\pm$ S.E.M. (n = 6). Values in the same parameter not sharing a common superscript differ significantly ($p < 0.05$).

the urine (Wada *et al.*, 1983). Accordingly, the decreased urinary excretion rate of Cr in rats given large amounts of CrCl₃ indicates that excess Cr saturates the urinary Cr excretion process *via* LMWCr. Thus, not Cr absorption but Cr excretion is thought to be higher in rats given CrPic than given CrCl₃. Picolinic acid increases urinary excretion as well as absorption of zinc and results in the increased turnover of endogenous zinc (Seal and Heaton, 1985). Similarly to zinc, Cr excretion may have been accelerated by picolinate, resulting in low Cr accumulation in the femur.

Anderson *et al.* (1997) observed no change of body, liver and kidney weights as well as serum biochemical tests, including transaminase activities in weanling rats fed diets supplemented with CrCl₃ or CrPic up to 100 μg Cr/g, and emphasized the lack of toxicity of trivalent Cr. In addition, they observed that rats consuming CrPic-supplemented diets had several-fold higher Cr concentrations in both the liver and kidney than those fed CrCl₃ and concluded that the absorption of CrPic was higher than CrCl₃. The reason for the inconsistency between the studies of Anderson *et al.* (1997) and ours could not be sufficiently

Cr accumulation and excretion in rats given CrCl₃ or CrPic**Table 3.** Cr concentration in organs of growing rats fed experimental diets containing graded level of CrCl₃ or CrPic

Dietary Cr level (µg/g)	Liver (µg/g)		Kidney (µg/g)		Femur (µg/g)	
	CrCl ₃	CrPic	CrCl ₃	CrPic	CrCl ₃	CrPic
1	0.46 ± 0.11	0.31 ± 0.03	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01 ^a	0.03 ± 0.01 ^a
10	0.87 ± 0.01	0.85 ± 0.04	0.12 ± 0.01	0.11 ± 0.01	0.16 ± 0.02 ^a	0.17 ± 0.01 ^a
100	1.86 ± 0.21	1.96 ± 0.24	0.81 ± 0.22	1.00 ± 0.11	1.79 ± 0.20 ^c	0.61 ± 0.06 ^b
Two-way ANOVA followed by the Scheffe multiple range test						
CrCl ₃ vs. CrPic	NS		NS		<i>p</i> < 0.001	
Cr level	<i>p</i> < 0.001		<i>p</i> < 0.001		<i>p</i> < 0.001	
1 µg/g vs. 10 µg/g	<i>p</i> = 0.007		NS		NS	
1 µg/g vs. 100 µg/g	<i>p</i> < 0.001		<i>p</i> < 0.001		<i>p</i> < 0.001	
10 µg/g vs. 100 µg/g	<i>p</i> < 0.001		<i>p</i> < 0.001		<i>p</i> < 0.001	
Interaction	NS		NS		<i>p</i> < 0.001	

Means ± S.E.M. (n = 6). Values in the femur not sharing a common superscript differ significantly (*p* < 0.05).

Table 4. Urinary Cr excretion of growing rats fed experimental diets containing graded level of CrCl₃ or CrPic

Dietary Cr level (µg/g)	Excretion amounts (µg/day)		Excretion rate (%)	
	CrCl ₃	CrPic	CrCl ₃	CrPic
1	0.41 ± 0.03 ^a	0.40 ± 0.02 ^a	1.65 ± 0.12 ^b	1.47 ± 0.09 ^b
10	2.89 ± 0.42 ^a	3.71 ± 0.48 ^a	1.15 ± 0.17 ^{ab}	1.52 ± 0.16 ^b
100	14.65 ± 0.99 ^b	33.35 ± 5.69 ^c	0.59 ± 0.04 ^a	1.36 ± 0.22 ^b
Two-way ANOVA				
CrCl ₃ vs. CrPic	<i>p</i> = 0.002		<i>p</i> = 0.013	
Cr level	<i>p</i> < 0.001		<i>p</i> = 0.002	
Interaction	<i>p</i> < 0.001		<i>p</i> = 0.011	

Means ± S.E.M. (n = 6). Excretion rate was calculated as follows: (excretion amounts)/[(dietary Cr level) × (feed intake)] × 100. Values in the same parameter not sharing a common superscript differ significantly (*p* < 0.05).

clarified. Differences between the two studies exist in the strain of rats, the feeding period and the basal diet used. Among these, differences in the basal diet may influence the manifestation of Cr toxicity and Cr metabolism. In the present study, we used a standard semi-purified diet, called AIN93G diet, as the basal diet. This diet is composed of purified casein and starch, sucrose, soybean oil, and mineral and vitamin mixtures (Reeves *et al.*, 1993). On the other hand, Anderson *et al.* (1997) used a commercial stock diet (RMH 3200, Agway Inc., Waverly, NY,

USA). This diet is composed of non-purified feed materials, such as cereal and grass, and contains various fibers and flavonoids. Since these substances are known to inhibit the intestinal absorption of several kinds of metals (Hurrell *et al.*, 1999; Sandberg, 2002; Zijp *et al.*, 2000) and prevent liver impairment (Ibrahim *et al.*, 2008; Park *et al.*, 2008), intake of the stock diet may have modified Cr absorption and CrPic-induced liver impairment. However, because raw materials and composition of these commercial stock diets including the RMH 3200 are not

constant, it is impossible to evaluate Cr toxicity under the same condition as the Anderson's study. Nevertheless, it is necessary to examine the effect of difference in basal diet (stock diets or semi-purified diets) on the manifestation of Cr toxicity.

A general human diet is not a semi-purified diet, but it is impossible to evaluate the effects of numerous unknown components contained in the human diets on Cr toxicity. Accordingly, due to the least unknown dietary factor, the results on Cr toxicity, tissue accumulation and urinary excretion obtained from our study using a semi-purified diet are available for a risk assessment of trivalent Cr. As for CrPic, since a dietary level of 100 µg Cr/g decreased the liver weight and elevated serum transaminase activities, the lowest observed adverse effect level (LOAEL) in the diet can be estimated to be below 100 µg Cr/g. Similarly, the LOAEL for dietary CrCl₃ was also below 100 µg Cr/g since saturation of the urinary Cr excretion process was observed at this dose level. Thus, it is concluded that the LOAEL for dietary Cr is less than 100 µg/g, irrespective of the chemical species of Cr. Since rats eat about 10% of their body weight every day, the dietary level of 100 µg/g corresponded to about 10 mg/kg. However, it is known that trivalent Cr shows mutagenicity or damages chromosomes in animal cells (Stearns *et al.*, 1995; 2002); therefore, research extend over the second generation is necessary for the risk assessment of trivalent Cr.

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REFERENCES

- Anderson, R.A., Bryden, N.A. and Polansky, M.M. (1997): Lack of toxicity of chromium chloride and chromium picolinate in rats. *J. Am. Coll. Nutr.*, **16**, 273-279.
- Bagchi, D., Stohs, S.J., Downs, B.W., Bagchi, M. and Preuss, H.G. (2002): Cytotoxicity and oxidative mechanisms of different forms of chromium. *Toxicology*, **180**, 5-22.
- Balk, E.M., Tatsioni, A., Lichtenstein, A.H., Lau, J. and Pittas, A.G. (2007): Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. *Diabetes Care*, **30**, 2154-2163.
- Cerulli, J., Grabe, D.W., Gauthier, I., Malone, M. and McGoldrick, M.D. (1998): Chromium picolinate toxicity. *Ann. Pharmacother.*, **32**, 428-431.
- Evans, G.W. and Pouchnik, D.J. (1993): Composition and biological activity of chromium-pyridine carboxylate complexes. *J. Inorg. Biochem.*, **49**, 177-187.
- Food and Nutrition Board, Institute of medicine, National Academy of Sciences (2001): Chromium. In *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc*. pp.197-223, National Academy Press, Washington DC.
- Hurrell, R.F., Reddy, M. and Cook, J.D. (1999): Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *Br. J. Nutr.*, **81**, 289-295.
- Ibrahim, W.H., Habib, H.M., Chow, C.K. and Bruckner, G.G. (2008): Isoflavone-rich soy isolate reduces lipid peroxidation in mouse liver. *Int. J. Vitam. Nutr. Res.*, **78**, 217-222.
- Lamson, D.W. and Plaza, S.M. (2002): The safety and efficacy of high-dose chromium. *Altern. Med. Rev.*, **7**, 218-235.
- Martin, W.R. and Fuller, R.E. (1998): Suspected chromium picolinate-induced rhabdomyolysis. *Pharmacotherapy*, **18**, 860-862.
- Mertz, W.H. (1969): Chromium occurrence and function in biological systems. *Physiol. Rev.*, **49**, 163-239.
- Mertz, W.H. (1993): Chromium in human nutrition: A review. *J. Nutr.*, **123**, 626-633.
- Ministry of Health, Labour, and Welfare (2009): *Dietary Reference Intakes for Japanese 2010*, pp.218-275, Dai-ichi shuppan, Tokyo.
- Nutrition Business Journal (2009): NBJ's Supplement Business Report 2009, pp.169-188, Penton Media Inc., San Diego.
- Ogata, S., Inoue, K., Iwata, K., Okumura, K. and Taguchi, H. (2001): Apoptosis induced by picolinic acid-related compounds in HL-60 cells. *Biosci. Biotechnol. Biochem.*, **65**, 2337-2339.
- Otsuka, Y., Isomoto, S. and Noda, H. (2000): Dietary intake of trace elements in the general population, estimated from a regional nutritional survey, and comparison with recommended dietary allowances and tolerable upper intake levels. *Nippon Koshu Eisei Zasshi (Jpn. J. Public Health)*, **47**, 809-818 (in Japanese).
- Park, S.W., Lee, C.H., Kim, Y.S., Kang, S.S., Jeon, S.J., Son, K.H. and Lee, S.M. (2008): Protective effect of baicalin against carbon tetrachloride-induced acute hepatic injury in mice. *J. Pharmacol. Sci.*, **106**, 136-143.
- Rabinovitz, H., Friedensohn, A., Leibovitz, A., Gabay, G., Rocas, C. and Habet, B. (2004): Effect of chromium supplementation on blood glucose and lipid levels in type 2 diabetes mellitus elderly patients. *Int. J. Vit. Nutr. Res.*, **74**, 178-182.
- Reeves, P.G., Nielsen, F.H. and Fahey, G.C. (1993): AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.*, **123**, 1939-1951.
- Sandberg, A.S. (2002): Bioavailability of minerals in legumes. *Br. J. Nutr.*, **88**, S281-285.
- Scientific Committee on Food, European Commission (2003): *Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Trivalent Chromium*, European Commission, Brussels (expressed on 4 April 2003).
- Seal, C.J. and Heaton, F.W. (1985): Effect of dietary picolinic acid on the metabolism of exogenous and endogenous zinc in the rat. *J. Nutr.*, **115**, 986-993.
- Stearns, D.M., Wise, J.P.Sr., Patierno, S.R. and Wetterhahn, K.E. (1995): Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J.*, **9**, 1643-1648.
- Stearns, D.M., Silveira, S.M., Wolf, K.K. and Luke, A.M. (2002):

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- Chromium(III) tris(picolate) is mutagenic at the hypoxanthine (guanine) phosphoribosyltransferase locus in Chinese hamster ovary cells. *Mutat. Res.*, **513**, 135-142.
- Vincent, J.B. (2004): Recent advances in the nutritional biochemistry of trivalent chromium. *Proc. Nutr. Soc.*, **63**, 41-47.
- Wada, O., Wu, G.Y., Yamamoto, A., Manabe, S. and Ono, T. (1983): Purification and chromium-excretory function of low-molecular-weight, chromium-binding substances from dog liver. *Environ. Res.*, **32**, 228-239.
- Wani, S., Weskamp, C., Marple, J. and Spry L. (2006): Acute tubular necrosis associated with chromium picolinate-containing dietary supplement. *Ann. Pharmacother.*, **40**, 563-566.
- Wasser, W.G., Feldman, N.S. and D'Agati, V.D. (1997): Chronic renal failure after ingestion of over-the-counter chromium picolinate. *Ann. Intern. Med.*, **126**, 410.
- Yamamoto, A., Wada, O. and Ono, T. (1987): Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. *Eur. J. Biochem.*, **165**, 627-631.
- Yoshida, M., Takada, A., Hirose, J., Endô, M., Fukuwatari, T. and Shibata, K. (2008): Molybdenum and chromium concentrations in breast milk from Japanese women. *Biosci. Biotechnol. Biochem.*, **72**, 2247-2250.
- Zijp, I.M., Korver, O. and Tijburg, L.B. (2000): Effect of tea and other dietary factors on iron absorption. *Crit. Rev. Food Sci. Nutr.*, **40**, 371-398.