Trace Nutrients Research 29 : 110－116 (2012)

Original Article

A Nutrition Survey of Atherosclerosis Patients to Study the Effects of Iron, Zinc, and Copper on Atherosclerosis

Naoki SHIDA[1),3)], Yoko TSUCHIYA[1)], Fumiyuki YAMAKURA[2)], Tatsuji KANO[3)], Yuji NAKAZATO[3)], Kazuko TAKADA[4)], Sachio KAWAI[5)], Keiichi IKEDA[6)], Yuko AMAMIYA[6)], Shigeru NAKAJIMA[7)], Kazuhiko MACHIDA[8)], Hideaki IWAI[6)]

[1)] *Graduate School of Human Sciences, Waseda University*,*

[2)] *Department of Chemistry, School of Health Care and Nursing, Juntendo University**,*

[3)] *Department of Cardiology, School of medicine, Juntendo University**,*

[4)] *National Institute of Health and Nutrition***,*

[5)] *Laboratory of Sports Medicine, School of Health and Sports Sciences, Juntendo University**,*

[6)] *Department of Health Science, School of Health and Sports Sciences, Juntendo University**,*

[7)] *Department of Health and Nutrition, Bunkyo University Women's College****,*

[8)] *Faculty of Human Sciences, Waseda University**

Summary

To investigate the differences in the trace mineral intake of atherosclerosis patients at different stages. , the nutrition survey was made in 80 atherosclerosis patients and 184 healthy individuals. Student's t-tests were performed to determine the differences in the mineral intake between atherosclerosis patients and healthy individuals, and the differences among the different stages of the disease. All subjects were divided into 2 groups according to body mass index above 25 (obese group) and below 25 (non-obese group). The Student's t-test was performed for groups comprising only obese group and only non-obese group too.

In Serum measurements, the subjects included 16 male atherosclerosis patients and 35 healthy men. The differences between the average concentrations of each mineral were determined between the obese and non-obese groups; the atherosclerosis and non-obese groups; and the atherosclerosis and healthy groups. Student's t-test was used to determine significance.

The results revealed that a significantly higher value of Fe intake was observed for the atherosclerosis group compared to the healthy group. Also Zn intake of the obese individuals of the non-atherosclerosis group was higher than that of the atherosclerosis group. The serum Zn level in the healthy and obese groups was higher than that in the atherosclerosis group.

Keywords Atherosclerosis, Nutrition survey, Iron, Zinc, Copper, Serum, Obesity

Introduction

Atherosclerosis is one of the causes of cardiovascular and cerebrovascular diseases, which account for nearly 50% deaths in developed nations[1)]. Metabolic syndrome is known to contribute to the development and progression of atherosclerosis[2)]. Metabolic syndrome includes the symptoms of visceral obesity and insulin resistance, which are caused by several risk factors such as overeating, lack of exercise, and aging[3)]. As overconsumption of fat and high calorie diets lead to visceral obesity or insulin resistance, health promotion activities such as creating awareness about healthy diet or providing exercise education have been undertaken elsewhere. According to epidemiological and animal studies, in addition to high fat or high calorie diet, consumption of higher levels of trace minerals such as iron (Fe), zinc (Zn), and copper (Cu) are reported to have an effect on atherosclerosis. Some studies suggest that the serum Fe levels of patients with atherosclerosis are higher than those of healthy adults[4, 5)]. This suggests

* Address : 2-579-15, Mikajima, Tokorozawa-shi, Saitama, 359-1192, Japan
** Address : 1-1, Hiragagakuenndai, Inzai-shi, Chiba, 270-1695, Japan
*** Address : 1-23-1 Toyama, Shinjuku-ku, Tokyo, 62-8636, Japan
**** Address : 1100, Namegaya, Chigasaki-shi, Kanagawa, 253-8550, Japan

that higher levels of serum Fe may be a risk factor for development of atherosclerosis. Other studies suggest that lower levels of Zn can be observed in patients with atherosclerosis than in healthy subjects. This suggests that Zn has a potential role in preventing atherosclerosis[5-11]. Studies investigating the relationship between serum Cu levels and atherosclerosis have produced inconsistent results, i.e., both high[8, 9, 12] and low[6, 7, 11] levels of Cu have been detected in the serum of patients with atherosclerosis.

Many studies have compared the amounts of trace minerals deposited in the tissue or blood of patients with atherosclerosis and those of healthy subjects. However, there are few reports of a causal association between the consumption of dairy products with trace minerals and atherosclerosis. With this background, we decided to study the relationship between dietary intake of trace minerals and atherosclerosis in patients at different stages of atherosclerosis. Informed consent was provided, and a popular questionnaire, the brief-type self-administered diet history questionnaire (BDHQ)[13], was used to determine the effect of dietary consumption of trace minerals on the progression of atherosclerosis. BDHQ is a questionnaire that can calculate the percentage of adequate intake of each nutriments. "Adequate intake" is a standard which the Ministry of Health, Labour and Welfare of Japan established[14]. In addition, we measured the concentrations of trace minerals by collecting blood samples from the participants in order to evaluate the results obtained from the questionnaire.

Material and Methods

1. Nutrition survey

Subjects

A total of 264 potential atherosclerosis patients were enrolled. These patients were ambulatory or were in-patients at the Department of Cardiovascular Internal Medicine of the Urayasu hospital of Juntendo University. Informed consent for participation in the study was provided by the patients who were enrolled between March and August 2006. Among the 264 candidate patients, 80 patients were diagnosed with atherosclerosis and were assigned to the "atherosclerosis group." The remaining 184 individuals who were not diagnosed with atherosclerosis and the healthy subjects were assigned to the "non-atherosclerosis control group." The youngest subject in the atherosclerosis group was 38 years old, and in order to adjust the age between these 2 groups, the 2 youngest subjects under the age of 35 years in the non-atherosclerosis group were eliminated. A total of 182 subjects were assigned to the control group. Among the subjects assigned to the atherosclerosis group, 38 individuals who experienced infarctions in the small arteries were assigned to an advanced atherosclerosis (ADV) group. The other 42 individuals who did not experience infarctions in the small arteries were grouped into an initial atherosclerosis group (INI).

Nutrition items

The levels of trace minerals were calculated according to the results of the BDHQ questionnaire-based survey; the survey was based on the diet of the patients in the month preceding the measurements. A total of 27 items were surveyed including Fe, Zn, Cu, total energy (cal), protein (Pro), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), manganese (Mn), vitamins A, B_1, B_2, B_6, B_{12}, C, D, E, and K, niacin, folic acid (FA), pantothenic acid (PA), cholesterol (Cho), dietary fiber (DF), dietary salt, n-3 polyunsaturated fatty acid (n-3), and n-6 polyunsaturated fatty acid (n-6) to determine the basic dietary intake of each patient.

Statistical analysis

First, for all data, Student's t-tests were performed to determine the differences in the percentage of adequate intake between the atherosclerosis and control groups and between the INI and ADV groups. Second, all subjects were divided into 2 groups: one with a body mass index (BMI) over 25 (obese group) and the other with a BMI under 25 (non-obese group); the Student's t-test was performed for groups comprising only obese group and only non-obese group too. The statistical significance level was set at $p < 0.05$.

2. Measurement of trace minerals in serum

Subjects

Among the 80 subjects assigned to the atherosclerosis group for the questionnaire survey, 16 subjects provided consent for collection of blood samples. Thirty-five healthy subjects, whose ages ranged from 35 to 65 years, were recruited from the patients who had undergone routine health examinations at Juntendo University. Furthermore, the healthy control subjects were reclassified into 2 groups: BMI over 25 (obese group) and BMI under 25 (non-obese group).

Measurements

The trace minerals Zn, Cu, and Fe were measured by atomic absorption spectrometry. This measurement was performed by SRL, Inc. (Japan).

Statistical analysis

The difference in the average concentrations of each mineral between the obese and non-obese groups, the atherosclerosis and non-obese groups, and the atherosclerosis and healthy groups were analyzed by Student's t-test, us-

ing Excel 2007. The significance level was set at $p<0.05$.

3. Ethics consideration

This study conformed to the Declaration of Helsinki. After explaining this study to the participants, including the objective and ethical considerations followed by a Q&A session, informed consent was obtained from each of the participants. The Ethics Committee of Health and Sports Science of Juntendo University approved this study.

Results

1. Nutrition survey

Characteristics of participants

A total of 182 individuals (94 men and 88 women; mean age, 63.1 ± 9.08 years) were assigned to the non-atherosclerosis group. Of these, 71 individuals (35 men and 36 women) were assigned to the obese group, and 111 individuals (59 men and 52 women) were assigned to the non-obese group.

A total of 80 subjects (66 men and 14 women; average age, 65.4 ± 8.06 years) were assigned to the atherosclerosis group. Of these, 38 individuals (30 men and 8 women) were assigned to the obese group, and 42 individuals (36 men and 6 women) were assigned to the non-obese group. Among the 42 subjects in the INI group (32 men and 10 women; mean age, 66.0 ± 8.33 years), 20 individuals (13 men and 7 women) were assigned to the obese group and 22 (19 men and 3 women) were assigned to the non-obese group. Among the 38 subjects in the ADV group (34 men and 4 women; average age, 64.7 ± 8.96 years), 18 subjects (17 men and 1 woman) were assigned to the obese group, and 20 individuals (17 men and 3 women) were assigned to the non-obese group. The statistical analysis was carried out on both male and female.

Statistical analysis

In this study, the percentage of adequate intake of Fe among the members of the atherosclerosis group was significantly higher than the percentage of adequate intake of Fe in the non-atherosclerosis group including (Fig. 1; $p=0.020$). However, for other items, no significant differences were noted between these 2 groups. For the comparison between the ADV and INI groups, 4 measure-

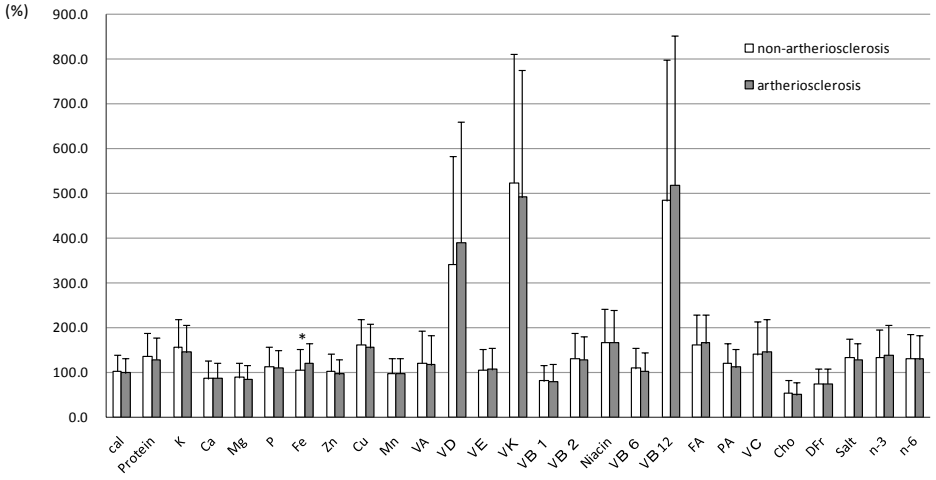

Fig. 1 Difference of intake percentage valuebetween arterioscrelosis group and non-Atherosclerosis group
*: $p<0.05$

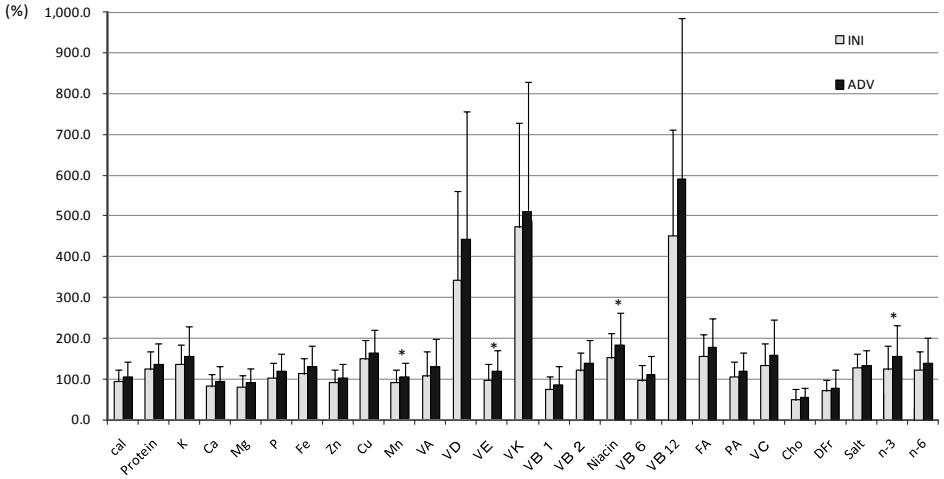

Fig. 2 Difference of intake percentage valuebetween INI and AVD
*: $p<0.05$

ments, including levels of Mn, niacin, VC, and n-3, were significantly higher in the ADV group than in the INI group (Fig. 2; $p=0.049$, $p=0.047$, $p=0.050$, and $p=0.035$, respectively). There were no significant differences for any of the other measurements between these 2 groups. However, the average percentages of adequate intake of all items in the ADV group were higher than those in the INI group.

In the non-obese group, the percentages of adequate intake of Fe in the atherosclerosis and ADV groups were significantly higher than in the non-atherosclerosis group (Fig. 3; $p=0.024$ and $p=0.019$, respectively). There were no significant differences among other measurements for the non-obese group.

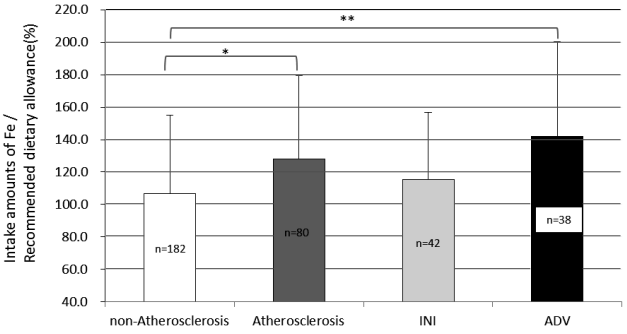

Fig. 3 The intake rate of Fe in non-obese
mean ± SD *: $p<0.05$, **: $p<0.01$

In the obese group, the percentage of adequate Zn intake in the non-atherosclerosis group was significantly higher than that in the atherosclerosis group, and was higher than that in the INI group (Fig. 4; $p=0.034$ and $p=0.049$, respectively). However, comparisons of the other measurements among the obese subjects did not indicate any significant differences.

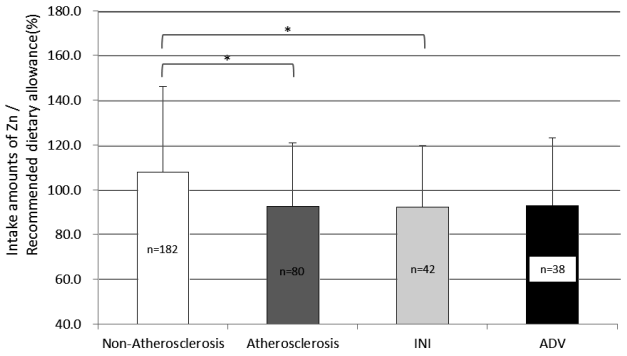

Fig. 4 The intake rate of Zn in the obese group
mean ± SD *: $p<0.05$

2. Traces of minerals in serum measurement

Characteristics of participants

Among the 35 healthy subjects, 12 subjects were assigned to the non-obese group, and 23 subjects, to the obese group. All the 16 subjects of the atherosclerosis group had BMI values higher than 25.

Results of the analysis

The serum Zn levels of the atherosclerosis group were significantly lower than that of obese subjects or healthy subjects ($p=0.024$ and $p=0.023$, respectively). However, the serum Zn levels of the non-obese and atherosclerosis groups did not differ significantly (Fig. 5). The serum Cu levels of the non-obese group were significantly higher than those of the obese and atherosclerosis groups ($p=0.008$ and $p=0.020$, respectively). However, no difference was observed between the serum Cu levels of the healthy and atherosclerosis groups (Fig. 6). The serum Fe level did not differ among any of the groups.

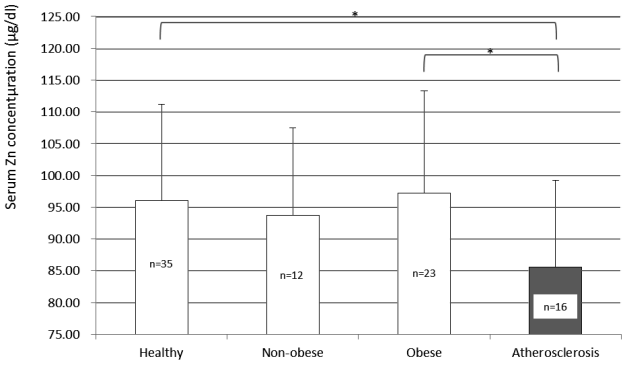

Fig. 5 Serum Zn concentration
mean ± SD *: $p<0.05$

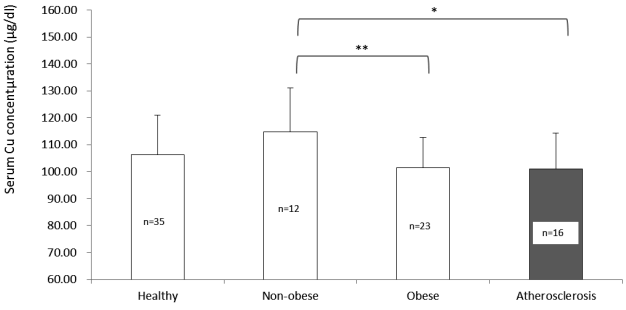

Fig. 6 Serum Cu concentration
mean ± SD *: $p<0.05$, **: $p<0.01$

Discussion

Atherosclerosis is caused by vessel wall inflammation, and metabolic syndrome induces atherosclerosis, induces atherosclerosis, which trigger the inflammatory process[1, 15, 16]. According to this theory, an increase in the active oxygen species in the blood or animal tissue may be one of the risk factors for the development of atherosclerosis. Knowles et al. reported an increase in atherosclerosis in mice that are hypertensive due to lack of endothelial nitric oxide synthase[17].

From the results of a nutrition survey, comparisons between the ADV and INI groups found that the average

values of all measurements of the ADV group exceeded those of the INI group. This may be due to overeating, which is one of the causes of metabolic syndrome of this group. Therefore, in order to eliminate the confounding effect of overeating, and to evaluate the effect of trace minerals on atherosclerosis, we additionally reclassified and assigned the subjects to the obese and non-obese groups.

Aging is said to be one of the risk factors for atherosclerosis. However, no age-related differences were observed among the groups. We, therefore, did not perform age-adjusted analysis.

Association between Fe and atherosclerosis

A previous study had showed that a high serum Fe concentration is a risk factor for coronary heart disease[4]. Another report has indicated that patients with coronary artery diseases have high serum Fe levels[5]. These studies suggest that high levels of Fe may be a risk factor for the development of atherosclerosis-related diseases. Stanley et al. identified a correlation between protein oxidation markers and serum Fe concentration in the artery tissues, and hypothesized that high concentrations of Fe is a risk factor for atherosclerosis[18].

In this study, significantly higher Fe intake was observed in the atherosclerosis group (including men and women) relative to the non-atherosclerosis group. Fe mineral consumption of the atherosclerosis and ADV groups of non-obese subjects including men and women was significantly higher than that of the non-atherosclerosis group. These findings showed concordance with previous study. As a strong correlation between the Fe consumption rate and atherosclerosis was observed in the non-obese group and not in the obese group, high intake of Fe was concluded to be an independent risk factor for atherosclerosis. Stanley et al. say that Fe in artery tissue (not in serum) may promote oxidation of vessel tissue which will lead atherosclerosis. That might be a reason we could not find out any correlation between the serum Fe levels and atherosclerosis in blood tests.

Association between Zn and atherosclerosis

Studies have reported an association between Zn and cardiovascular diseases. One study reported low serum Zn levels and Zn mineral consumption in coronary artery disease patients[5]. Another report indicated low serum Zn concentrations among coronary heart disease patients[19]. Another report indicated low urine Zn levels in cardiovascular disease patients, and suggested that increasing serum Zn levels can reduce the risks for coronary heart disease[6]. Additional reports identified low Zn levels in the heart tissue in individuals with advanced atherosclerosis[7], and stated that the serum Zn levels in the organs and artery tissues among congestive heart failure patients are lower than those in the organs and artery tissues of the healthy subjects[8-10]. An additional study suggests that the Zn concentration in hair and the Zn/Cu ratio are higher in the survivors of myocardial infarction than those in healthy subjects[11]. These studies show a clear association between low levels of Zn and atherosclerosis-related diseases. One animal study reported an increase in the serum Cho levels in animals fed without Zn supplementation[20]. Another report indicated that arteriosclerotic lesions can be ameliorated by Zn supplementation[21]. This suggests that Zn has an anti-atherosclerosis effect. However, other studies report that Zn supplements can lower high-density Cho when administered at doses of 50 mg and 75 mg[22] and 160 mg per day[23]. This suggests the possibility that oversupply of Zn may enhance the development of atherosclerosis.

In this study, Zn intake among the obese members of the non-atherosclerosis group was found to be higher than in the atherosclerosis group. With regard to serum measurement, Zn levels in the healthy and obese groups were higher than that in the atherosclerosis group. Reiterer et al. reported that in LDL receptor-deficient mice that were fed a cholesterol-rich zinc-deficient diet, a significant increase was observed in VLDL, LDL, and HDL. When Zn was added to this diet, the levels of VLDL, LDL, and HDL decreased. In contrast, in LDL-receptor-deficient mice, only VLDL and HDL levels were altered upon Zn supplementation[24].

Uza et al. also reported that significantly higher serum Zn levels were noted in individuals with hyperlipoproteinemia and in a non-atherosclerosis group relative to a control group or a hyperlipoproteinemia group with atherosclerosis[25]. Our study provided supporting evidence for these 2 studies, which showed a positive correlation between Zn levels and atherosclerosis in the obese group. Hennig et al. suggested that the antioxidative effect of Zn may inhibit the disruption of endothelial cell integrity and provide protection from inflammation[26, 27]. Zn may also exert antioxidative effects on fat cells.

Association between Cu levels and atherosclerosis

Several studies suggest an association between Cu and cardiovascular diseases. Alissa et al.[6] reported that low levels of Cu in the urine and an increase in the Cu/Zn ratio in the serum decrease the risk of coronary heart diseases. Another report has indicated that Cu levels in the heart tissues are lower in an ADV group than in an INI

group[7]. Cu levels in the arterial tissues of coronary heart disease patients were lower than in healthy volunteers[12]. Another study reports that increase in the serum Cho were observed among hypercholesterolemic rabbits fed a Cu-deficient diet, which led to anti-atherogenic effects[20]. In contrast, other reports are inconsistent with those described above, which indicate the presence of significantly higher levels of Cu in the serum or organs of patients with congestive heart failure relative to the healthy subjects[8, 9]. Cu levels in the ischemic cardiomyopathy patients were significantly higher than those in the healthy subjects[12]. This indicates that Cu is one of the risk factors for atherosclerosis. Thus, these studies are inconsistent with the former studies, which showed that Cu has an anti-atherogenesis effect.

In the present study, which includes both an atherosclerosis group and a healthy group, no significant differences were observed between these groups with respect to Cu levels for both the nutrition study and the blood tests.

Study limitations

In this study, the number of male and female subjects was similar, and there were 94 men and 88 women in the non-atherosclerosis group. However, in the atherosclerosis group, there were 66 male subjects and only 14 female subjects. The gender difference in the prevalence of atherosclerosis and other vascular diseases appears to be responsible for this difference[28].

Biological effects of trace minerals such as Zn are related to various factors[29]. One of these known factors is the gender of the patient. A same-gender-sample study may be more appropriate for a more precise evaluation. In case control studies, which include both genders, the male:female ratio is preferably equivalent. However, in this study, sample selection was limited, because only inpatients and outpatients who had provided informed consent could be enrolled in this study. This may have caused a biased male:female ratio between the case and the control groups. One of the objectives of a future study will be to enroll a sample size large enough to avoid gender bias.

Conclusion

We found that high intake of Fe is one of the risk factors for developing atherosclerosis. On the contrary, Zn can reduce the risk of development of atherosclerosis in obese individuals. However, no correlation was identified between Cu and the risk of development of atherosclerosis. This study contributes to the knowledge regarding the relationship between trace elements and atherosclerosis, and indicates correlations between the levels of Fe and Zn and the development of atherosclerosis on the basis of the results of a simple nutrition survey. Collection of more data in the future will be useful for nutrition education, which can be used as a strategy to prevent the progression of atherosclerosis.

Acknowledgments

This study was funded in part by the Health, Medical and Welfare Fund of Chiba Prefecture Serum Laboratories from 2005 to 2007.

References

1) Lusis A J (2000) Atherosclerosis. Nature 407: 233–241
2) Hioki C, Arai M (2007) Bofutsushosan use for obesity with IGT: search for scientific basis and development of effective therapy with Kampo medicine. J Trad Med 24(4): 115–127
3) Yokote K (2008) Risk factors for atherosclerosis. Japanese Soc Apheresis 27(1): 3–8 (in Japanese)
4) Danesh J, Appleby P (2009) Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 99: 852–854
5) Singh RB, Gupta UC, Mittal N, Niaz MA, Ghosh S, Rastogi V (1997) Epidemiologic study of trace elements and magnesium on risk of coronary artery disease in rural and urban Indian populations. J Am Coll Nutr 16: 62–67
6) Alissa EM, Bahjri SM, Ahmed WH, Al-Ama N, Ferns GA (2006) Trace element status in Saudi patients with established atherosclerosis. J Trace Elem Med Biol 20: 105–114
7) Aalbers ThG, Houtman JPW (1985) Relationships between trace elements and atherosclerosis. Sci Total Environ 43: 255–283
8) Atlihan F, Soylemezoglu T, Gokce A, Guvendik G, Satici O (1990) Zinc and copper in congestive heart failure. Turk J Pediatr 32(1): 33–38
9) Kosar F, Sahin I, Taskapan C, Kucukbay Z, Gullu H, Taskapan H (2006) Trace element status (Se, Zn, Cu) in heart failure. Anadolu Kardiyol Derg 6(3): 216–220
10) Vlad M, Caseanu E, Uza G, Petrescu M (1994) Concentration of copper, zinc, chromium, iron and nickel in the abdominal aorta of patients deceased with coronary heart disease. J Trace Elem Electrolytes Health Dis 8(2): 111–114
11) Białkowska M, Hoser A, Szostak WB, Dybczyński R, Sterliński S, Nowicka G, Majchrzak J, Kaczorowski J,

Danko B (1987) Hair zinc and copper concentration in survivors of myocardial infarction. Ann Nutr Metab 31(5): 327–332

12) Shokrzadeh M, Ghaemian A, Salehifar E, Aliakbari S, Soheil S, Saravi S, Ebrahimi P (2009) Serum zinc and copper levels in ischemic cardiomyopathy Biol Trace Elem Res 127: 116–123

13) Science and Technology Agency (2000) Standard tables of food composition in Japan 5th revised edn. Tokyo: Bureau of the Ministry of Finance (in Japanese)

14) Daiichi Shuppan Publishing Co. (2009) Dietary reference intake for Japanese, 2010.

15) Libby P (2002) Inflammation in atherosclerosis. Nature 420: 868–874

16) Viviane Z, Libby P (2009) Obesity, inflammation, and atherosclerosis. Nature Rev Cardiol 6: 399–409

17) Knowles JW, Reddick RL, Jennette JC, Shesely EG, Smithies O, Maeda N (2000) Enhanced atherosclerosis and kidney dysfunction in eNOS(–/–) apoE(–/–) mice are ameliorated by enalapril treatment. J Clin Invest 105: 451–458

18) Stanley N, Stadler N, Woods AA, Bannon P G, Davies M J (2006) Concentrations of iron correlate with the extent of protein, but not lipid, oxidation in advanced human atherosclerotic lesions. Free Radic Biol Med 40: 1636–1643

19) Kerkeni M, Added F, Ben Farhat M, Miled A, Trivin F, Maaroufi K (2008) Hyperhomocysteinaemia and parameters of antioxidative defence in Tunisian patients with coronary heart disease. Ann Clin Biochem 45(2): 193–198

20. Rashtchizadeh N, Ettehad S, DiSilvestro RA, Mahdavi R (2008) Antiatherogenic effects of zinc are associated with copper in iron-overloaded hypercholesterolemic rabbits. Nutrition Research 28: 98–105

21) Ren MQ, Rajendran R, Pan N, TK Huat BTK, Halliwell B, Watt F (2005) The protective role of iron chelation and zinc supplements in atherosclerosis induced in New Zealand white rabbits: A nuclear microscopy study. Nucl Instrum Meth B 231: 251–256

22) Black MR, Medeiros DM, Brunett E, Welke R (1988) Zinc supplements and serum lipids in young adult white males. Am J Clin Nutr 47: 970–975

23) Hooper PL, Visconti L, Garry PJ, Johnson GE (1980) Zinc lowers high density lipoprotein-cholesterol concentrations. J Am Med Assoc 244: 1960–1961

24) Reiterer G, MacDonald R, Browning JD, Morrow J, Matveev SV, Daugherty A, Smart E, Toborek M, Hennig B (2005) Zinc deficiency increases plasma lipids and atherosclerotic markers in LDL-receptor–deficient mice. J Nutr 135: 2114–2118

25) Uza G, Gabor S, Kovats A, Vlaicu R, Cucuianu M (1985) Serum zinc and copper in hyperlipoproteinemia. Biol Trace Element Res 8: 167–172

26) Hennig B, Toborek M, McClain CJ (1996) Antiatherogenic properties of zinc: Implications in endothelial cell metabolism. J Am Coll Nutr 15(4): 345–358

27) Hennig B, Meerarani P, Toborek M, McClain CJ (1999) Antioxidant-like properties of zinc in activated endothelial cells. J Am Coll Nutr 18(2): 152–158

28) Vitale C, Mendelsohn ME, Rosano GM (2009) Gender differences in the cardiovascular effect of sex hormones. Nature Rev Cardiol 6(8): 532–542

29) Mocchegiani E (2007) Zinc and ageing: third Zincage Conference. Immunity & Ageing 4(5): 2–7