# COMPOSITIONAL CHANGES OF THE CORONARY ARTERIES WITH AGING IN JAPANESE, THAI, AND MONKEYS

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*Abstract* : To examine whether there were differences between different races and between different species in regard to age-related changes of elements and the relationships among element contents in the coronary arteries, the authors investigated the left coronary arteries of Japanese, Thai, and monkeys by direct chemical analysis. After ordinary dissections at Nara Medical University and Chiang Mai University were finished, the left coronary arteries were resected from the subjects. The anterior interventricular branch was used as the left coronary artery. The anterior interventricular branches were also resected from rhesus and Japanese monkeys bred in the Primate Research Institute, Kyoto University. After ashing of the arteries with nitric acid and perchloric acid, the element content was determined by inductively coupled plasma-atomic emission spectrometry.

In the left coronary arteries of Japanese, the average content of Ca increased remarkably in subjects in their seventies and thereafter increased in the nineties. In the left coronary arteries of Thai, the average content of Ca increased progressively from the forties to the seventies.

The average content of Ca in the seventies was two times higher in the left coronary arteries of Thai than in those of Japanese. The accumulation of Ca in the left coronary arteries of Thai occurred at least 10 years earlier in comparison with Japanese. In contrast, the accumulation of Ca hardly occurred at all in the left coronary arteries of rhesus and Japanese monkeys at old age. Regarding the relationships among element contents in the left coronary arteries of Japanese and Thai, significant direct correlations were found among the contents of Ca, P, Mg, Zn, and Na, whereas significant inverse correlations were found between S and element contents, such as Ca, P, Mg, Zn, and Na.

**Key words** : coronary artery, atherosclerosis, calcium, phosphorus, aging

## INTRODUCTION

To elucidate compositional changes of the arteries with aging, the authors investigated age-related changes of elements in almost all of the arteries of human[1-20] and monkey[21-25]. It is established that with regard to the human arteries, there are the following two types of arteries: The first type is one where a significant accumulation of Ca and P occurs with aging, whereas the second type is one in which an accumulation of Ca and P hardly occurs with aging[6]. The thoracic and abdominal aortas, coronary, common carotid, splenic, common iliac, internal iliac, external iliac, uterine, internal pudendal, femoral, popliteal, posterior tibial, and dorsalis pedis arteries belonged to the first type, whereas the internal thoracic,

cerebral, pulmonary, axillary, brachial, radial, ulnar, and obturator arteries belonged to the second type.

In the present paper, the authors focus on the left coronary arteries, in which a high accumulation of Ca and P occurs with aging, and describe the differences between different races and between different species in regard to age-related changes of elements in the left coronary arteries and the relationships among element contents.

## MATERIALS AND METHODS

### *Sampling of Arteries*

Japanese cadavers were treated by injection of a mixture of 36% ethanol, 13% glycerin, 6% phenol, and 6% formalin through the femoral artery[26]. Thai cadavers were treated by injection of a mixture of 26% methanol, 14% glycerin, 3% phenol, 14% formalin, 0.34 M potassium nitrate, and 14 mM arsenic oxide through the femoral artery[27]. After ordinary dissections by medical students at Nara Medical University and Chiang Mai University were finished, the left coronary arteries were resected from the subjects.

Rhesus and Japanese monkeys were bred in the Primate Research Institute, Kyoto University. Monkeys were pretreated with an intramuscular injection of ketamine hydrochloride (10 mg/kg) and were deeply anesthetized by intravenous administration of pentobarbital sodium (nembutal, 30 mg/kg). They were then perfused through the left ventricle with 0.5 l of ice-cold saline containing 2 ml (2,300 units) of heparin sodium, followed by 1-2 l of ice-cold fixative consisting of 2% paraformaldehyde and 0.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4)[21]. After the perfusion, the left coronary arteries were resected from the monkeys.

The anterior interventricular branch of the left coronary artery was used as the left coronary artery in the present study.

### *Determination of Elements*

The samples of the coronary arteries were thoroughly washed with distilled water and were dried at 80°C for 16 h. After 1 ml concentrated nitric acid was added to the dried samples, the mixtures were heated at 100°C for 2 h. After the addition of 0.5 ml concentrated perchloric acid, they were heated at 100°C for an additional 2 h. The samples were adjusted to a volume of 10 ml by adding ultrapure water and were filtered through filter paper (No. 7; Toyo Roshi, Osaka, Japan). The resulting filtrates were analyzed with an inductively coupled plasma-atomic emission spectrometer (ICPS-7510; Shimadzu, Kyoto, Japan)[1]. The conditions were 1.2 kW of power from a radio-frequency generator, a plasma argon flow rate of 1.2 l/min, a cooling gas flow of 14 l/min, a carrier gas flow of 1.0 l/min, an entrance slit of 20 μm, an exit slit of 30 μm, a height of observation of 15 mm, and an integration time lapse of 5 s. The element amount was expressed on a dry-weight basis.

### *Statistical Analysis*

Statistical analyses were performed using the GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, CA, USA). Pearson's correlation was used to investigate the association between parameters. A p value of less than 0.05 was considered to be

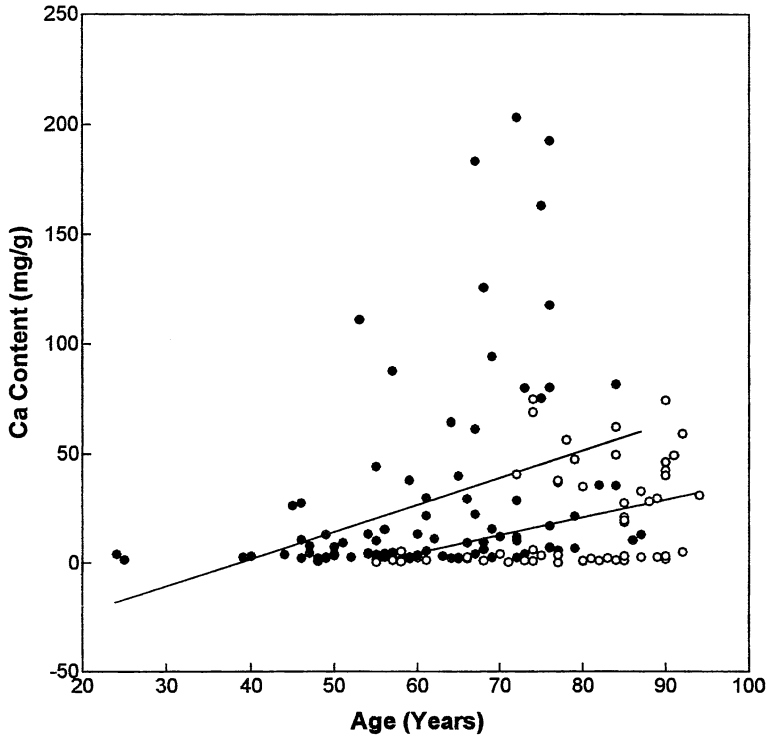


Fig. 1. Age-related changes of Ca in the left coronary arteries of Japanese (open circle) and Thai (closed circle).

statistically significant. Data were expressed as the mean ± standard deviation.

## RESULTS

### *Age-Related Changes of Ca in the Left Coronary Arteries of Japanese*

Japanese subjects consisted of 29 men and 23 women, ranging in age from 55 to 94 years (average age=79.4±10.1 years). Figure 1 shows age-related changes of the Ca content in the left coronary arteries of Japanese. The correlation coefficient was estimated to be 0.352 ($p=0.010$) between age and Ca content, indicating that there was a significant direct correlation between them. Figure 2 shows the average content of Ca in the left coronary arteries of Japanese and Thai by age group. The average content of Ca increased remarkably in the seventies in the left coronary arteries of Japanese and thereafter increased in the nineties.

### *Age-Related Changes of Ca in the Left Coronary Arteries of Thai*

Thai subjects consisted of 63 men and 28 women, ranging in age from 24 to 87 years (average age=61.4±12.5 years). Figure 1 also shows age-related changes of the Ca content in the left coronary arteries of Thai. The correlation coefficient was estimated to be 0.350 ($p=0.0007$) between age and Ca content in the left coronary arteries of Thai. An extremely significant direct correlation was found between age and Ca content in the left coronary

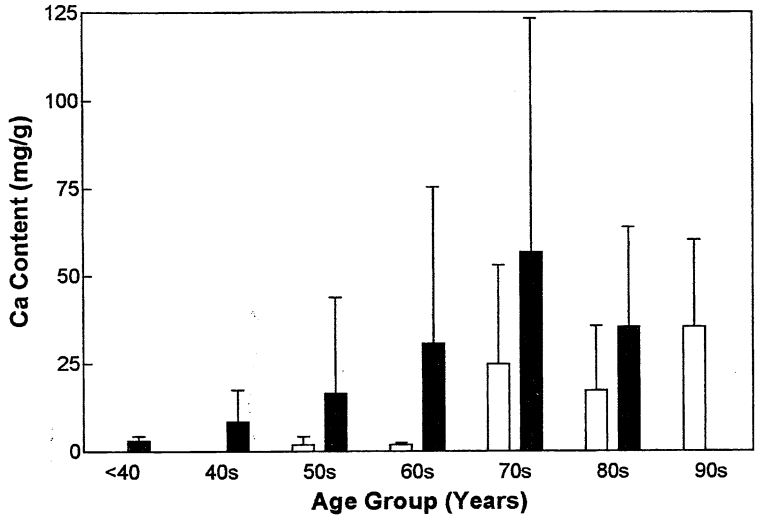


Fig. 2. Comparison in the average content of Ca in the left coronary arteries of Japanese (open bar) and Thai (shaded bar) by age group.

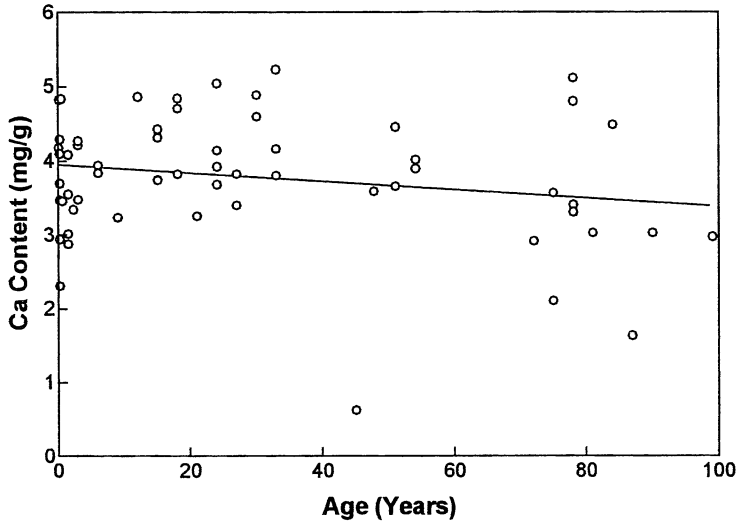


Fig. 3. Age-related changes of Ca in the left coronary arteries of rhesus and Japanese monkeys.

arteries of Thai. Figure 2 also shows the average content of Ca in the left coronary arteries of Thai by age group. The average content of Ca increased progressively from the forties to the seventies in the left coronary arteries of Thai.

The average content of Ca in the seventies was two times higher in the left coronary arteries of Thai than in those of Japanese (Fig. 2). The accumulation of Ca in the left coronary arteries of Thai occurred at least 10 years earlier in comparison with Japanese.

### *Age-Related Changes of Ca in the Left Coronary Arteries of Monkeys*

Monkey subjects consisted of 38 rhesus and 23 Japanese monkeys. Rhesus monkeys were composed of 16 males, 16 females, and 6 unknown, whereas Japanese monkeys were composed of 9 males and 14 females. The monkeys ranged in age from newborn to 33 years (average age=9.8±10.0 years). Rhesus and Japanese monkeys were treated as one monkey group in the present study.

Figure 3 shows age-related changes of the Ca content in the left coronary arteries of monkeys. All of the Ca content were less than 5.3 mg/g in the left coronary arteries of rhesus and Japanese monkeys. The correlation coefficient between age and Ca content was estimated to be −0.199 (p=0.125) in the left coronary arteries of monkeys. No significant correlation was found between age and Ca content in the left coronary arteries of monkeys.

Table 1 shows the average content of Ca in the left coronary arteries of monkeys below and over 20 years of age. It is generally said that the age of 20 years in rhesus and Japanese monkeys corresponds to the age of 60 years in human[21]. The average content of Ca did not increase in the left coronary arteries of monkeys at old age and reversely decreased by 13% in the left coronary arteries of monkeys more than 20 years of age in comparison with those below 20 years of age.

### *Comparison in the Ca Content of the Left Coronary Arteries Among Japanese, Thai, and Monkeys*

Table 2 shows the average content of Ca in the left coronary arteries of Japanese, Thai,

Table 1. Comparison in the Average Content of Ca in the Left Coronary Arteries of Monkeys Between Below and Over 20 Years of Age

| Age Group (Years) | Average Content of Ca (mg/g) |
|---|---|
| <20 | 3.88±0.79 |
| ≥20 | 3.37±1.03 |

Table 2. Comparison in the Average Content of Ca in the Left Coronary Arteries of Japanese, Thai, and Monkey Over 60 Years of Age

| Subject | Average Content of Ca (mg/g) |
|---|---|
| Japanese | 21.98±23.67 |
| Thai | 41.32±53.67 |
| Monkey* | 3.37±1.03 |

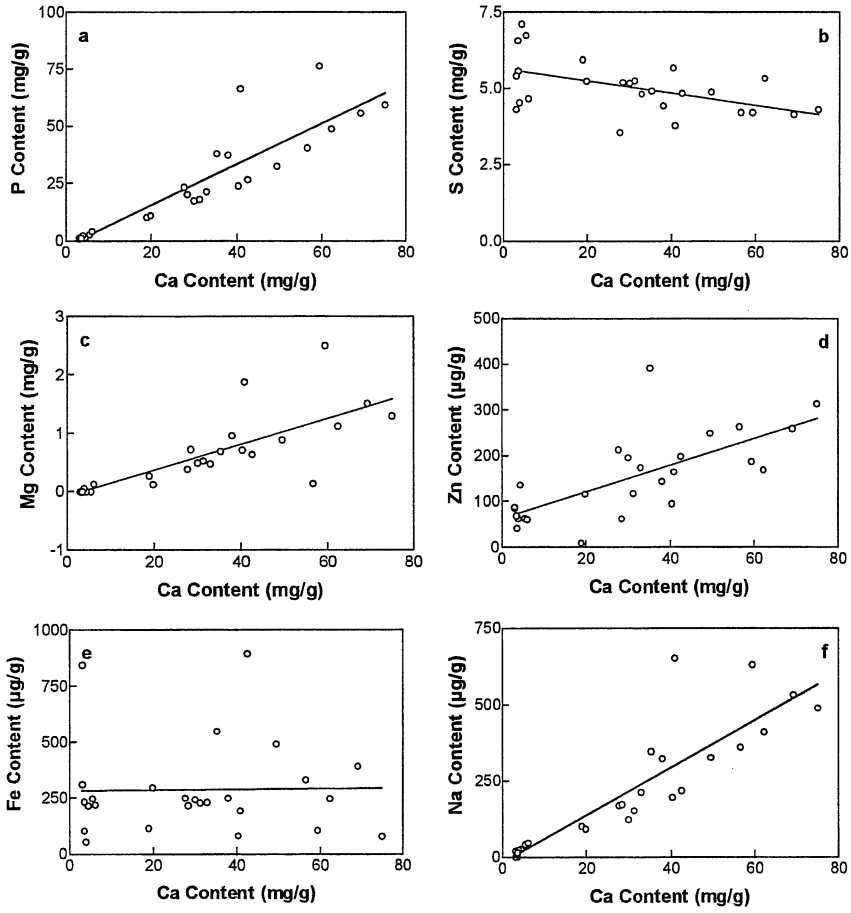


Fig. 4. Relationships between Ca and element contents, such as P (a), S (b), Mg (c), Zn (d), Fe (e), and Na (f), in the left coronary arteries of Japanese.

and monkeys more than 60 years of age(or the equivalent in monkeys). The average content of Ca in the left coronary arteries of Japanese and Thai corresponded to 6.5- and 12.3-fold the amount of that in the left coronary arteries of monkeys.

### *Relationships Among Elements in the Left Coronary Arteries*

Figure 4 shows the relationships between Ca and element contents, such as P (Fig. 4a), S (Fig. 4b), Mg (Fig. 4c), Zn (Fig. 4d), Fe (Fig. 4e), and Na (Fig. 4f), in the left coronary arteries of Japanese. The correlation coefficients between Ca and other element contents are shown in Table 3. Extremely significant direct correlations were found between Ca and element contents, such as P, Mg, Zn, and Na, in the left coronary arteries of Japanese, whereas a very significant inverse correlation was found between Ca and S contents. No significant correlation was found between Ca and Fe contents in the left coronary arteries of Japanese.

Table 3 summarizes the relationships among the element contents in the left coronary

Table 3. Relationships Among the Elements in the Left Coronary Arteries of Japanese

| | Correlation Coefficient and *p*-Value | | | | | |
|---|---|---|---|---|---|---|
| Element | P | S | Mg | Zn | Fe | Na |
| Ca | 0.899 | -0.507 | 0.772 | 0.712 | 0.017 | 0.880 |
| | (<0.0001) | (0.008) | (<0.0001) | (<0.0001) | (0.935) | (<0.0001) |
| P | | -0.589 | 0.931 | 0.657 | -0.059 | 0.992 |
| | | (0.002) | (<0.0001) | (0.0003) | (0.775) | (<0.0001) |
| S | | | -0.500 | -0.466 | -0.070 | -0.580 |
| | | | (0.009) | (0.016) | (0.733) | (0.002) |
| Mg | | | | 0.453 | -0.117 | 0.924 |
| | | | | (0.020) | (0.569) | (<0.0001) |
| Zn | | | | | 0.292 | 0.651 |
| | | | | | (0.147) | (0.0003) |
| Fe | | | | | | -0.048 |
| | | | | | | (0.817) |

Note : *p*-Values are indicated in parentheses.

Table 4. Relationships Among the Elements in the Left Coronary Arteries of Monkeys

| | Correlation Coefficient and *p*-Value | | | | | |
|---|---|---|---|---|---|---|
| Element | P | S | Mg | Zn | Fe | Na |
| Ca | 0.607 | 0.732 | 0.482 | 0.383 | 0.238 | -0.094 |
| | (<0.0001) | (<0.0001) | (<0.0001) | (0.002) | (0.065) | (0.471) |
| P | | 0.270 | 0.076 | 0.146 | 0.203 | 0.039 |
| | | (0.035) | (0.562) | (0.262) | (0.116) | (0.769) |
| S | | | 0.595 | 0.186 | 0.328 | -0.112 |
| | | | (<0.0001) | (0.152) | (0.010) | (0.389) |
| Mg | | | | 0.279 | 0.038 | -0.136 |
| | | | | (0.030) | (0.770) | (0.296) |
| Zn | | | | | -0.054 | -0.114 |
| | | | | | (0.679) | (0.382) |
| Fe | | | | | | -0.018 |
| | | | | | | (0.890) |

Note : *p*-Values are indicated in parentheses.

arteries of Japanese. Significant direct correlations were found among the contents of Ca, P, Mg, Zn, and Na in the left coronary arteries of Japanese, whereas significant inverse correlations were found between S and element contents, such as Ca, P, Mg, Zn, and Na. Namely, as Ca increased in the left coronary arteries of Japanese, P, Mg, Zn, and Na also increased in the arteries, whereas S decreased reversely in the arteries.

In the left coronary arteries of Thai, similar relationships among the element contents were obtained.

Table 4 summarizes the relationships among the elements in the left coronary arteries of monkeys. Extremely significant direct correlations were found between Ca and element contents, such as P, S, and Mg, and a very significant direct correlation was found between Ca and Zn contents. Likewise, an extremely significant direct correlation was found between S and Mg contents, and significant direct correlations were found between S and either P or Fe contents and between Mg and Zn contents. Significant direct correlations were found among the contents of Ca, P, S, and Mg, with one exception between P and Mg contents in the left coronary arteries of monkeys. Namely, as Ca increased in the left coronary arteries of monkeys, P, S, and Mg also increased in the arteries.

Regarding S, Zn, and Na, there were some differences in the relationships among the elements between the left coronary arteries of monkeys and Japanese or Thai.

## DISCUSSION

The present study revealed that a high accumulation of Ca occurred earlier in the left coronary arteries of Thai than in those of Japanese.

Matsuda-Inoguchi et al.[29] investigated the nutrient intakes of working women in Bangkok by the total food duplicate method and reported that although about a half of the women had insufficient energy intakes, lipid intakes (vegetable oil, butter, and lard) was excess in more than a half of the women. There is a possibility that the excess lipid intake is related to earlier and higher accumulation of Ca in the left coronary arteries of the Thai compared with that of the Japanese.

Regarding the human coronary artery, it is known that the accumulation of Ca and P does not occur uniformly in the left and right coronary arteries with aging, and a higher accumulation of Ca and P occurs in the proximal sites of both the coronary arteries compared with the distal sites[16]. The left and right coronary arteries ramify orthogonally from the ascending aorta and, in consequence, a mechanical (hemodynamic) stress occurs at the original sites of the coronary arteries[28]. It is presumed that this difference might result from a difference in mechanical stress between the proximal and distal sites of the coronary arteries. The heart beats regularly. It is unclear whether the heartbeat affects an accumulation of Ca, P, and Mg occurring in the coronary artery.

The present study revealed that an accumulation of Ca did not occur in the left coronary arteries of rhesus and Japanese monkeys at old age.

There are several pathological reports[30-38] on athero-sclerosis of the coronary artery in nonhuman primates. Prathap[35] and Prathap and Lau[36] examined wild adult male cynomolgus monkeys and reported that fatty streaks in the aortic arch and thoracic aorta in

90% of these animals were observed and rare fibrous plaques without complicated lesions were seen. Chawla et al.[37] reported that the incidence of naturally occurring atherosclerosis in the coronary artery was low in rhesus monkeys. Our finding of the left coronary arteries of monkeys was consistent with the finding by Chawla et al.[37].

The authors[21] examined age-related changes of the Ca content in various arteries of Japanese monkeys and found that the Ca content increased progressively in the axillary, brachial, radial, subclavian, common carotid, common iliac, and femoral arteries. It is generally said that the age of the Japanese monkey multiplied by three corresponds to the human age. In comparison between two groups of Japanese monkeys below and over 20 years of age, the Ca content increased to two times higher in the thoracic aorta, common, internal and external iliac, common carotid, and subclavian arteries more than 20 years of age in comparison with those below 20 years of age. In subjects more than 20 years of age, the average content of Ca in the left coronary arteries of rhesus and Japanese monkeys was similar to those of the radial and ulnar arteries[21], in which the average content of Ca was the lowest, within the limits of the arteries of Japanese monkeys analyzed by us.

Kramsch and Hollander[38] were the first to describe diet-induced atherosclerosis of wild adult, male cynomolgus monkeys. Clarkson et al.[39,40] reported that atherosclerosis was induced in rhesus monkeys by feeding them with a cholesterol-containing diet and atherosclerotic lesions were more severe in the coronary arteries and at the carotid bifurcation. However, it is ambiguous whether the induction of atherosclerosis occurs in the coronary arteries of Japanese monkeys by feeding them with a cholesterol-containing diet.

It should be noted that atherosclerosis occurs frequently in the left coronary arteries of Japanese and Thai, but it hardly occurs naturally in the left coronary arteries of rhesus and Japanese monkeys.

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