

原著

## The Effect of Non-substituting Dietary Intake of Almonds on the Serum Lipid Profile for Healthy Younger Women

Tatsuo Kai<sup>\*</sup> Kiyohisa Motoki<sup>\*\*</sup> Kazue Tatsuguchi<sup>\*\*\*</sup>

### < Abstract >

Previous studies undertaken in the U.S. demonstrated that almonds have beneficial effect to control serum cholesterol level in such a manner that low-density lipoprotein cholesterol (LDL-C) level decreases while high-density lipoprotein cholesterol (HDL-C) level is maintained. This study was conducted to examine the effect of almonds on serum cholesterol profile for Japanese people who have different eating culture from the U.S. people. The experimental conditions performed in this study were carefully designed to reflect the usual eating habit of nuts in Japan. The significant differences of experimental conditions in this study from the previous works are summarized as following four features: (1) lower amount of almonds was fed (20 g in a day, while 60-100 g of almonds were fed in foreign studies), (2) commercial cooked and seasoned almonds were fed (while raw almonds were fed in previous studies), (3) usual diet was not substituted with almonds intake since almonds are taken as a snack food which is additional food over usual diet in Japan, (4) non-blinded test was performed to take the psychological effect into account. As a result of this study, it was observed that LDL-C level decreased while HDL-C level was preserved as same as shown in previous studies. An interesting finding is that almond intake seems to elevate the level of HDL-C when it is in lower level of normal range. This may indicate that almonds may have the buffering effect to control the serum cholesterol to the standard level, that is to say, it reduces the comparatively higher level of cholesterol and also it elevates the comparatively lower level of cholesterol. This buffering effect was possibly observed also for HDL-C / LDL-C ratio. On the other hand, serum triglyceride increased remarkably upon almonds intake. This increase may be due to the additional intake of triglyceride contained in almonds to the usual diet, but the exact reason is unclear. In the previous studies done in the U.S., although dietary fat intake went over the standard level due to almonds intake where the daily energy intake was adjusted to standard level, the serum triglyceride level showed no significant difference during the test period. Therefore, the reason for the remarkable increase in serum triglyceride by almonds intake may be possibly explained from the standpoint of the cooking method of almonds or the difference in lipid metabolism of people, or the difference in eating habit between Japanese and American that may affect lipid metabolism.

Key words: Almonds, Serum cholesterol, Serum triglycerides

### INTRODUCTION

Physiological advantage of nuts consumption is initially investigated on the function for the lowering risk of coronary heart disease (CHD) with five large

epidemiologic studies<sup>1-5)</sup> which had been undertaken between 1977 and 1994 in the U.S. These cohort studies involved totally 195,215 subjects and have demonstrated the clear inverse relationship between nut consumption and the risk of CHD. According to

\* Associate Professor, Department of Nutritional Sciences, Faculty of Health and Welfare, Seinan Jo Gakuin University

\*\* Director, Motoki Clinic

\*\*\* Professor, Department of Nutritional Sciences, Faculty of Health and Welfare, Seinan Jo Gakuin University

the results of these prospective researches, traditional perception, that is, nuts are an unhealthy food because of their high fat content causing higher blood cholesterol and risk of CHD, is scientifically denied. The beneficial effects of nuts consumption underscore the importance of distinguishing different types of fat<sup>6)</sup> as one of the possible causative factors. Most fats in nuts are mono- and polyunsaturated fats that lower low-density lipoprotein cholesterol (LDL-C) level. This significant finding initiated the further studies to investigate the other physiological function of nut intake and some efforts are resulting in that nuts are effective to avoid cancer.<sup>7)</sup> The anti-carcinogenic effect of nuts is presumed to be derived from the anti-oxidant Vitamin E<sup>8)</sup> and the dietary fiber<sup>9)</sup> contained in most of nuts.

The almond is the most consumed nuts in Japan and also in the world. But the reason why we focused on the almond in this study among variable kinds of nuts is its distinguished nutritional properties as described below. One of the reasons is its remarkably high content of alpha-tocopherol as shown in Figure 1 which possesses the strongest anti-oxidative power among the Vitamin E family.

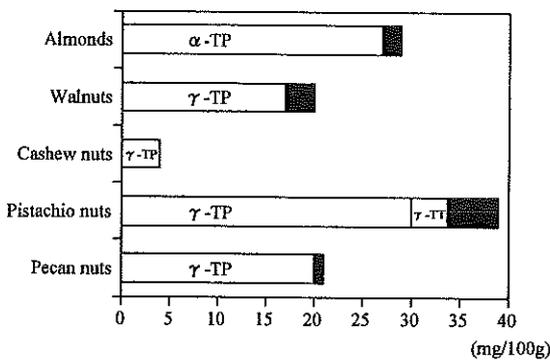


Figure 1 Comparison of Vitamin E contents of nuts, data from Papas<sup>10)</sup>

□ Major component TP: Tocopherols  
 ■ Others TT: Tocotrienols

In addition unknown endogenous anti-oxidant besides Vitamin E is predicted to exist in almonds as a strong anti-carcinogen.<sup>11)</sup> Modification of LDL-C by oxidation creates a LDL particle that is more rapidly taken up into macrophages via specific receptors, which do not recognize unoxidized LDL-C. Since in a crossover randomized dietary trial, the relationship between oxidation and the ratio of oleic acid to linoleic acid of the LDL particle is inverse<sup>12)</sup>, it may be possible that almonds are more beneficial in reducing the risk of CHD although almonds are regarded as the hypocholesterolemic agent.

The fatty acid composition, rather than the total amount of fat consumed, is the greater predictor of cholesterolemic effects. Most commonly eaten nuts, including almonds, are high in monounsaturated fatty acid (MUFA) (oleic acid), while walnuts and pine nuts are exceptionally high in polyunsaturated fatty acid (PUFA) (linoleic and linolenic acid) as shown in Figure 2. It is well known<sup>13,14)</sup> that MUFA lowers LDL-C and TC (total cholesterol) levels, without significantly altering HDL-C (high-density-lipoprotein cholesterol) levels. The physiological function of almonds on lowering the serum LDL-C level preserving HDL-C level was demonstrated by epidemiologically well designed study<sup>15-17)</sup> and the beneficial effect is considered to be due to the high MUFA content.

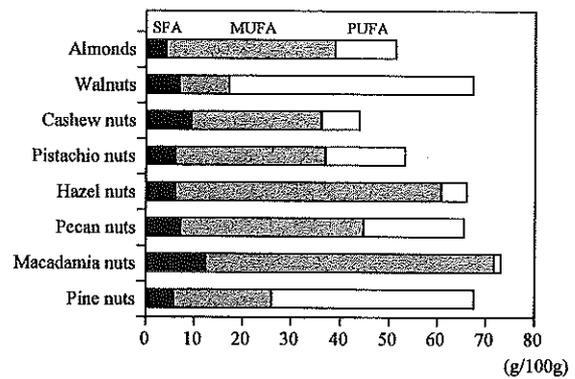


Figure 2 Comparison of fatty acids composition of nuts, data from "Standard tables of food composition in Japan, 5th ed"

□ SFA: Saturated Fatty Acid  
 ▨ MUFA: Mono Unsaturated Fatty Acid  
 ■ PUFA: Poly Unsaturated Fatty Acid

The purpose of this study is to confirm whether almond intake has beneficial effect on Japanese people who has its own tradition in the food culture. We focused on younger generation and women in normal health condition to avoid the influence of age, gender and disease for proper understanding of the experimental results. Also we very cared that this experiment should be conducted under the similar condition to our usual eating habit of nuts. We designed the experimental condition such that commercial almonds snack is taken as non-blinded, additionally over the usual meal without substituting the total energy intake. Furthermore, all the previous works undertaken in the U.S. do not reflect the Japanese eating standard of nuts from the point of the daily dose and the cooking condition. The biggest difference is the amount of the nut daily intake. Japanese annual consumption of nuts is about 300 g per person, while the four times larger amount is consumed in the U.S.<sup>18)</sup> In the previous epidemiological studies, the daily dose was mostly from 50 g to 100 g per person, but in Japan suitable daily intake of nuts is considered to be 20 g per day (personal information from Blue Diamond Almond Growers Japan), thus most nut snack food of individual package is designed on 20 g based in Japanese market. Therefore, we took 20 g daily dose in this study which is a fairly lower dose compared with the previous studies. The second important problem is that nuts are fed as uncooked raw form in the former studies. Japanese people eat nuts as roasted and seasoned style. So we used commercial snack almonds that are fried and seasoned with salts, and packed on 20 g individually. The last care was paid as that subjects did not change the usual diet style and they took the almonds once a day whenever they like to eat as a snack, and almonds are additionally taken over the usual meal.

## METHODS

### Subjects

Women responded to the advertisement in Motoki clinic were screened by two investigators. Persons who ate nuts frequently, had known food allergies, had a history of hypertension or atherosclerotic or

metabolic disease, were taking any medication on a regular basis, or were considered unable to comply with the study protocol were excluded.

Of the 27 subjects selected, 3 withdrew from the study during the trial, because they showed slight symptom of atopic dermatitis during the first experimental week, thus 24 completed the experiment. The 24 women were between 18 and 26 years old (mean,  $20 \pm 3$ ), weighed between 41 and 72 kg (mean,  $51 \pm 8$ ), and had body-mass indexes (BMI, defined as the weight in kilograms divided by the square of the height in meters) ranging from 17.2 to 28.9 (mean,  $21.0 \pm 2.7$ ), body fat percentage ranging from 17.9 to 36.5 % (mean,  $25.0 \pm 4.5$ ). Their fasting levels of serum total cholesterol before the experiment began ranged from 126 to 242 mg/dl (mean,  $184 \pm 26$ ). Triglyceride levels before the experiment began ranged from 25 to 107 mg/dl (mean,  $67 \pm 22$ ).

### Experimental Design

An uncontrolled non-blinded design was used without calorie substitution. All subjects consumed the non-blinded almond snack of 20 g package everyday at one time whenever they like to take. No reference diet is prepared. Experimental period was four weeks. All the measurements were conducted at a day before the experiment starts (noted as day 0 in this issue), and at a day after the experiment finished (noted as 4 weeks in this issue).

The study protocol was prepared under the direction of the Medical Ethics Committee of The Japanese Society of Nutrition and Food Science (5-16-9, Honkomagome, Bunkyo-Ku, Tokyo, 113-8622 Japan), and all subjects gave informed consent.

### Diets

The subjects received the 28 packs of commercial almonds snack at the day 0 of the experiment. Each package contained 20 g of almond snack. The almond snack used in this study is the product of Blue Diamond Almond Growers Japan (1-22-15, Toranomom, Minatoku, Tokyo, 105-0001 Japan).

That commercial brand name is Kaori No.1 Almonds which is seasoned almonds with salt after oil-roasted.

The nutritional values per 20 g pack of the almond snack are as follows: energy; 124kcal, protein; 4.2 g, fat; 10.9 g, carbohydrate; 3.4 g, dietary fiber; 2.7 g, sodium; 72 mg, calcium; 64 mg, phosphate; 95 mg, iron; 0.63 mg, zinc; 0.40 mg, magnesium; 64 mg, Vitamin B<sub>1</sub>; 0.02 mg, Vitamin B<sub>2</sub>; 0.23 mg, Vitamin E; 5.7 mg.

The subjects were required to maintain their activities and lifestyle habits including eating habits and record in diaries the time of almonds intake, any signs of illness, medication used, and any deviation from their experimental diets. Inspection of their diaries every week revealed that none deviated from the protocol.

### Measurements

Blood was drawn at Motoki clinic between 14 and 15 o'clock. Serum and lipoprotein sub-fractions were analyzed to determine concentrations of cholesterol<sup>19)</sup> and triglyceride<sup>20)</sup> with the use of enzyme reagent kits (Ciba-Corning Diagnostics, Oberlin, Ohio) and the automated analyzer (Ciba-Corning) at the Medical Research Center of RINTEC Co., Ltd. (13-1, shin-machi, shimosone,

kokuraminamiku, 800-0221 Japan). HDL-C was measured directly after the precipitation of other lipoproteins by dextran sulfate<sup>21)</sup>. LDL-C was calculated by subtraction with the Friedewald algorithm<sup>22)</sup>.

The instrumental measurements for body weight, BMI and body fat percentage were conducted just before drawing blood. Body weight was measured without shoes or heavy clothing was recorded. BMI and body fat percentage was measured with Body Fat Percentage Analyzer OMRON HBF-306-A.

### Statistical Analysis

Descriptive values are expressed as means  $\pm$  SD. Statistical analysis included two-tailed t-test for the comparison of changes in outcome variables in response to diet period with methods described by Fleiss<sup>23)</sup>.

## RESULTS

Figure 3 illustrates changes in serum cholesterol and triglyceride (TG) at day 0 and 4 weeks. Figure 3, A shows the mean serum concentration of TG,

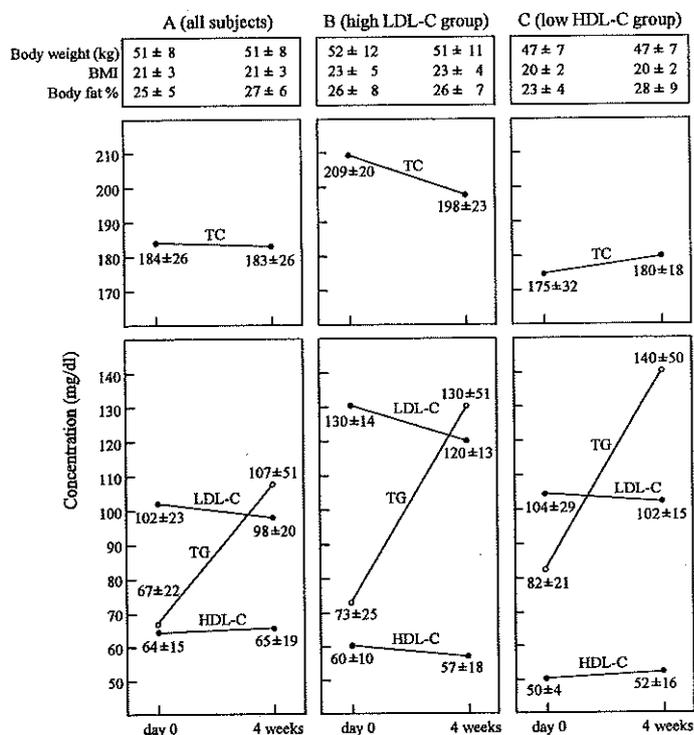


Figure 3 Mean serum concentrations of cholesterol and triglyceride

TG (○): Triglyceride  
 TC (●): Total cholesterol  
 LDL-C (●): low-density lipoprotein cholesterol  
 HDL-C (●): high-density lipoprotein cholesterol

TC, HDL-C and LDL-C, respectively for all the subjects. Figure 3, B indicates the mean data from high LDL-C group who has initial LDL-C level (that is the value of day 0) of more than 112 mg/dl. The value of 112 mg/dl is the 80% of the normal LDL-C highest limit (=140 mg/dl). Therefore, this group is considered to have the higher risk of excessive LDL-C. 7 subjects were adopted in this group. Figure 3, C indicates the mean data from low HDL-C group who has initial HDL-C level less than 56 mg/dl. The value of 56 mg/dl is the number that 20% (=16) of normal ranged value (120-40=80) was added to the lowest limit of HDL-C (=40). Thus, this group is considered to have the higher risk of lacking in HDL-C. 7 subjects were adopted in this group.

#### All subjects

LDL-C level decreased by 3.9% from  $102 \pm 23$  mg/dl to  $98 \pm 20$  mg/dl ( $p < 0.05$ ), while TC and HDL-C level showed no change. TG level increased significantly by 59.7% from  $67 \pm 22$  mg/dl to  $107 \pm 51$  mg/dl ( $p < 0.001$ ). Although there were no significant changes in body weight and BMI over the study period, body fat percentage increased slightly ( $p < 0.05$ ) from  $25 \pm 5$  to  $27 \pm 6$ .

#### High LDL-C group

Cholesterol level decreased significantly in this group. LDL-C level decreased by 7.7% from  $130 \pm 14$  mg/dl to  $120 \pm 13$  mg/dl ( $p < 0.001$ ). TC level decreased by 5.3% from  $209 \pm 20$  mg/dl to  $198 \pm 23$  mg/dl ( $p < 0.1$ ). HDL-C level decreased by 5.0% from  $60 \pm 10$  mg/dl to  $57 \pm 18$  mg/dl ( $p < 0.1$ ). TG level showed remarkable increase by 78.1% from  $73 \pm 25$  mg/dl to  $130 \pm 51$  mg/dl ( $p < 0.001$ ). There were no significant changes in body weight, BMI and body fat percentage over the study period.

#### Low HDL-C group

LDL-C level decreased slightly by 1.9% from  $104 \pm 29$  mg/dl to  $102 \pm 15$  mg/dl, but this decrease is not statistically significant. TC level slightly increased by 2.9% from  $175 \pm 32$  mg/dl to  $180 \pm 18$  mg/dl ( $p < 0.5$ ). HDL-C level showed very slight increase by 4.0% from  $50 \pm 4$  mg/dl to  $52 \pm 16$  mg/dl

( $p < 0.5$ ). TG level showed remarkable increase by 70.7% from  $82 \pm 21$  mg/dl to  $140 \pm 50$  mg/dl ( $p < 0.001$ ). There were no significant changes in body weight and BMI, while body fat percentage showed significant increase by 21.7% from  $23 \pm 4\%$  to  $28 \pm 9\%$  ( $p < 0.001$ ) over the study period.

## DISCUSSION

There are many studies undertaken previously<sup>6,24)</sup> showing the effect of nuts on reducing abnormal level of serum LDL-C and the risk on CHD. Eminent works focused on almonds were conducted by Spiller *et al.*<sup>16,17)</sup> in the U.S. where the world best almonds grows in amount. Figure 3 shows such a same beneficial effect of almonds intake on serum cholesterol with the previous studies<sup>16,17)</sup> that serum LDL-C level reduced significantly while serum HDL-C level showed no significant change except low HDL-C group. In this study, realistic amount of almonds as 20 g per day was dosed. That is fairly lower amount compared with the experiments done by Spiller *et al.*<sup>16,17)</sup>. It is notable to see almonds intake shows good effect on serum cholesterol even when the dose is fairly reduced. This beneficial effect is considered in general to be due to the following three constituents contained in almonds: MUFA, alpha-tocopherol and dietary fiber. These three constituents are found in almonds with comparatively high level among variable food sources. The role of these constituents on serum cholesterol is already well known<sup>12,24-28)</sup>. A possible explanation is also employed by the presence of lipid-altering phytochemicals such as plant sterols<sup>29)</sup> and saponins<sup>30)</sup> that are minor constituents of almonds.

Interesting phenomenon was observed in high LDL-C group and low HDL-C group. High level of serum TC and LDL-C observed in high LDL-C group decreased significantly during experimental period as shown in Figure 3, B. On the other hand, in low HDL-C group as shown in Figure 3, C, comparatively lower level of HDL-C and TC both increased, where the statistical significance of the increase in HDL-C is very low ( $p < 0.5$ ). Here, LDL-C which is quite in normal level decreased. The ratio of HDL-C to

LDL-C seems to be improved by the effect of almond intake. It might be possible to say that almonds may act as the buffering agent to control the serum cholesterol to adjust their present levels to ideal level and to improve the ratio of HDL-C/ LDL-C. We call this Chinese kampo-like effect of almonds as the buffering effect on serum cholesterol. Further study is expected to demonstrate and clarify this buffering effect of almonds.

Serum TG increased tremendously in all three groups by the almond intake. The increasing ratio is much higher in the order of low HDL-C group (78.1%), high LDL-C group (70.7%) and all subjects group (67.2%), respectively. This results disagrees with the experiment conducted by Spiller *et al.*<sup>17)</sup> in which 100 g of raw almonds diet was dosed for 4 weeks as same as our study. In their results, serum TG reduced from 120 mg/dl to 110 mg/dl. They involved 18 subjects of men and women whose mean age was 53±10 years old, mean body weight was 66±13 kg, mean TC at day 0 was 251±30 mg/dl, mean LDL-C at day 0 was 166±26 mg/dl and mean HDL-C at day 0 was 59±16 mg/dl. There were no reports that showed such a drastic increase of serum TG when nuts were fed. This disagreement is difficult to be explained by the total fat content of the experimental diet, since the daily diet that contained 100 g of raw almonds had 34-39g fat content<sup>17)</sup>, whereas our snack almond of 20 g pack contained much lower fat content of 10.9 g. This disagreement may be due to the cooking method of almonds or the difference in lipid metabolism of Japanese younger women, or the difference in eating habit that may affect lipid metabolism. The exact reason remained unknown.

Though there were no significant changes both in body weight and BMI over the study period, body fat percentage showed significant increase in low HDL-C group that seemed to reflect the result of all subject group. The result may concern the fact that the mean of body weight and BMI of low HDL-C group was the lowest among the three groups, but the explanation in detail is difficult to do.

## CONCLUSIONS

This study supports in a hand the benefits of almonds to control the serum cholesterol even under the usual eating habit of nuts in Japan. Interesting finding is that almonds intake seems to control both higher and lower serum cholesterol level into the ideal level for TC, LDL-C and HDL-C, and also seems to balance the HDL-C/ LDL-C ratio. This buffering effect introduced by almonds may be due to interactive or additive effects of the numerous bioactive constituents existing in almonds. It is the significant problem that serum triglyceride increased tremendously which has not been seen in the previous studies. This disagreement may be due to the cooking method of almonds or the difference in lipid metabolism of Japanese people, or the difference in eating habit that may affect lipid metabolism. The exact reason has to be studied to know the proper manner of almond intake as a snack food that is one of the important sources of various nutritional components.

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## 健康な若い女性におけるアーモンド摂取が血清脂質に及ぼす影響

甲斐 達男 元木 清久 竜口 和恵

### <要 旨>

米国で実施されてきた数多くの研究成果によって、アーモンド摂取が血清中の HDL- コレステロール濃度に影響を与えないまま LDL- コレステロール濃度を下げるという効果が証明されている。本研究は、日本人においてもこのような効果が期待できるのかを検証するために実施した。但し本実験では、日本におけるナッツの食習慣を十分に反映するよう十分に考慮して実験条件を設定した。つまり、米国で実施された研究とは、次の 4 項目について実験条件が異なる。(1) 米国では 1 日の投与量を 60~100 g としているが、本実験では、現実的な摂取量である 20g を投与した、(2) 米国での実験では生のアーモンドを用いているが、本実験では、加熱調理後に味付けした市販の製品を用いた、(3) 日本ではアーモンドをスナックとして常食に上乘せする形で摂取するため、アーモンドによる摂取カロリーを常食から差し引かなかった、(4) 心理的な影響も含めて結果を検討するために盲検を入れなかった。結果として、米国での研究結果と同様に、HDL- コレステロールに影響しないまま LDL- コレステロール濃度を低減させる効果が確認された。さらに興味深い結果として、正常値の範囲内でも低めの HDL- コレステロール濃度を示す場合、やや濃度を引き上げる効果が示唆された。これが事実であれば、アーモンドの血清コレステロールに対する緩衝効果とも言うべき作用であり、正常値の範囲内にあっても、高めや低めのコレステロール濃度を理想的なレベルに制御しようとする効果があることになる。この緩衝作用については、HDL / LDL 比についても同様の効果が示唆された。一方、血清中の中性脂肪濃度がアーモンド摂取によって急激に増加した。この増加は、アーモンド摂取による中性脂肪摂取量の増加によるものと推測されるが、明確な原因は明らかではない。米国の研究では 1 日の摂取カロリーを標準量に調整してはいるものの、脂質摂取量は標準摂取量を上回っており、そこでの結果では、アーモンド摂取が血清中の中性脂肪濃度に影響を与えていない。今回の研究において血清中性脂肪が急増した原因は、アーモンドの調理方法や、日本人の脂質代謝形態の問題、もしくは食習慣の違いが脂質代謝に影響を与えたことなどが考え得る。