

Fatty acid profiles among individuals in Nagano, Japan: potential effects on health

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Summary. *Aim:* Increased life expectancy has long been linked to a healthy diet. Although individuals from the Nagano Prefecture have the longest life expectancy in Japan, the characteristics of the Nagano diet are unclear. While marine-derived n-3 free fatty acids (FFA) have traditionally been thought to decrease mortality, Nagano has no seashore; hence, we hypothesized that non-marine dietary constituents in the diet may contribute to the decreased death rate. We thus aimed to characterize the Nagano diet with respect to serum triglycerides, FFA, cholesterol, and lipoprotein concentrations. *Methods:* This was a cross-sectional study, where 106 Nakano city residents completed a health and lifestyle questionnaire. Additionally, we calculated body mass index and analyzed non-fasting blood samples for triglycerides, FFA, total cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations. *Results:* Participants had significantly higher oleic acid and alpha-linolenic acid concentrations, and significantly lower docosahexaenoic and linoleic acid concentrations than Japanese reference values. *Conclusions:* Our results suggest that the Nagano diet may alter FFA composition in Nagano residents, potentially contributing to better maintenance of health.

Key words: diet, oleic acid, omega-3 fatty acids, omega-6 fatty acids

Introduction

According to the World Health Organization, Japan ranks among the top 10 countries in overall life expectancy worldwide. In Japan, life expectancy is highest in the Nagano Prefecture for both men and women (1, 2). While the percentages of individuals that suffer from any fatal disease are 0.54% (men) and 0.27% (women) in all of Japan, the all-cause mortality rates in Nagano Prefecture are the lowest at 0.48% and 0.25% for men and women, respectively. Lower mortality rates have been attributed to a proper diet (3), along with factors such as exercise and a healthy lifestyle. Although several studies have focused on the Japanese diet, the beneficial effects of the Nagano diet, in particular, remain unclear.

Recent studies have focused on the overall quality of the diet, taking into account the complexity of different foods and the interaction between various components of the diet. To this end, several guidelines have characterized a healthy diet and examined the relationship between dietary factors and disease risk (4–6). The Japanese Ministry of Health, Labor and Welfare, along with the Ministry of Agriculture, Forestry and Fisheries, has developed a guide for a balanced daily diet, taking into account differing food groups and portion sizes for each food group (7). Adherence to these proper dietary guidelines has also been associated with decreased overall mortality (8), predominantly from cardiovascular disease (9). Interestingly, Nagano's population exhibits the lowest rates of cardiovascular disease and cancer-related mortality in Japan (1).

Given the increased life expectancy in the Nagano Prefecture, we hypothesized that a Nagano-style diet may play an important role. Geographically, the Nagano Prefecture is surrounded by high mountains and isolated from the seashore. Therefore, the Nagano diet consists mainly of plant-derived foods, with lower seafood consumption than other parts of Japan (Supplementary Figure 1). Thus, marking Nagano as the highest consumer of vegetables among the 47 prefectures (10). Hence, we hypothesized that non-marine dietary components of the Nagano diet are associated with good health. Indeed, previous studies have shown that a diet with higher consumption of fruits, vegetables, nuts, and long grains is associated with decreased mortality (11). We chose to focus on the middle-aged population of Nagano given previous observations made by the Framingham Heart Study, which demonstrated that risk factor measurements conducted between 40 and 60 years of age can predict survival later in life. Lower levels of key cardiovascular risk factors in middle age predict overall survival and major morbidity-free survival up to the age of 85 years (12).

Dietary patterns have been shown to influence the composition of triglycerides, cholesterol, and free fatty acids (FFA) in patient serum (13). Among the FFAs, n-3, n-6, and n-9 FFAs are of particular nutritional interest for potential beneficial effects (14, 15). The n-3 and n-6 polyunsaturated fatty acids have been shown to have beneficial effects against prostate and breast cancer (16). The n-3 polyunsaturated fatty acids decrease the risk of myocardial infarction and coronary heart disease (17). Thus, there is an increased interest in dietary sources of these fatty acids (18).

In this study, we aimed to characterize the Nagano diet with respect to serum triglycerides, FFA, cholesterol, and lipoprotein concentrations.

Materials and Methods

The appropriate hospital ethics committee approved this cross-sectional study, and participants provided written informed consent. This study complied with the STROBE guidelines for observational studies. Nakano City, with a population of approximately 44,000, is located in the northern part of the Nagano

Prefecture, and has a mean life expectancy (men: 80.4 years; women: 87.4 years) similar to that of the Nagano Prefecture as a whole (men: 80.9 years; women: 87.2 years) (1, 19). Farmers living in Nakano City were invited to participate through announcements to the local farmers group. In addition, the families of farmers who were factory laborers or had desk jobs were also invited to participate. Participants with uncontrolled liver, renal, heart, or other chronic diseases were excluded. Candidates with a past and/or present history of controlled hypertension, controlled diabetes mellitus, myocardial infarction, cerebrovascular disease, or cured and/or controlled cancer were included. After excluding 16 individuals, a total of 106 individuals (mean age: 45 ± 12 years [range: 21–78 years; men: 22–78 years; women: 21–61 years]; of the men, 44 were in the age range 40–59 years) participated in and completed the study.

All individuals completed a health and lifestyle questionnaire (Supplementary File 1). This questionnaire was designed to confirm that the dietary intake was mainly plant-derived and that other characteristics of the population were relatively uniform. Height and weight were measured while the participants were wearing lightweight clothing without shoes, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Non-fasting venous blood collection was performed with the participants in a seated position between February 18 and February 22, 2013. Samples were centrifuged at $3,500 \times g$ for 15 minutes at room temperature in a clinical centrifuge. Serum was separated and frozen at -80°C for 15 minutes before analysis for FFA content. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA), arachidonic acid (AA), and oleic acid (OA) were measured by gas-liquid chromatography at Kotobiken Medical Laboratories Inc., Ltd. (Tokyo, Japan). The Folch procedure was used to extract total serum lipids. Following methylation of the FFAs with boron trifluoride/methanol, the transesterified FFAs were analyzed with a gas chromatograph (GC-17A; Shimadzu, Kyoto, Japan) and capillary column (Omegawax 250; Supelco, Bellefonte, PA, USA) (20). Serum concen-

trations of total cholesterol, triglycerides, and high-density lipoprotein cholesterol were measured using standard enzymatic methods (Kainos, Tokyo, Japan), and low-density lipoprotein (LDL) cholesterol values were calculated using the Friedewald formula (21).

Some participants were required to work from the early hours of the morning, and thus, in consideration of their health, samples were collected under non-fasting conditions. These participants were, however, instructed to limit ingestion where possible, and to avoid food for several hours before the examination.

To represent reference values for the average Japanese population, we used values from typical healthy Japanese residents in Nagoya City, Aichi Prefecture (Aichi reference values). These values were taken from residents who were randomly selected from age-stratified and sex-stratified individuals from neighborhoods of the satellite city of Obu and the Higashiura suburb of Nagoya City, whom had been included in a National Institute for Longevity Sciences study. These reference values were provided by the National Centre for Geriatrics and Gerontology, Aichi Prefecture, Japan (22–24).

All statistical analyses were conducted using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Continuous data are presented as a mean \pm standard deviation. Comparisons between the Nakano individuals and Aichi reference values were performed

using nonparametric one-sample Wilcoxon tests. Since the data were not normally distributed, median values for the FFAs were used in the analysis. In all analyses, a two-tailed *P*-value <0.05 was considered significant.

Results

This study primarily focused on Nagano men aged 40–59 years ($n = 44$). However, we also examined men and women of other ages (ranges: 21–78 years, 21–61 years, respectively) to determine whether the Nakano citizens had any unique physical or other characteristics. Our results showed that the BMI values of male participants (24.3 ± 3.0 kg/m²) were relatively higher than those of female participants (22.4 ± 4.0 kg/m²) (Table 1). The BMI values of the entire sample were comparable to Japanese reference values (Figure 1).

We next examined the triglycerides, cholesterol, and serum FFA profile in these individuals. The mean triglycerides value was higher, while the LDL cholesterol and EPA/AA ratios were within the normal range of the reference values provided by our clinical laboratory (Table 1). In Nagano men aged 40–59 years, the weight percentage of OA to total FFA was significantly higher than Aichi reference values ($21.63 \pm 3.72\%$, $19.23 \pm 2.80\%$, respectively) (Table 2). Similarly, ALA ($0.95 \pm 0.386\%$) was significantly higher

Table 1. Characteristics of participants in Nakano City, Japan

	Men 40–59 years		All men		Women		Entire sample	
	n = 44		n = 80		n = 26		n = 106	
	Value or number (SD)	%	Value or number (SD)	%	Value or number (SD)	%	Value or number (SD)	%
Age (years)	49.0 (5.97)		44.6 (12.7)		44.7 (10.8)		44.6 (12.2)	
Body weight (kg)	71.7 (8.2)		70.9 (9.3)		55.4 (9.6)		67.1 (11.5)	
Height (cm)	1.71 (0.05)		1.71 (0.06)		1.57 (0.05)		1.67 (0.08)	
BMI (kg/m ²)	24.6 (2.8)		24.3 (3.0)		22.4 (4.0)	4.0	23.9 (3.3)	
BMI ≥ 25 kg/m ²	15	34%	24	30%	5	19%	29	27%
BMI < 18.5 kg/m ²	0	0%	0	0%	3	12%	3	3%
LDL-C (mg/dL)	115.6 (28.5)		116.1 (26.0)		109.7 (28.1)		114.5 (27.3)	
HDL-C (mg/dL)	59.4 (16.0)		59.2 (15.8)		77.3 (14.5)		63.6 (17.3)	
TG (mg/dL)	195.8 (147.0)		183.3 (124.2)		96.7 (66.5)		162.0 (118.6)	
EPA/AA	0.34 (0.22)		0.34 (0.21)		0.28 (0.17)		0.32 (0.20)	

SD, standard deviation; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; EPA/AA, eicosapentaenoic acid/arachidonic acid ratio

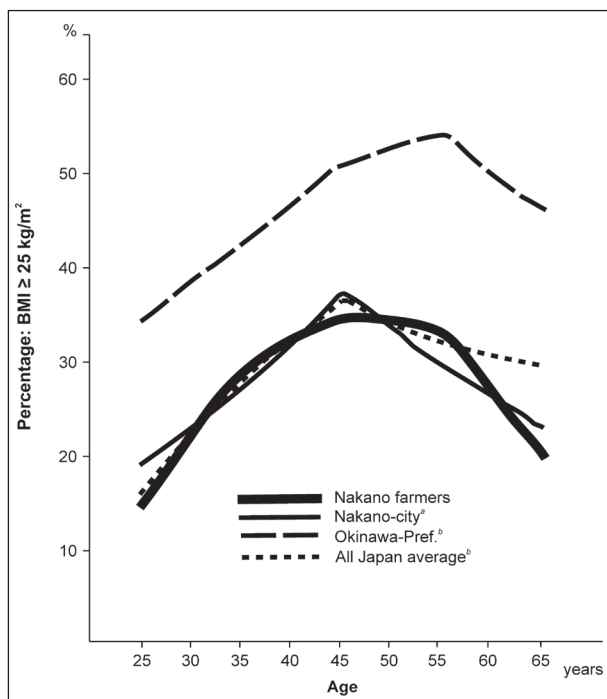


Figure 1. Percentage of individuals with body mass index (BMI) ≥ 25 kg/m² at each age, compared by district in 2012. Men in Okinawa had higher BMIs at all ages, especially those aged between 40 and 59 years, where more than 50% had excess body weight.^a *Kenko Nagano Life Plan 21* (second edition), Nagano City government (21).^b Comparisons between Okinawa and Japan are provided in the annual report of the health survey in Okinawa, *Kenko-Okinawa 21w*, from the Department of Welfare and Health, The Government of Okinawa Prefecture” (22).

than the Aichi reference value ($0.85 \pm 0.27\%$) (Table 2). While the DHA and EPA levels were lower than reference values, the difference for EPA did not reach the level of statistical significance (Tables 1 and 2). The ratio between the Nakano and Aichi reference values revealed that plant-based FFAs, such as OA and ALA, tended to be higher in Nakano men aged 40–59 years (Table 2, Figure 2, and Supplementary Table 1).

Discussion

This study examined the dietary intake, BMI, and serum FFA profile of individuals in the Nagano Prefecture. The relationship between a lower BMI and diet is important for obesity prevention, and it is likely to be part of a major public health strategy to promote

Table 2. Mean serum free fatty acid values in men from Nagano aged 40–59 years compared with Aichi reference values^(a)

Free fatty acid	Nagano individuals		Aichi reference		<i>P</i> -value
	wt%	SD	wt%	SD	
ALA	0.95	0.39	0.85	0.27	0.013
EPA	1.93	1.15	2.23	1.22	0.288
DHA	4.27	1.21	5.00	1.32	0.001
LA	27.98	4.31	29.40	4.06	0.033
GLA	0.33	0.13	0.34	0.16	0.243
DGLA	1.12	0.22	1.17	0.28	0.243
AA	6.01	1.49	5.70	1.17	0.363
OA	21.63	3.72	19.23	2.80	<0.001

ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linoleic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; OA, oleic acid; SD, standard deviation. ^(a)Reference values were provided by the National Institute for Development of Preventive Medicine, National Institute for Longevity Science, National Centre for Geriatrics and Gerontology, Aichi, Japan. Comparisons of median values were made using the non-parametric Wilcoxon test (Cf. Supplementary Table 1). wt% = weight of each FFA as a percentage of the total free fatty acid.

healthy ageing (25, 26). While the BMI levels of men in this study were higher than those of women (Table 1), taken together, they were significantly lower than BMI levels in the Okinawa Prefecture, which historically had the highest life expectancy in Japan (Figure 1, Supplementary Table 2). In Okinawa, BMI appears to have significantly increased around the 1960s, presumably due to increased consumption of Western foods. While the BMI of Nagano residents was similar to the Japanese reference value, the serum FFA profile was different, suggesting that the FFA profile in Nagano residents is most likely influenced by diet, even in middle-aged men with the highest BMI.

Currently, the Nagano Prefecture has the highest life expectancy of the 47 prefectures in Japan for both sexes, and a higher than average proportion of residents aged more than 65 years (Supplementary Figure 2) (27). Dietary patterns have been thought to play an important role in changes in mortality rates. Healthy diets are associated with a lower risk of cardiovascular diseases, hypertension, and metabolic syndrome, as well as cancer-related mortality (25, 28–29). Both plant-derived and marine-derived dietary components have been shown to have beneficial health-promoting

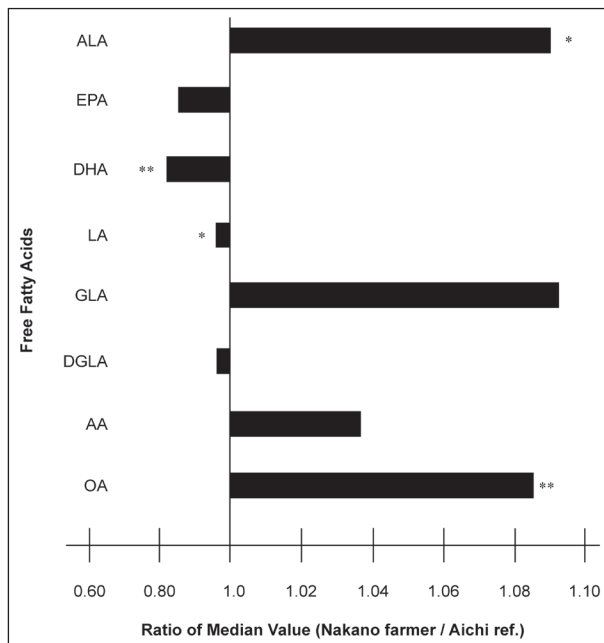


Figure 2. Ratio of median serum free fatty acid concentrations in male participants (40–59 years) in Nagano compared to the Aichi reference values (Nagano/Aichi ref.). Levels of plant-derived free fatty acids, such as alpha-linolenic acid and oleic acid, are significantly higher, and docosahexaenoic acid and linoleic acid levels are significantly lower in the Nagano population. * $P < 0.05$; ** $P < 0.01$. ALA, alpha-linolenic acid; LA, linoleic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; OA, oleic acid.

effects (14, 29). Recent studies and nutrition surveys have shown that the Nagano diet is lower in fish and characteristically rich in vegetables, mushrooms, and milk products compared with the average Japanese diet (Supplementary Tables 3 and 4, Supplementary Figure 1) (10, 30). For example, *oyaki* is a popular dish that contains mainly vegetables and is cooked typically with plant-based oils, such as flaxseed oil or sesame oil, which are rich in OA and ALA. The results of our study provide support to the hypothesis that plant-derived constituents of the Nagano diet may contribute to the longevity in this population.

The Nagano diet is very similar to the traditional Okinawa diet, which consists of low glycemic index carbohydrates, grains, more fish than meat, and plenty of vegetables and fruits (31). Suzuki *et al.* reported that elderly Okinawa individuals had healthier cardiovascular systems, good endothelial function, and well-compensated ventricular function, potentially because of a

plant-based diet that was low in salt and fat, with monounsaturated fats as the principal fat (32). Willcox *et al.* found the diet of Okinawa centenarians was high in antioxidant-rich, calorie-poor foods that were thought to contribute to a healthy fat profile, high phytonutrient intake, and less inflammation; in addition, the diet involved a mild form of caloric restriction, resulting in low lipid peroxide levels, high superoxide dismutase activity, and high serum hydroxyproline levels (31). The Mediterranean diet is another example of a diet associated with longer survival, mainly due to cardio-protection and relatively low rates of cancer (33). The Nagano-style diet is similar to the Mediterranean diet, which consists mainly of vegetables, olive oil, wheat, grapes, and products derived from these food items. The common elements of the Nagano diet, Mediterranean diet, and traditional Okinawa diet include plenty of vegetables and mushrooms, which provide fiber, vitamins, and other micronutrients. The intake of vegetable oils, a major source of polyunsaturated fatty acids, has been shown to be inversely associated with mortality in humans (34).

Our cross-sectional study demonstrated that plant-derived FFAs, namely OA and ALA, were higher in men aged 40–59 years in Nagano, compared with the Aichi reference values (Table 2). OA is a well-known monounsaturated fatty acid that exhibits protective functions for blood vessels, including reducing oxidative stress, lowering serum LDL cholesterol concentrations, and reducing inflammation (35). Virgin olive oil is rich in OA, and a characteristic component of the Mediterranean diet. Virgin olive oil is thought to improve risk factors for cardiovascular disease, including the lipoprotein profile, blood pressure, glucose metabolism, antithrombotic profile, and body weight (36). In countries where the Mediterranean diet is common, it has been observed that mortality from coronary heart disease and the incidence of cancer are lower than those in northern Europe and the United States (37). ALA is an intermediate-chain n-3 polyunsaturated fatty acid that promotes health by reducing the risk of heart diseases. The primary mechanism is thought to be suppression of ventricular fibrillation, which may reduce the risk of abnormally prolonged repolarization (38). In addition, it was reported that ALA-rich flaxseed oil supplementation elevates DHA

and reduces body weight, BMI, systolic blood pressure, and glycosylated hemoglobin levels (39). ALA may also exert favorable effects on endothelial function, inflammation, and the risk of thrombosis by suppressing platelet aggregation, thereby reducing the risk of coronary heart disease and sudden cardiac death (40). Hence, higher levels of ALA may contribute to the good health of the people of Nagano.

Several studies have suggested that marine-based diets result in a healthier FFA profile and thus exhibit health-promoting effects (20). Hirai *et al.* investigated dietary fatty acid composition, the distribution of plasma fatty acids, and platelet function in people living in fishing and farming villages in Chiba Prefecture, Japan. While EPA, DHA, AA, and EPA/AA levels were higher in people from the fishing village, platelet aggregation was higher in individuals from the farming village (41). Subsequent studies by the same group showed that the risk of cardiovascular and ischemic cerebral diseases was lower in individuals from the fishing village than in those from the farming village (42). In contrast to these results, our studies from the Nagano Prefecture, which is dominated by farming communities, suggest that these individuals have lower levels of EPA and DHA, and yet have a low mortality rate. Indeed, results from a previous study suggested that plant sources of n-3 fatty acids may also be important for coronary heart disease prevention in individuals who do not regularly consume fish oils (43). Our results showed that individuals from Nagano, who follow a largely plant-derived diet, had significantly lower DHA levels as compared to the Aichi reference. EPA levels were also lower but failed to reach statistical significance. DHA and EPA are metabolic products of the n-3 polyunsaturated fatty acid ALA; however, previous studies have shown that conversion of ALA to EPA and DHA is relatively low in adult men (44). In addition, it has been shown that while conversion of dietary ALA to DHA and EPA decreases when dietary EPA and DHA availability increase, conversion remains unaffected by an increase in dietary ALA consumption (44). This suggests that in Nagano residents, lower DHA and EPA levels are reflective of a decreased dietary intake of DHA and EPA.

There were several limitations in this study. First, the sample size was relatively small. However, we col-

lected information regarding medical history, medications, vitamin and mineral supplementation, basic dietary intake, alcohol intake, smoking, and physical activity from the participants (data not shown), and the data indicated that the lifestyle of the recruited group was mostly similar to that of the Japanese population. Second, the subjects from Nagano were not sampled in the same way as those in the control population, and it remains likely that the recruited population was healthier or agreed to participate since they were more health conscious. Third, we did not examine the serum FFA profile under fasting conditions. Fourth, since the present study involved only questionnaires on dietary intake and medical history, other details, such as the presence of inflammation, glucose intolerance, or any other factors potentially influencing the FFA profile, including renal dysfunction (which could be determined by a urine study), were not examined. In addition, no investigations on caloric intake or glycemic index were performed. Finally, we were unable to compare lipoprotein levels in different areas of Japan, as a nationwide investigation of Japanese lipoprotein levels would be necessary to obtain such data. Future larger epidemiological studies are needed to further identify other factors, such as genetic and psychosocial factors, that were not accounted for in this study.

In conclusion, we characterized the Nagano diet with respect to serum FFA levels to gain insight into the longevity of individuals in the Nagano Prefecture. Consistent with a diet rich in vegetables, middle-aged men in Nagano, at a stage of life where their BMI is highest, had higher levels of plant-derived FFAs, such as OA and ALA, and lower levels of marine-derived FFAs, such as EPA and DHA. Our results suggest that the characteristics of the Nagano-style diet, particularly in middle-aged men, likely contribute to the maintenance of health in the latter half of life.

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