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

Hiroshi Harada, MD[1,2,3], Yoshiaki Kanai [3,4], Mikiyuki Katagiri, PhD[3], Takuji Shirasawa, MD, PhD[3]

[1]Department of Neurosurgery, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan

[2]Department of Neurosurgery, Iiyama Red Cross Hospital, 226-1 Iiyama, Iiyama-city, Nagano 389-2253, Japan

[3]Shirasawa Anti-aging Medical Institute, 3-5-4-501 Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan

[4]Kotobiken Medical Laboratories, Inc., 4-12-12 Honmachi, Tatebayashi-city, Gunma 374-0024, Japan

Designations:

Hiroshi Harada: Graduate Student; Assistant Director of the Inspection Department

Yoshiaki Kanai: Research Associate

Mikiyuki Katagiri: Research Associate

Takuji Shirasawa: Chief of Laboratory

Running title:

Fatty acid profiles among individuals in Nagano

Authorship declaration:

TS designed and supervised the research; HH, MK, and YK conducted the research and

analyzed data; HH performed the statistical analysis and wrote the manuscript and also had primary responsibility for final content of the manuscript; all authors directly participated in the planning and execution of the study and read and approved the final manuscript.

Correspondence:

Dr. Hiroshi Harada

Department of Neurosurgery, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-Ku, Tokyo 113-8421, Japan

Tel: 03-3868-2041, Fax: 03-3868-2042

E-mail: h-harada@juntendo.ac.jp

Funding:

This study was supported by a grant from the Japan Agricultural Cooperatives-Nakano city (IBSD49G). The funding was received by Prof. Takuji Shirasawa.

Conflicts of interest:

The authors declare no conflict of interest

Acknowledgments:

We thank Rei Otsuka (National Center for Geriatrics and Gerontology) for the median values of the Aichi reference group. Editorial support, in the form of medical writing based on authors' detailed directions, collating author comments, copyediting, fact checking, and referencing, was provided by Cactus Communications.

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

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 × 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-linoleic 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 concentrations 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 ± 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/m^2) were relatively higher than those of female participants (22.4 ± 4.0 kg/m^2) (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 ± 3.72%, 19.23 ± 2.80%, respectively) (Table 2). Similarly, ALA (0.95 ± 0.386%) was significantly higher than the Aichi reference value (0.85 ± 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 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 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 collected 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.

References

1. Nagano prefectural government. Nagano, 2016. Available from: http://www.pref.nagano.lg.jp/kenko-fukushi/kenko/kenko/toukei/hokeneisei/index.html. (Retrieved on: October 14, 2016). [table; Excel 6, Japanese]
2. Nakano city health plan. Nakano: Department of Health Promotion Division (Nakano Health Centre), 2016. Available from: http://www.city.nakano.nagano.jp/docs/2014031400065/file_contents/plan.pdf. (Retrieved on: October 14, 2016). [table, page 5, Japanese]
3. Fraser GE, Shavlik DJ. Ten years of life: Is it a matter of choice? Arch Intern Med 2001; 161(13): 1645-52.
4. Guenther PM, Casavale KO, Reedy J, et al. Update of the healthy eating index: HEI-2010. J Acad Nutr Diet 2013; 113(4): 569-80.
5. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012; 142(6): 1009-18.
6. Wirt A, Collins CE. Diet quality--what is it and does it matter? Public Health Nutr 2009; 12(12): 2473-92.
7. Yoshiike N, Hayashi F, Takemi Y, Mizoguchi K, Seino F. A new food guide in Japan: the Japanese food guide Spinning Top. Nutr Rev 2007; 65(4): 149-54.
8. Kurotani K, Akter S, Kashino I, et al. Japan Public Health Center based Prospective Study Group. Quality of diet and mortality among Japanese men and women: Japan Public Health Center based prospective study. BMJ 2016; 352: i1209.
9. Ministry of Internal Affairs and Communications. Family income and expenditure survey in each year. Tokyo: Ministry of Internal Affairs and Communications, 2015 (Pre-2000: available from the Statistics Library, Statistics Bureau, Ministry of Internal Affairs and Communications, Shinjuku-ku Tokyo; after 2000: available from:

http://www.stat.go.jp/data/kakei/npsf.htm. (Retrieved on: July 14, 2016). [Japanese]

10. National Institute of Health and Nutrition, editor. The National Health and Nutrition Survey in Japan, 2012. Available from: http://www.e-stat.go.jp/SG1/estat/GL08020103.do?_toGL08020103_&listID=000001118468&requestSender=dsearch: (Accessed on: September 29, 2017). [Japanese]
11. Wang JB, Fan JH, Dawsey SM, et al. Dietary components and risk of total, cancer and cardiovascular disease mortality in the Linxian Nutrition Intervention Trials cohort in China. Sci Rep 2016; 6: 22619.
12. Terry DF, Pencina MJ, Vasan RS, et al. Cardiovascular risk factors predictive for survival and morbidity-free survival in the oldest-old Framingham Heart Study participants. J Am Geriatr Soc 2005; 53(11): 1944-50.
13. Raatz SK, Bibus D, Thomas W, Kris-Etherton P. Total fat intake modifies plasma fatty acid composition in humans. J Nutr 2001; 131(2): 231-4.
14. Siriwardhana N, Kalupahana NS, Moustaid-Moussa N. Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Adv Food Nutr Res 2012; 65: 211-22.
15. Klek S. Omega-3 fatty acids in modern parenteral nutrition: A review of the current evidence. J Clin Med 2016; 5(3): pii: E34.
16. Azrad M, Turgeon C, Demark-Wahnefried W. Current evidence linking polyunsaturated Fatty acids with cancer risk and progression. Front Oncol 2013; 3: 224.
17. Nordøy A, Marchioli R, Arnesen H, Videbaek J. n-3 polyunsaturated fatty acids and cardiovascular diseases. Lipids 2001; 36 Suppl: S127-9.
18. Williams CM, Burdge G. Long-chain n-3 PUFA: plant v. marine sources. Proc Nutr Soc 2006; 65(1): 42-50.
19. Handbook of Health and Welfare Statistics 2013, Part 1 Population and Households,

Chapter 2 Vital Statistics, Statistics and Other Data. Tokyo: Ministry of Health, Labour and Welfare, 2016. Available from: http://www.mhlw.go.jp/english/database/db-hh/1-2.html. (Retrieved on: October 14, 2016).

20. Otsuka R, Tange C, Nishita Y, et al. Serum docosahexaenoic and eicosapentaenoic acid and risk of cognitive decline over 10 years among elderly Japanese. Eur J Clin Nutr 2014; 68(4): 503-9.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18(6): 499-502.
22. Otsuka R, Kato Y, Imai T, Ando F, Shimokata H. Higher serum EPA or DHA, and lower ARA compositions with age independent fatty acid intake in Japanese aged 40 to 79. Lipids 2013; 48(7): 719-48.
23. Nagano city health plan. Nagano, 2016. Available from: https://www.city.nagano.nagano.jp/uploaded/attachment/66056.pdf. (Retrieved on: October 14, 2016). [Japanese]
24. Department of Welfare and Health, Okinawa prefectural government, 2016. Available from: http://www.kenko-okinawa21.jp/090-docs/2015121500016/files/2012tyukanhyouka.pdf. (Retrieved on: October 14, 2016). [Japanese]
25. Mathers JC. Impact of nutrition on the ageing process. Br J Nutr 2015; 113 Suppl: S18-22.
26. Moriguchi EH, Moriguchi Y, Yamori Y. Impact of diet on the cardiovascular risk profile of Japanese immigrants living in Brazil: contributions of World Health Organization CARDIAC and MONALISA studies. Clin Exp Pharmacol Physiol 2004; 31 Suppl: S5-7.

27. Statistics Bureau, Ministry of Internal Affairs and Communications, E-Stat, 2016. Available from: http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001132435. (Retrieved on: October 14, 2016.) [Table 12]
28. Reedy J, Krebs-Smith SM, Miller PE, et al. Higher diet quality is associated with decreased risk of all-cause, cardiovascular disease, and cancer mortality among older adults. J Nutr 2014; 144(6): 881-9.
29. Malik VS, Li Y, Tobias DK, Pan A, Hu FB. Dietary protein intake and risk of type 2 diabetes in US men and women. Am J Epidemiol 2016; 183(8): 715-28.
30. Kobayashi M, Kobayashi Y. Dietary habit of the people of Nagano prefecture based on nutrition surveys (Japanese). Shinshu J Public Health 2014; 8: 89-95.
31. Willcox DC, Willcox BJ, Todoriki H, Suzuki M. The Okinawan diet: health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. J Am Coll Nutr 2009; 28 Suppl: S500-16.
32. Suzuki M, Wilcox BJ, Wilcox CD. Implications from and for food cultures for cardiovascular disease: longevity. Asia Pac J Clin Nutr 2001; 10(2): 165-71.
33. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. BMJ 2008; 337: a1344.
34. Nagata C, Nakamura K, Wada K, et al. Total fat intake is associated with decreased mortality in Japanese men but not in women. J Nutr 2012; 142(9): 1713-9.
35. Perona JS, Cabello-Moruno R, Ruiz-Gutierrez V. The role of virgin olive oil components in the modulation of endothelial function. J Nutr Biochem 2006; 17(7): 429-45.
36. Perez-Jimenez F, Alvarez de Cienfuegos G, Badimon L, et al. International conference on the healthy effect of virgin olive oil. Eur J Clin Invest 2005; 35(7): 421-4.
37. Lopez-Miranda J, Perez-Jimenez F, Ros E, et al. Olive oil and health; Summary of the

II international conference on olive oil and health consensus report, Jaen and Cordoba (Spain) 2008. Nutr Metab Cardiovasc Dis 2010; 20(4): 28-94.

38. Djouss L, Rautaharju PM, Hopkins PN, et al. Dietary linolenic acid and adjusted QT and JT intervals in the National Heart, Lung, and Blood Institute Family Heart study. J Am Coll Cardiol 2005; 45(10): 1716-22.
39. Amir K, Watanabe M, Yokotsuka M, Kobayashi Y. Changes in serum fatty acids and effects of metabolic syndrome induced by intermittent flaxseed-oil supplementation. Ningen Dock 2012; 26(6): 927-34.
40. Prasad K. Flaxseed and cardiovascular health. J Cardiovasc Pharmacol 2009; 54(5): 369-77.
41. Hirai A, Hamazaki T, Terano T, et al. Eicosapentaenoic acid and platelet function in Japanese. Lancet 1980; 2(8204): 1132-3.
42. Hirai A. An epidemiological study on dietary ingestion of eicosapentaenoic acid (EPA) and platelet function in Japanese. Nihon Naika Gakkai Zasshi 1985; 74(1): 13-20. [Japanese]
43. Mozaffarian D, Ascherio A, Hu FB, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 2005; 111(2): 157-64.
44. Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C] alpha-linolenic acid to longer-chain fatty acids and partitioning towards beta-oxidation in older men. Br J Nutr 2003; 90(2): 311-21.

Tables

Table 1. Characteristics of participants in Nakano City, Japan

	Men 40–59 years n = 44		All men n = 80		Women n = 26		Entire sample 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

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

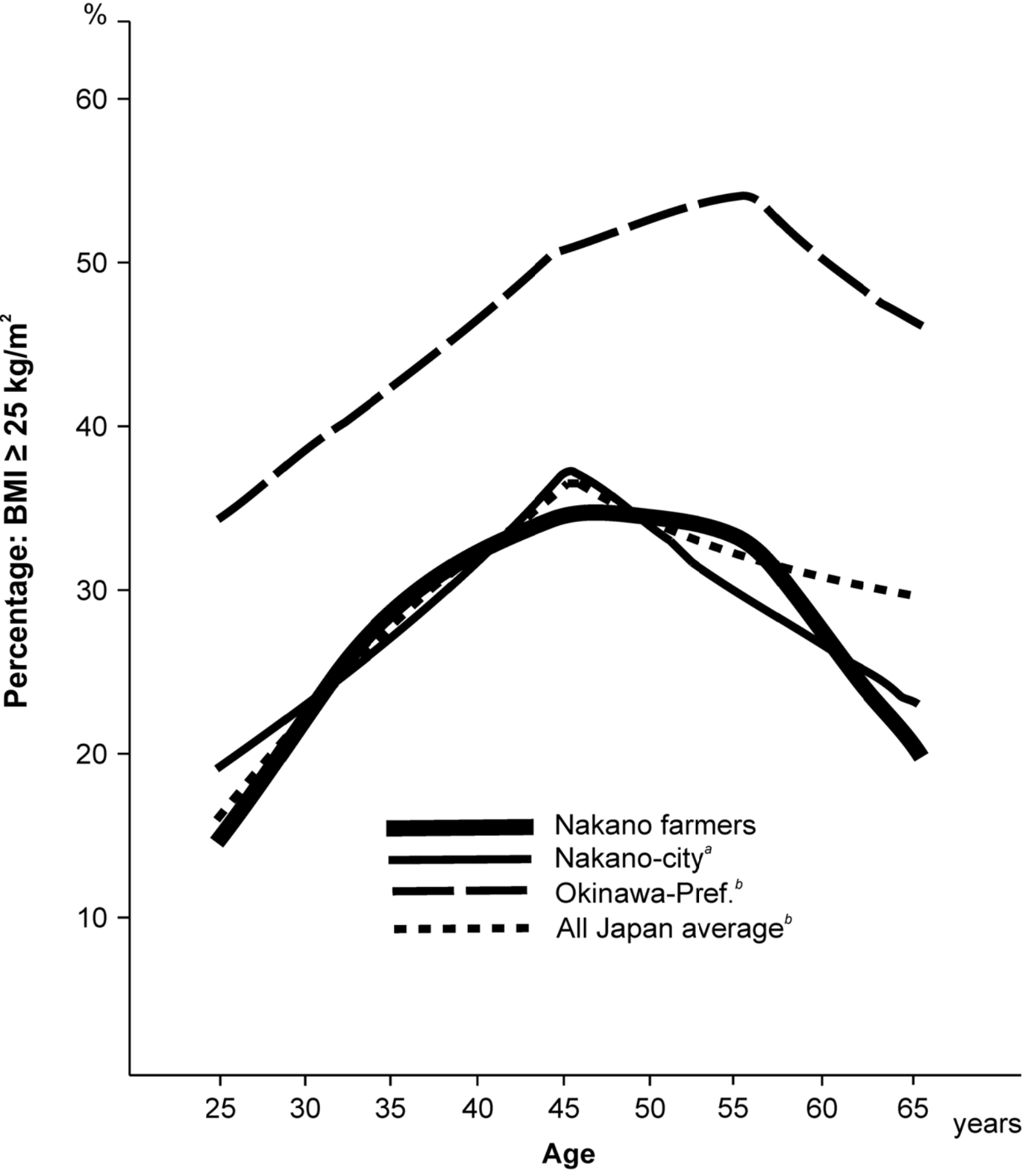
Free fatty acid	Nagano individuals		Aichi reference		*P*-value
	wt%	SD	wt%	SD	
ALA	0.95	0.39	0.85	0.27	0.013
EPA	1.93	1.15	2.23	1.22	0.288
DHA	4.27	1.21	5.00	1.32	0.001
LA	27.98	4.31	29.40	4.06	0.033
GLA	0.33	0.13	0.34	0.16	0.243
DGLA	1.12	0.22	1.17	0.28	0.243
AA	6.01	1.49	5.70	1.17	0.363
OA	21.63	3.72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

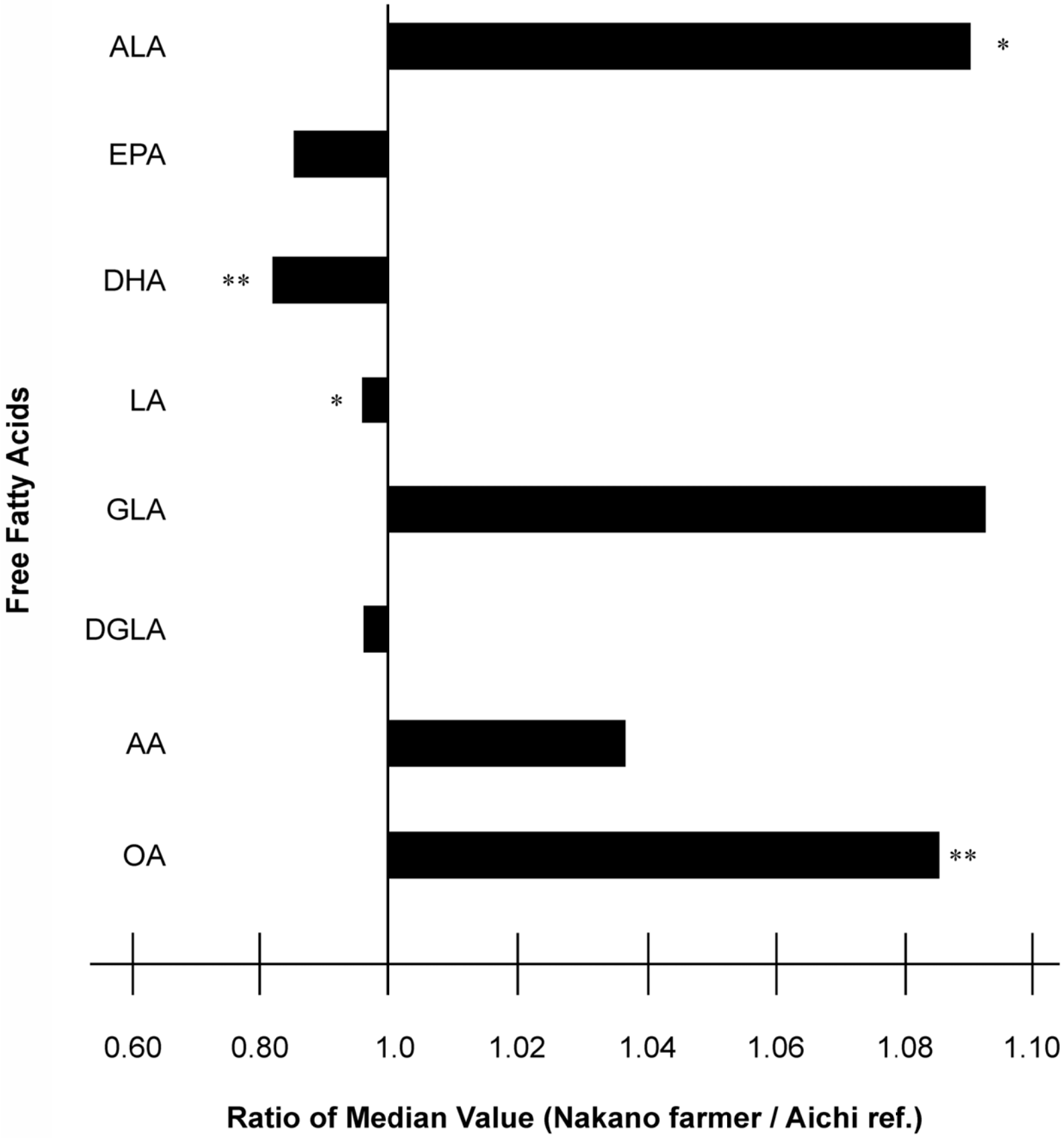
ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linoleic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; OA, oleic acid; SD, standard deviation. [(a)]Reference values were provided by the National Institute for Development 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.

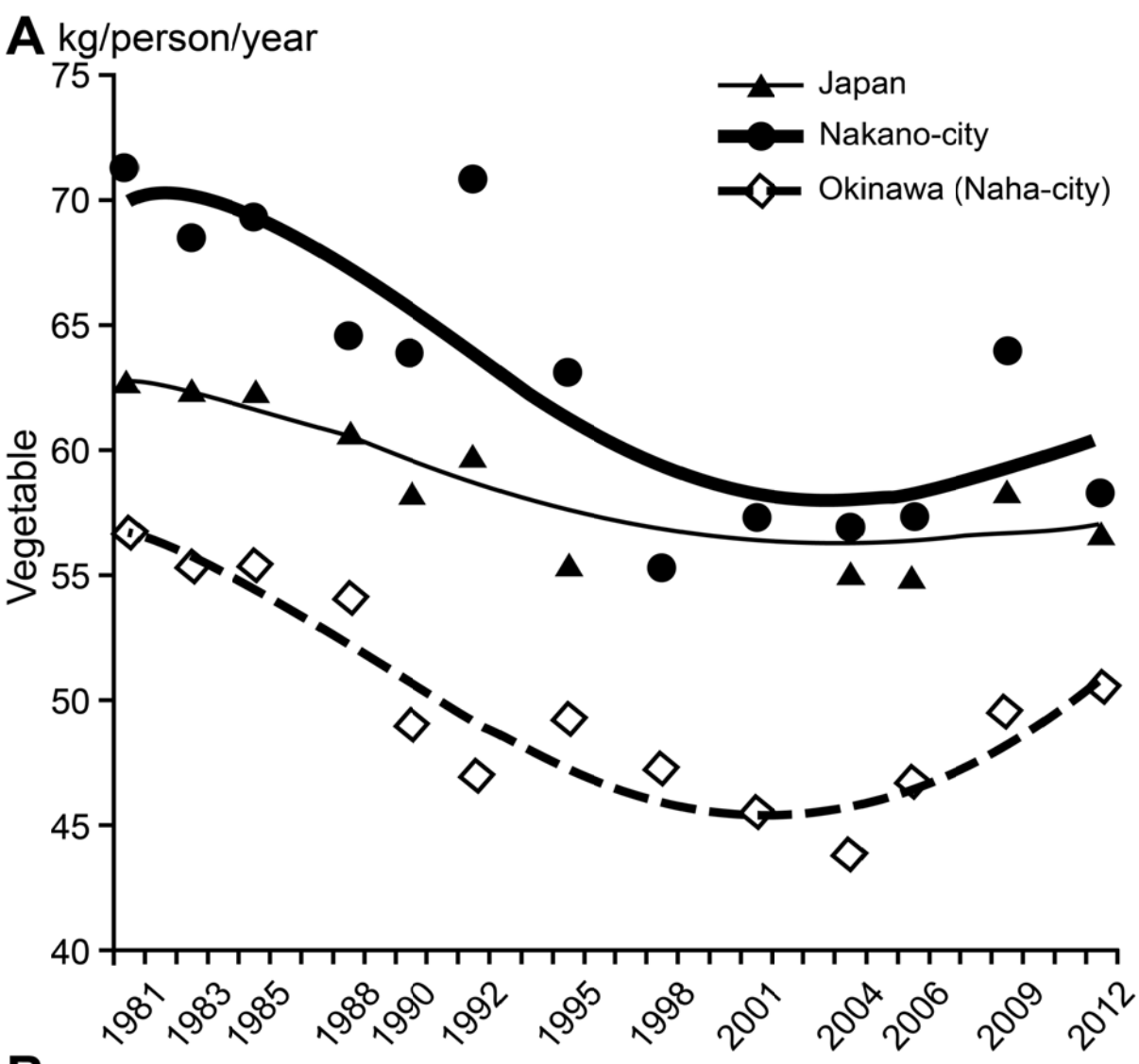
Figure Legends

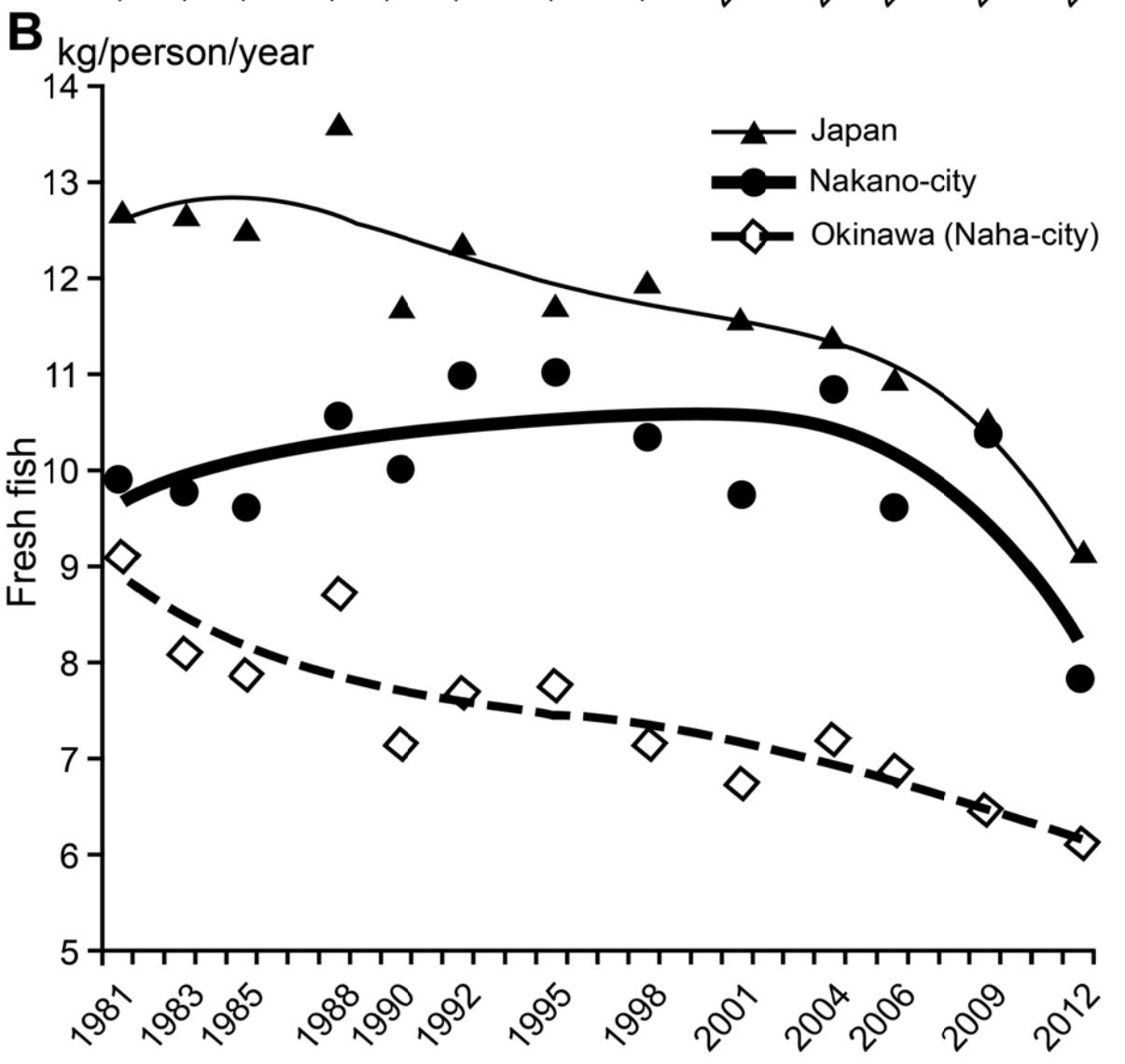
Figure 1. Percentage of individuals with body mass index (BMI) ≥25 kg/m^2 at each age, compared by district in 2012. Men in Okinawa had higher BMIs at all ages, especially those aged between 40 and 59 years, where more than 50% had excess body weight.[a] *'Kenko Nagano Life Plan 21'* (second edition), 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).

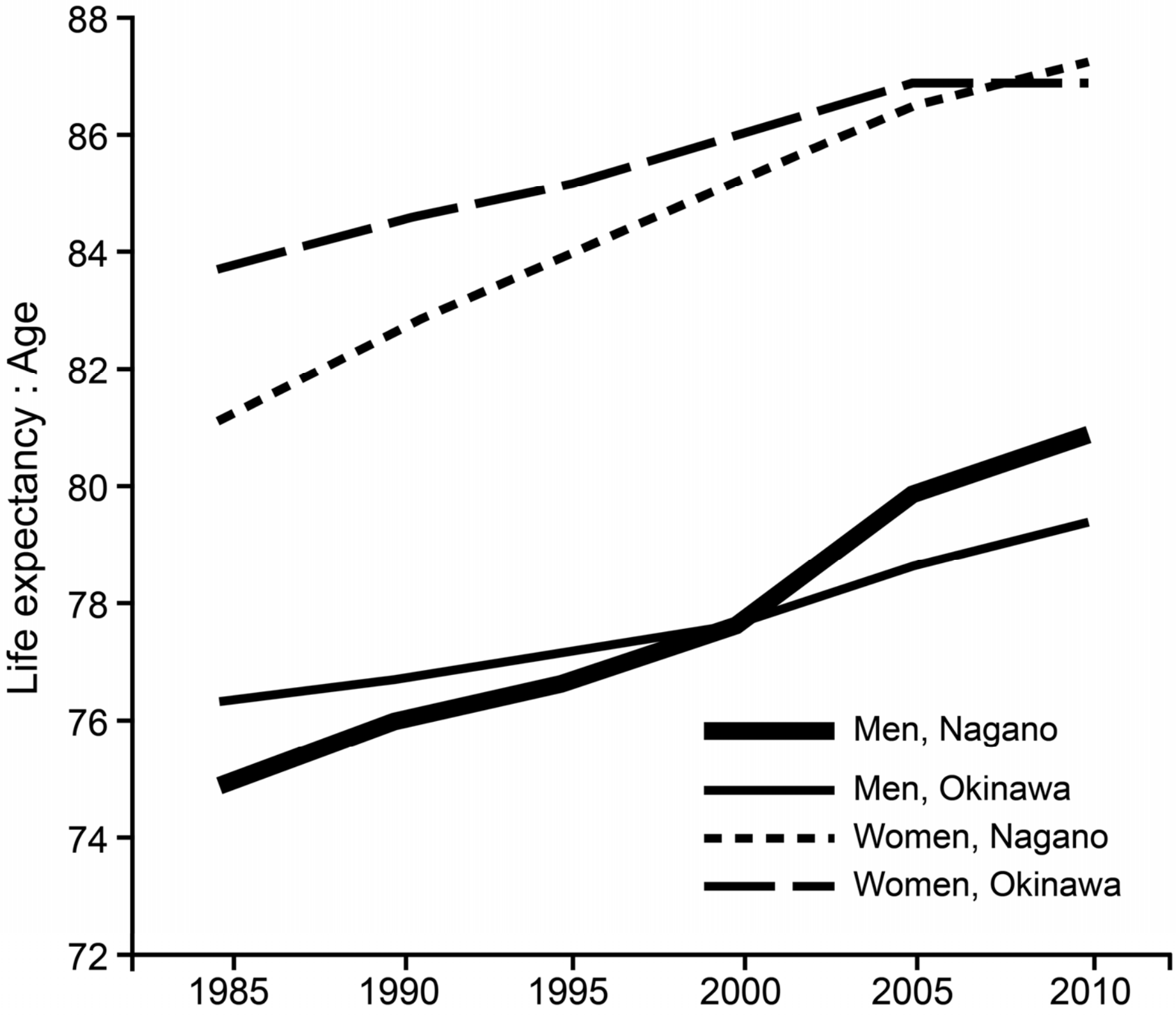
Figure 2. Ratio of median serum free fatty acid concentrations in male participants (40-59 years) in Nagano compared to the Aichi reference values (Nagano/Aichi ref). Levels of plant-delivered free fatty acids, such as alpha-linolenic acid and oleic acid, are significantly higher, and docosahexaenoic 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.


%
60
50
40
30
20
10
Percentage: BMI ≥ 25 kg/m²
Nakano farmers
Nakano-city[a]
Okinawa-Pref.[b]
All Japan average[b]
25
30
35
40
45
50
55
60
65
years
Age


ALA
EPA
DHA
LA
GLA
DGLA
AA
OA
*
**
*
**
Free Fatty Acids
0.60
0.80
1.0
1.02
1.04
1.06
1.08
1.10
Ratio of Median Value (Nakano farmer / Aichi ref.)


A
kg/person/year
Japan
Nakano-city
Okinawa (Naha-city)
Vegetable
75
70
65
60
55
50
45
40
1981
1983
1985
1988
1990
1992
1995
1998
2001
2004
2006
2009
2012


B
kg/person/year
Japan
Nakano-city
Okinawa (Naha-city)
Fresh fish
14
13
12
11
10
9
8
7
6
5
1981
1983
1985
1988
1990
1992
1995
1998
2001
2004
2006
2009
2012


Life expectancy : Age
88
86
84
82
80
78
76
74
72
1985
1990
1995
2000
2005
2010
Men, Nagano
Men, Okinawa
Women, Nagano
Women, Okinawa

ID : JN_____

Questionnaire on lifestyle

Name	(　　) years	Date	Year　Month　Date
Address		Phone	
Profession	Engaged in mushroom cultivation　Family business of mushroom cultivation　Engaged in grape cultivation　Family business of grape cultivation		
Years in the profession	Years		
Breed cultivated			
Frequency of eating at home	Mushrooms: ( ) times a week　,　grapes: ( ) times a week [Period ( ) month to ( ) month]		

Ⅰ　At present, do you have any health concerns?

1. No　　2. Yes　(Details:

Ⅱ　Besides the above Ⅱ, have you had any serious illness until now?

1. No　　2. Yes　Disease:　Around (　　) years of age

Disease:　Around (　　) years of age

Ⅲ　Are you presently under treatment for any disease?

1. No　　2. Yes　Disease:　Since when (　　)　…oral medications ① yes ② no　Medicine name (　　)

Disease:　Since when (　　)　… oral medication ① yes ② no　Medicine name (　　)

Ⅳ　Is there any medicine you take at present? (including the ones commercially available)

1. Yes

What is it:_____

Since when:_____

2. No

Ⅴ　At present, are you on any dietary supplements?

1. Yes

ID : JN_____

What is it:_____

_____tablets, capsules, packets in a day

2. No

Ⅵ At present, whom do you live with

1. Transfer without family 2. Staying alone 3. Spouse 4. Children （ ） 5. Father
6. Mother 7. Father in law 8. Mother in law 9. Brother(s) （ ） 10. Sister(s) （ ）
11. Others （ ） （ ） people

Ⅶ Are any of your blood relations or dependants under treatment or have died due to any of the below ailments?

1～9 Please chose ※ and enter data

Cerebral haemorrhage, cerebral infarction	Hypertension	Myocardial infarction, angina pectoris	Diabetes
Cancer	Hyperlipidaemia (arteriosclerosis)	Other （please enter the disease）	

※ 1. Paternal grandfather 2. Paternal grandmother 3. Maternal grandfather 4. Maternal grandmother 5. Father
6. Mother 7. Brothers 8. Sisters 9. Spouse

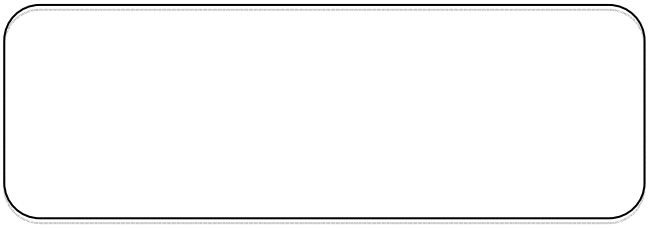
PTO

ID : JN

Ⅷ	Please insert a number in the ( ), and letters in the ☐ (the English translation is written under the Japanese counterpart.)

MEAL	Breakfast	Lunch	Dinner
	・ I eat () times per week ・ Meal time ( ) o'clock ・ Meal details ・ Please circle the below 1.Staple food (rice, bread, noodles) 2 Main dish (meat, fish, eggs, soy bean products) 3. Sub meal (veg, potato, mushroom, seaweed) 4. Others	・ I eat () times per week ・ Meal time ( ) o'clock ・ Meal details Please circle the below 1. Staple food (rice, bread, noodles) 2. Main dish (meat, fish, eggs, soy bean products) 3. Sub meal (veg, potato, mushroom, seaweed) 4. Others	・ I eat () times per week ・ Meal time ( ) o'clock ・ Meal details Please circle the below 1. Staple food (rice, bread, noodles) 2. Main dish (meat, fish, eggs, soy bean products) 3. Sub meal (veg, potato, mushroom, seaweed) 4. Others
	Day snacks	Night snacks	
	1. I don't eat 2. _ I d o () times per week () times per day When? (time) ( ) what and how much?	1. I don't eat 2. I d o () times per week () times per day When? (time) ( ) what and how much?	1. Thin 2. Regular 3. Thick Sweet drinks, canned coffee and juice 1. I don't drink 2. I do () times per week () times per day When? (time) ( ) what and how much?
	seafood		fruits

ID : JN

	1. I do eat 2. I do not eat ↓ () times per week ・ what do you eat often (you can be approximate) 	1. 食べる I do eat 2.食べない I do not eat → ・times per week When  What do you eat 1. Breakfast 2. lunch 3. dinner 4. snacks 5. dinner, and how much? *please tell client that the "に"in the " 1 に日"is not necessary.

alcohol	1. I don't drink 2. I quit → When did you give up( )() months ago or () years ago 3. (I do drink) ↓ () times per week, () times per month → How much do you drink per day (can choose multiple, it can be an approximation) ① sake ( ) ② beer( )ml ③ ( ) (shochu) spirits ④ whisky ( )ml ⑤ others 	
cigarettes	1. I do not smoke 2. ~~I quit~~ → When did you quit? () months ago OR () years ago 3. I smoke ↓ ・( ) the age which I started to smoke ・ the number of cigarettes smoked per day Do you want to quit smoking? ① yes ② no ・Do you know the evil effects smoking has ① yes ② no	
stimulants	Spicy food	Coffee

ID : JN

	1. I eat often 2. I eat sometimes 3. I almost never eat () times per week What do you eat and how much (approximation is fine)	1. I drink 2. I do not drink () times per week () times per day When? How often?
Rest	Sleep	I sleep between () and (), for () hours Quality of sleep: 1. good 2. can't sleep sometimes 3. can't sleep at all The fatigue you have when waking up: 1. none 2. sometimes 3. yes
	Stress level	Stress ① I don't feel it ② I feel it slightly ③ I tend to feel it Ways of dealing with stress ① I have ② I do not have

Do

EXERCISE	1. Regularly Kind of sport () of times () minutes () times per week	2. Sometimes Kind of sport () minutes per work out () times per year, month, week () please circle one of the durations	3. Not at all

Thank you.

Supplementary Table 1. Median values and ratio for Nakano residents and the Aichi prefecture reference, analysed using the Wilcoxon nonparametric sample t-test

Fatty acid (wt%)	Median value, Nakano residents	Median value, Aichi reference	Nakano/reference	*P*-value
ALA	0.86	0.789	1.090	0.013
EPA	1.66	1.933	0.859	0.288
DHA	4.08	4.925	0.828	0.001
GLA	0.34	0.311	1.093	0.243
DGLA	1.11	1.153	0.963	0.243
AA	5.85	6.00	1.028	0.363
LNA	28.42	29.61	0.960	0.033
OA	20.41	18.81	1.085	< 0.001

Reference values were provided by Rei Otsuka, National Institute for Development of Preventive Medicine, National Institute for Longevity Science, National Center for Geriatrics and Gerontology, Aichi Japan (personal communication). ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; LNA, linoleic acid; OA, oleic acid. * statistically significant at $P < 0.05$; ** statistically significant at $P \leq 0.001$.

Supplementary Table 2. Average BMI (kg/m^2) for men (age 20–69 y) in given prefectures and in Japan as a whole as reported in 2012

	Nagano	Okinawa	Aichi	Japan
BMI	23.4	24.3	23.5	23.6
(95% CI)	(22.9, 23.9)	(23.4, 25.2)	(22.8, 24.1)	(23.5, 23.7)

The national health and nutrition survey in Japan, 2012 (10). Data have been adjusted for age.

BMI, body mass index; CI, confidence interval.

Supplementary Table 3. Japanese current nutritional profiles (age > 20 y, per capita per day, not statistically analysed between each place in this table)

	Nagano (2013) [a]			Okinawa (2011) [b]		All Japan (2013) [c]		
	Mean	SD	Median	Mean	SD	Mean	SD	Median
Energy, kcal	1877	508	1806	1680.1[d]	529.6[d]	1887	555	1834
All protein, g	70.0	22.3	68.0	63.0	23.4	69.8	23.2	67.6
Animal protein, g	37.8	17.7	35.2	33.7	19.0	37.3	18.3	35.2
All fat, g	53.7	21.6	51.1	51.3	21.7	54.3	23.5	51.4
Animal fat, g	27.5	15.2	25.2	26.8	15.7	27.4	16.1	24.9
Total saturated fatty acid, g	14.00	6.54	13.05	13.3	6.5	14.51	7.36	13.37
Total monounsaturated fatty acid, g	18.46	8.36	17.22	17.8	8.5	18.60	9.11	17.22
Omega-6 fatty acids, g	9.40	4.47	8.55	9.2	4.7	9.32	4.71	8.63
Omega-3 fatty acids, g	2.43	1.53	2.08	2.0	1.5	2.25	1.47	1.94
Cholesterol, mg	308	170	299	275.5	175.2	308	184	286
Carbohydrates, g	262	78	253	224.8	74.0	260.8	81.9	253.1

Fibre, g	15.4	6.3	14.7	12.5	5.8	14.7	6.5	13.7
Vitamin A, µg RE	527	661	413	645.4	1130.5	522	663	401
Vitamin D, µg	9.3	9.6	5.6	5.5	6.4	7.9	8.9	4.6
Vitamin E, mg	6.9	3.3	6.3	7.0	13.7	6.5	3.3	6.0
Vitamin K, µg	248	174	207	190.5	160.8	230	178	179
Vitamin B1, mg	0.93	0.8	0.81	1.1	4.4	0.85	0.40	0.78
Vitamin B2, mg	1.22	0.77	1.10	1.3	1.9	1.14	0.51	1.06
Vitamin B3 mg NE	15.9	7.7	14.4	14.0	8.1	15.1	7.0	13.8
Vitamin B6, mg	1.26	0.89	1.12	1.6	5.9	1.14	0.48	1.07
Vitamin B12, µg	7.22	7.07	5.06	5.5	6.8	6.5	6.8	4.3
Folic acid, µg	318	150	296	266.5	159.8	294	141	273
Pantothenic acid, mg	5.52	1.80	5.37	4.5	1.8	5.42	1.97	5.20
Vitamin C, mg	100	66	84	87.2	124.7	100	78	81
Sodium, mg	4173	1575	3976	3353.7	1399.6	4010	1575	3818
Potassium, mg	2355	845	2258	1949.4	781.6	2293	901	2185

Calcium, mg	503	257	478	428.8	247.9	498	255	456
Magnesium, mg	248	87	233	224.8	92.2	247	92	238
Phosphorus, mg	1012	325	977	860.9	320.7	985	338	954
Iron, mg	7.8	2.9	7.5	7.3	3.1	7.7	3.0	7.3
Zinc, mg	8.0	2.5	7.6	7.4	2.8	8.0	2.9	7.6
Copper, mg	1.19	0.42	1.13	1.0	0.4	1.15	0.40	1.11
Energy from protein, %	15.0	3.0	14.8	15.0	3.4	14.9[d]	-[d]	-[d]
Energy from fat, %	25.6	7.2	25.2	27.6	7.2	25.7[d]	7.4[d]	25.5[d]
Energy from carbohydrates, %	59.4	8.3	59.7	57.5	8.6	59.4[d]	8.5[d]	59.4[d]

SD, standard deviation; RE, retinol equivalents; NE, niacin equivalents.

[a]Nagano Prefecture health and nutritional survey, 2013. Data have been adjusted according to sample size and population composition of the prefecture (1).

[b]Okinawa Prefecture health and nutritional survey, 2011. Median values were not cited (22).

[c]The national health and nutrition survey in Japan, 2013 (10).

[d]In the 1980s, residents of Okinawa consumed more than 1900 kcal, approaching 2000 kcal in 1988.

[e]Derived from average of each individual's data. Energy from protein was not cited and was calculated after subtracting fat and carbohydrates from the total.

Supplementary Table 4. Dietary intake of each food group in current Japanese diets (age > 20 y per capita per day, not statistically analysed between each place in this table)

	Nagano (2013) [a]			Okinawa (2011) [b]		All Japan (2013) [c]		
	mean	SD	Median	Mean	SD	mean	SD	median
Grains: rice and processed foods	347.9	182.5	320.0	329.1	164.4	383.2	182.5	300.0
Grains: oat and processed foods	101.8	115.3	66.7	86.1	99.8	107.5	110.0	72.0
Tubers and roots	53.2	63.2	37.5	30.8	51.2	53.0	66.2	32.0
Sugar and sweeteners	6.4	8.8	3.8	4.3	7.0	6.8	9.6	4.0
Pulses	60.3	73.6	37.5	75.0	102.9	64.1	78.1	41.0
Nuts	3.3	12.0	0.0	0.6	3.3	2.0	8.4	0.0
Vegetables	319.0	178.6	293.8	282.6	167.9	283.1	172.9	254.2
Green and yellow vegetables	113.8	100.5	85.1	87.4	78.2	87.3	81.3	67.5
Other vegetables	205.3	134.6	183.5	181.8	131.0	172.1	121.0	150.3
Pickled products	17.3	32.9	0.0	3.5	8.2	10.8	22.9	0.0
Fruits and products	103.7	126.3	66.1	63.2	104.7	116.5	142.5	77.7

Mushrooms	21.0	31.9	10.0	10.7	21.9	17.3	28.0	3.3
Algae	12.1	22.7	2.1	14.2	27.1	10.7	20.6	1.5
Raw fish and seashells	46.4	58.1	25.0	46.3	62.9	47.8	62.0	24.0
Processed sea foods	36.5	44.6	21.0	17.6	29.1	31.0	44.7	10.0
Meat	81.6	70.6	65.5	89.4	78.8	86.8	74.3	72.3
Eggs	34.6	32.0	35.2	31.7	31.7	34.1	34.7	29.5
Milk and processed daily products	103.3	127.1	50.1	66.8	117.8	103.9	131.7	50.0
Oils and fats	9.9	8.9	8.2	10.4	8.3	10.3	9.4	8.0
Confectioneries	19.8	38.0	0.0	13.3	30.3	25.3	47.2	0.0
Alcoholic beverages	94.2	237.5	0.0	103.2	291.8	121.1	283.0	0.8
Other preference beverages	577.8	460.3	516.5	470.2	420.3	547.4	416.6	492.0
Flavouring agents	94.9	89.1	66.5	78.0	89.7	91.9	87.2	62.5

SD, standard deviation.

[a]Nagano Prefecture health and nutritional survey, 2013. Data have been adjusted according to sample size and population composition of the prefecture (1).

[b]Okinawa Prefecture health and nutritional survey, 2011. Median values were not cited (22).

[c]The national health and nutrition survey in Japan, 2013 (10).