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Review Article **Open Access**

Nutrigenetics of Japanese Vegetarians with Polymorphism in the Fatty Acid Desaturase

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Abstract

This review investigates how the genotype of vegetarians controls lipid metabolism responses to the dietary intake of n-3 fatty acids. While health indices of Japanese vegetarians are superior to those of omnivores, the effects of genetics have not been studied. The lipid intake of Japanese omnivores and vegans is less than that of Western vegans. Mongolian carnivores and Inuit piscivores have shorter lifespans compared to other Asians who consume a rice diet or taro-fish diet. Our genetic studies on Mongoloids in Asia Pacific regions revealed similar allele frequencies of genes related to diabetes and cardiovascular diseases, which are increasing with recent Westernization because of genetic differences between Mongoloids and Caucasoids. Long-chain n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), play a role in preventing these diseases, yet vegetarians consume nearly 0 g per day. Conversion of plant-derived α-linolenic acid (ALA) to EPA and DHA, and linoleic acid to arachidonic acid (AA) requires the Δ5 desaturase. Thus, plasma concentrations of EPA, DHA and AA in vegetarians are lower than those in omnivores, especially vegans with the C allele of the Δ5 desaturase polymorphism rs174547. The following hypotheses have been proposed to explain the health of C homozygote vegans: 1. They are unable to survive, 2. They cease the vegan diet, 3. They preferentially preserve EPA/DHA by lowered catabolism, 4. Their Δ5 desaturase is activated by specific factors, and 5. Their EPA/AA ratio is elevated and harmful LDL-C and inflammation etc. is reduced. This review does not support hypotheses 1 and 2, but partially accepts 3 and 4 and completely confirms 5. In future, genotype-specific personalized dietary recommendations for optimal health span based on telomere length will be developed. Currently, the pesco-vegetarian diet, which is similar to the traditional Japanese (Washoku) diet, is the healthiest for the majority of subjects.

Keywords: Vegetarian; DHA; Genetic polymorphism; Fish; Cardiovascular risk

Abbreviations

AA: Arachidonic Acid; ACO: Acyl-CoA Oxidase; AGT: Angiotensinogen; AI: Adequate Intake; ALA: α-Linolenic Acid; Apo E: Apolipoprotein E; β3AR: Beta 3 Adrenergic Receptor; DGLA: Dihomo-γ-Linolenic Acid; DHA: Docosahexaenoic Acid; DTA: Docosatetraenoic Acid; EPA: Eicosapentaenoic Acid; ETA: Eicosatetraenoic Acid; FAS: Fatty Acid Synthase; GLA: Gamma-Linolenic Acid; GWAS: Genome-Wide Association Atudy; HDL-C: High Density Lipoprotein Cholesterol; LA: Linoleic Acid; LCPUFA: Long-Chain PUFA; LDL-C: Low Density Lipoprotein Cholesterol; LEPR: Leptin Receptor; MCAD: Medium-Chain Acyl-CoA Dehydrogenase; MS: Metabolic Syndrome; MUFA: Monounsaturated Fatty Acids; PL: Phospholipid; PPAR: Peroxisome Proliferator-Activated Receptor; PUFA: Polyunsaturated Fatty Acids; SFA: Saturated Fatty Acids; SNP: Single Nucleotide Polymorphism; TC: Total Cholesterol; TFA: Total Fatty Acids; TG: Triglyceride; TL: Telomere Length; UCP: Uncoupling Protein; VL: Vegans +Lactovegetarians

Introduction

There are a number of variations of the vegetarian diet [1] (Table 1): a vegan diet excludes all animal products including eggs; an ovo-lacto vegetarian diet includes both eggs and dairy products; and a pesco-vegetarian diet, which closely resembles the traditional Japanese diet (Washoku), is described as including "fish with small amounts of egg and dairy products".

	Meat	Fish	Egg	Dairy
Vegan	X	X	X	X
Lactovegetarian	X	X	X	○
Ovolacto-vegetarian	X	X	○	○
Pesco-vegetarian	X	○	Δ	Δ
Semi-vegetarian	Δ	Δ	Δ	Δ

Table 1: Classification of vegetarians studied. The circles indicate fully include in diet, the triangles, partially include in diet and X, excludes in diet.

A semi-vegetarian diet includes all foods, but fish, meat, dairy and egg intakes are modest, resembling the flexitarian diet of the Shinshu party. The American Dietetic Association states that an appropriately planned vegetarian diet, including fully vegetarian or vegan diets, is healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases [1].

Variation within vegetarian diets makes absolute categorization of vegetarian dietary practices challenging. However, one notable difference relates to the type and amount of dietary fat. Western vegetarian diets are slightly lower in total fat than omnivorous diets as expressed by % total energy (28%-32% for vegans, 30%-34% for lacto-ovo-vegetarians, and 34%-36% for omnivores) [1]. However, Western vegetarians eat about one-third less saturated fat (vegans about one-half) and about one-half as much cholesterol (vegans consume none) as omnivores [1].

It is noteworthy that the Japanese omnivore's diet is lower in total fat as expressed by % total energy (3.4% that is 18.3 g in 1950, 25.5% that is 52.9 g in 2011) [2] than that of Western vegans [1]. The Japanese omnivore diet is typically comprised of rice (326 g), vegetables (281 g), fruits (110 g), milk (96 g), meats (80 g), fish (78 g) and beans (55 g) (2011, over 20 years, per day) [2]. The intakes of saturated, monounsaturated, n-6 polyunsaturated and n-3 polyunsaturated fatty acids (PUFAs) are 14.1 g, 18.1 g, 9.2 g, and 2.3 g per day, respectively [2].

The lipid intake of Japanese middle-aged vegetarians (men: 24.9% that is 54.9 g, women 28.2% that is 52.5 g) [3] is nearly equal to that consumed by Japanese omnivores [2]. The daily diet of Japanese vegans contains lipids (24.5% that is 37 g) and mainly depends on vegetables (309 g), rice (485 g), beans (174 g, mainly soy), and fruit (36 g) without meat and fish [4]. These dietary records were confirmed by daily photographs of the meals [4]. Since the main focus of this review is the effects of fish oil and related genetic polymorphisms on the metabolism and health of Japanese vegetarians, both vegans and lactovegetarians are combined together as the VL group.

The study of vegetarians requires investigation of their genetic background. Genome-wide association studies (GWASs) typically focus on associations between single-nucleotide polymorphisms (SNPs) and traits such as major diseases and metabolism. GWASs have revealed that the adaptation to diverse diets and environments during human evolution has resulted in the global distribution of ethnically diverse phenotypes [5].

Mongoloids are genetically different from Caucasoids as the result of dietary evolution [6]. Moreover, Westernization has resulted in an increase of non-communicable diseases, such as type 2 diabetes [7], in those who previously depended on a traditional diet [6,8] including the Japanese. Recently, based on genetic analysis of 20,004 genes in the Japanese, researchers identified the Δ5 fatty acid desaturase (gene name FADS1) polymorphism rs174547 (C allele) among the Japanese [9]. The copy number of rs174547 C allele is significantly associated with increased triglyceride levels ($P=1.5 \times 10^{-6}$) and decreased HDL-C levels ($P=0.03$) in the Japanese population [9]. The Δ5 desaturase activity of rs174547 CC homozygote, indirectly calculated fatty acid desaturase product to precursor ratios, of Japanese vegetarians [4] and omnivores [10] is lower than that of TT homozygotes, according to the fatty acid profiles of both plasma and erythrocyte membrane phospholipids (PL) [4,10].

Telomere length (TL) can be used to evaluate the effects of dietary habits and determine health span, which is defined as the period of life without limitations for health reasons in both physical and social activities [11]. Vegetarian subjects tend to have longer telomeres compared to subjects on a mixed diet (vegetarians 1.31 (1.25 to 1.38); mixed 1.25 (1.18 to 1.33); p=0.088) [12]. In a 10 year analysis of particular food items consumed by 2314 subjects (40-69 years of age), a higher consumption of legumes, nuts, seaweed and fruits, and a lower consumption of red meat or processed meat were associated with longer leucocyte TL in Asians [13].

In this context, the lipid metabolism of Japanese vegetarians with and without polymorphisms of the Δ5 fatty acid desaturase gene and genes associated with diabetes and cardiovascular diseases will be discussed. The purpose of the present review is to assess the fatty acid profile and health index of rs174547 C-carrier vegetarians according to their dietary patterns. The following hypotheses are presented to explain the health of C homozygous vegans: 1. They are unable to survive, and thus no EPA/DHA-deficient vegans exist, 2. They no longer follow a vegan diet, and thus there are no C homozygotes among vegans, 3. They preferentially preserve EPA/DHA by lowered catabolism of these fatty acids; thus, the deficiency is ameliorated, 4. Their Δ5 desaturase is activated by specific factors such as SREBP-1, increasing the synthesis of EPA, 5. While their EPA+DHA/AA ratio is elevated, compensatory mechanisms prevent associated increases in harmful HDL-C, inflammation, etc., thereby maintaining health. Finally, the excellent health outcomes of the traditional pesco-vegetarian diet (Washoku) of Japan are discussed.

Longevity of Japanese vegans

According to 6,889 epitaphs on Japanese gravestones between AD700 and AD1911 (Figure 1), strict vegans belonging to three Zen priest (Zensou) parties, i.e., Oubaku (74.2 ± 3.0 year), Soutou and Rinzai, lived much longer than flexitarians including Shinshu priests and omnivores including commoners (about 40 year), feudal lords (Daimyo) (48.3 year), and court nobles (Kuge) [14].

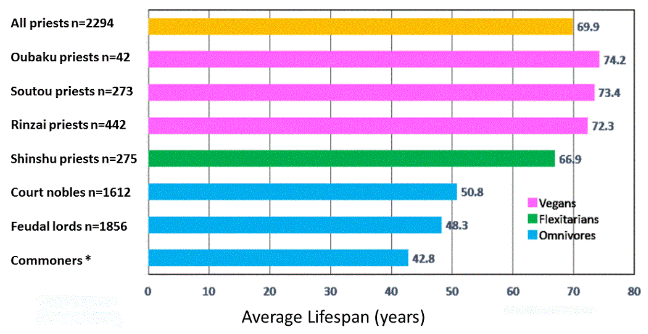

Figure 1: Historical average lifespan of Japanese vegans, flexitarians and omnivores (AD700-1911). The names of Buddhist party and social classes are indicated. Lifespans of ancient vegans (Oubaku, Soutou and Rinzai priests), flexitarians (Shinshu priests) and omnivores (Court nobles, Feudal lords and commoners) were estimated from 6,889 epitaphs on gravestones [14]. *Lifespan of commoners was obtained on all Japanese males in AD 1891.

Moreover, the standardized mortality ratio (SMR) of modern Zen priests (Rinzai, n=4352) is 0.82 ($p<0.001$), with few cases of diabetes and hypertension [15]. In fact, a vegetarian dietary pattern studied in 773 subjects (mean age 60 years) was associated with a more favorable profile of metabolic risk factors except HDL (P for trend<0.001 for those factors) and a lower risk for metabolic syndrome (OR 0.44, 95% CI 0.30-0.64, $P<0.001$) when compared with a non-vegetarian dietary pattern; this relationship persists after adjusting for lifestyle and demographic factors [16].

Japanese vegetarian men had a significantly lower body mass index ($p<0.05$), diastolic blood pressure ($p<0.001$), systolic blood pressure ($p<0.01$), and serum triacylglyceride level ($p<0.001$) than male

Japanese omnivores [3]. California Seventh-Day Adventists (n=34,192, mainly ovo-lacto vegetarians) have higher life expectancies at the age of 30 years than other white Californians by 7.28 years (95% confidence interval (CI), 6.59-7.97 years) in men and by 4.42 years (95% CI, 3.96-4.88 years) in women, possibly giving them the highest life expectancy of any formally described population [17]. The dietary patterns of vegetarians as well as their healthful lifestyle practices are thought to at least partly explain these differences.

Short lifespan of carnivores

The dietary habit of vegans is in stark contrast to that of carnivores. Our genetic studies on Mongoloids living in 6 Asia Pacific regions reveal similar allele frequencies of genes related to obesity and non-communicable diseases (Figure 2) [18,19].

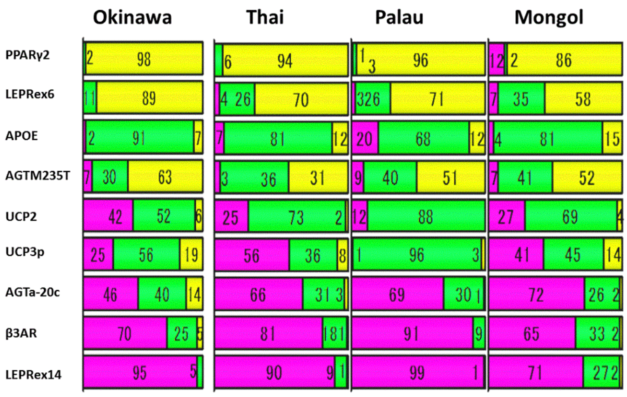

Figure 2: Variant frequencies (%) of single nucleotide polymorphisms among Asian populations. Genomic DNA was extracted from peripheral blood leukocytes and the polymorphisms were determined by the polymerase chain reaction fragment length method [18,19]. Yellow indicates disease-prone homozygotes, green indicates heterozygotes, and pink indicates disease-resistant homozygotes. PPARγ2, Peroxisome Proliferator-Activated Receptor γ2; LEPR ex, Leptin Receptor Exon; Apo E, Apolipoprotein E; AGT, Angiotensinogen; UCP, Uncoupling Protein; Δ3AR, Beta 3 Adrenergic Receptor.

However, comparative nutritional studies on Mongoloids reveal a marked dietary influence on non-communicable diseases [18-20]. Food intakes by Mongolian carnivores, Japanese vegans and Japanese omnivores are compared in the radar chart (Figure 3) [2,4,20]. The lifespan of Mongolian carnivores (68 year) was the shortest compared with peoples living on a rice diet (Japan 84 year, Thai 75 year, China 75 year) and those on a taro-fish diet known as Ubi agriculture (Palau 73 year, Tonga 71 year) (World Health Statistics 2015 of WHO). Detailed clinical, genetic and nutritional studies on nomadic Mongolians living around Murun (n=365) revealed a characteristic carnivorous daily dietary habit of large amounts of meat (122.7 g; mutton>beef>horse>goat>camel) and milk+dairy products (430.1 g; yogurt 119.1 g),but few vegetables (72.2 g), fruits (29.5 g), fish (5.5 g) and beans (0 g) (Figure 3) [19,20].

Prospective cohort studies revealed that red and processed meat intakes are associated with increased total mortality, cancer mortality, and cardiovascular disease mortality [21,22]. High intake of red meat increased the risk of mortality in 322,263 men and 223,390 women (aged 50 to 71 years); 47,976 male deaths and 23,276 female deaths were recorded during the 10 year follow-up [22]. The men and women in the highest vs lowest quintile of red meat (HR, 1.31 [95% CI, 1.27-1.35], and HR, 1.36 [95% CI, 1.30-1.43], respectively) intake showed an elevated risk for overall mortality [22].

The daily caloric intake of Murun adults was estimated to be 2,525 kcal (compared to 1,902 kcal in Japanese 50-69 year, 2011), and the fat/total caloric ratio was calculated as 33.7%, about 1.3-fold higher than that of Japanese (26.7%, 2011) [19,20], but it was not as high as that of Western omnivores because of low-fat grass-fed meat. The intake ratio of fatty acids from Mongolian foods, saturated: monounsaturated: polyunsaturated fatty acids (PUFA) ratio was 10.3:7.8:3.0 [19]. Results of clinical blood tests showed significantly higher levels of serum triglycerides, low-density lipoprotein cholesterol (LDL) and homocysteine, and lower levels of high-density lipoprotein cholesterol (HDL), n-3 PUFA, folic acid and adiponectin, compared with those of Japanese adults [20]. Oxidative stress was greater than that of the Japanese because of the low intake of vegetables (Figure 3) and the resulting deficiency of vitamins C and E; thus, reactive oxygen metabolites (ROM) levels were 429.7 ± 95.2 Carr U for Murun subjects, compared to 335.3 ± 59.8 (p<0.001) for Japanese subjects (n=220, 21-98 year) [20].

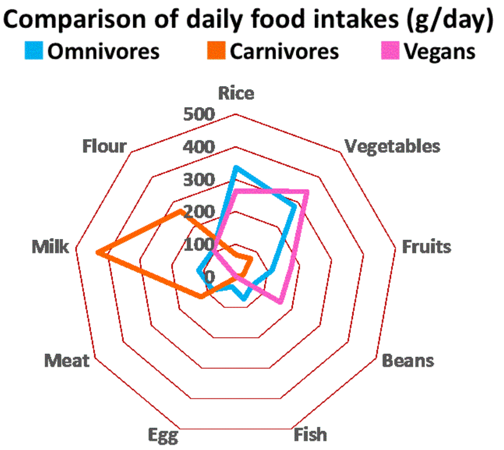

Figure 3: Radar chart displaying multivariate data of daily food intake (g/day) of Japanese omnivores and vegans, and Mongolian carnivores. Data from [2] for omnivores, from [4] for vegans, and from [20] for carnivores.

It is estimated that 9.3% of deaths in men and 7.6% in women in cohort studies (37,698 men from the Health Professionals Follow-up Study (1986-2008) and 83,644 women from the Nurses' Health Study (1980-2008) could have been prevented at the end of follow-up if all individuals consumed fewer than 0.5 servings per day (approximately 42 g/d) of red meat [21].

Long-chain polyunsaturated fatty acids

The American Dietetic Association points out that the fatty acids intake of vegetarians may be marginal in n-3 fatty acids [1]. Essential fatty acids are important constituents of all cell membranes and influence both inflammatory and atherosclerotic processes [23-25]. The essential fatty acids cis-linoleic acid (LA;18: 2 n-6) and α-linolenic acid (ALA; 18:3 n-3) are metabolized to arachidonic acid (AA; 20:4

n-6) and eicosapentaenoic acid (EPA; 20:5 n-3), respectively, by Δ5 and Δ6 desaturases [9,26] (Figure 4). AA and docosahexaenoic acid (DHA, 22:6n-3) derived from EPA are necessary components for brain development and related activities. AA, EPA and DHA are classified as long-chain polyunsaturated fatty acids (LCPUFA), with AA as n-6 LCPUFA, and EPA and DHA as n-3 LCPUFA [24-26] (Figure 4).

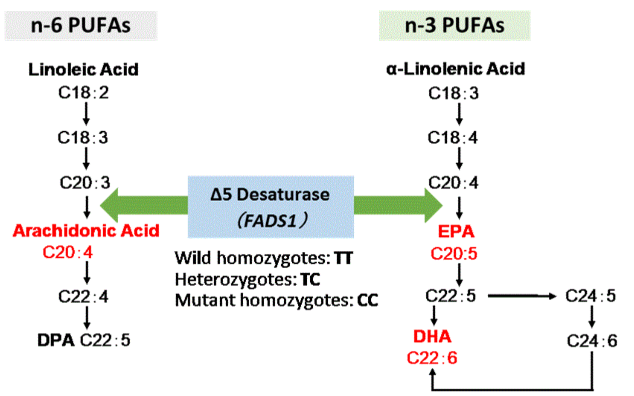

Figure 4: Metabolic pathways of n-3 and n-6 polyunsaturated fatty acids, and the Δ5 desaturase polymorphism rs174547. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; PUFA, polyunsaturated fatty acids [9,26].

Since AA is mostly derived from animal meat and eggs, and both EPA and DHA are of marine origin, these fatty acids are scarce in vegetarian diets [4]. The evidence indicates that the consumption of dietary fish oils containing n-3 LCPUFA is associated with a reduced risk of coronary heart disease in both epidemiologic and prospective randomized controlled trials [23-25]. An adequate intake (AI) of 1 g per day of EPA+DHA is recommended in the 2010 edition of Dietary Reference Intakes for Japanese [25]. The intake of fishery products was 0 g in VL and a median of 6.8 g/1000 kcal in pescovegetarians [4]. An omega-3 index (erythrocyte EPA+DHA) of >8% is associated with the greatest cardioprotection, whereas an index of <4% is associated with the least [27]; typical vegetarians (vegans+lacto-vegetarians:VL) consume nearly 0 g per day [4] (Figure 5 VL). The intake of semi-vegetarians is similar to that of young omnivores, whose consumption of fish has been decreasing, and with the more strict avoidance of animal products, there has been a further decrease in EPA+DHA intake (Figure 5).

Fatty acids in plasma and erythrocyte membrane PL of these subjects were analyzed by gas chromatography [4]. Differences in the dietary intake of EPA+DHA are reflected in the erythrocyte EPA+DHA composition (Figure 6) [4]; semi-vegetarians showed the highest levels and VL the lowest. EPA+DHA erythrocyte level of pesco-vegetarians was similar to that of young male omnivores, while that of semi-vegetarians was similar to young female omnivores. The omega-3 index is a biomarker of cardiovascular disease prevention [27]. According to the index, the EPA+DHA level of VL was at the level of least protection (Figure 6, VL) [4].

A lower serum EPA/AA ratio is associated with a greater risk of cardiovascular disease, especially coronary heart disease, among subjects with higher high-sensitivity C-reactive protein levels (>1 mg/L) in the general Japanese population (p for heterogeneity=0.007) [28]. The multivariable-adjusted risk of cardiovascular disease increased significantly, by 1.52 times (95% CI, 1.12-2.04) per 0.20 decrement in serum EPA/AA ratio [28], because of pro-inflammatory and blood coagulating effects of AA-derived prostaglandins on atherosclerosis [29].

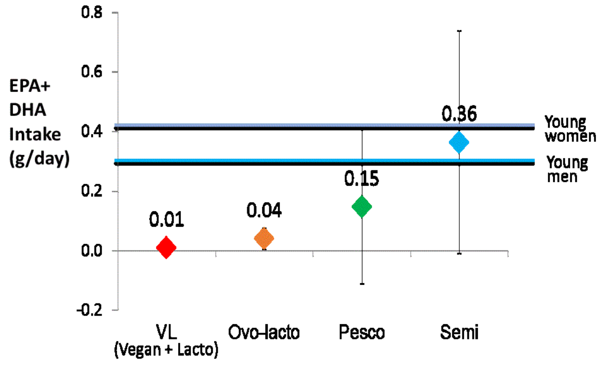

Figure 5: EPA and DHA intake levels (g/day) of Japanese vegetarians. VL (vegans and lacto-vegetarians) show the lowest intake [4]. Young men and women were 20 years old.

When DHA is deficient, it is synthesized from ALA via EPA, and AA from LA by Δ5 fatty acid desaturase [9,26] (Figure 4). Since most of the products of the AA pathway have pro-inflammatory effects [29], increased activity of both Δ5 and Δ6 desaturases in the n-6 pathway may promote systemic inflammation. Conversely, increased activity of these desaturases could stimulate the synthesis of EPA, which has anti-inflammatory properties, from ALA, counteracting the effects of AA synthesis [28]. However, EPA, DHA and AA are synthesized by these desaturases at low rates *in vivo*, as in the case of vegetarians with normal Δ5 desaturase [4] (Figure 6).

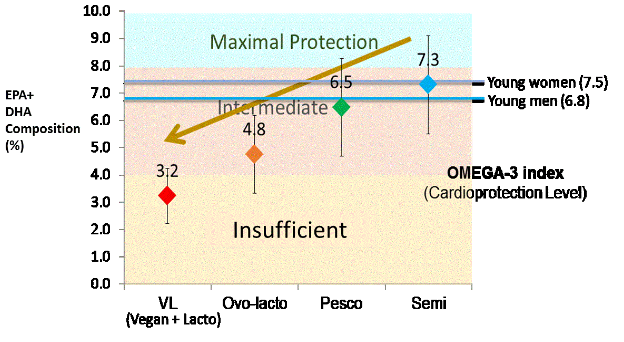

Figure 6: EPA and DHA blood levels of Japanese vegetarians. The omega-3 index is a biomarker of cardiovascular disease prevention [4]. Young men and women were 20 years old.

Polymorphism of Δ5 desaturase

In addition to decreased exogenous LCPUFAs from vegan diet, genetic variations influence endogenous plasma and erythrocyte fatty acids, particularly PUFAs. Polymorphism of Δ5 desaturase decreases the synthesis of LCPUFAs, including AA, EPA and DHA [9] (Figure 4). The genetic polymorphism was determined using a TaqMan Genotyping Assay System [4,9,10]. The genes for Δ5 desaturase

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(FADS1) exist in a cluster on chromosome 11 (11q12-q13.1) [9].The genotype frequencies of the FADS1 polymorphism rs174547 C-allele among Japanese were TT 32.3%, TC 54.0% and CC 13.7% [9]. A distribution map of this polymorphism is shown in Figure 7, in which peoples depending largely on fish, such as Palauans, show very high C-allele frequency (Figure 7).

As shown in Table 2, the numbers and frequencies of C-homozygotes (CC genotype) are not significantly different among all vegetarian types. This result excludes hypotheses 1 and 2 that postulate the selective absence of C-homozygotes in vegans.

rs174547		TT	TC	CC	Total	χ^2	P*
All subjects	N	54	84	23	161	0.57	0.75
	n (%)	-33.5	-52	-14.3			
	Allele frequencies	0.6		0.4			
VL	N	21	29	7	57	0.2	0.91
	n (%)	-36.8	-51	-12.3			
	Allele frequencies	0.62		0.38			
Ovo-lacto	N	7	15	1	23	2.1	0.35
	n (%)	-30.4	-65	-4.3			
	Allele frequencies	0.63		0.37			
Pesco	N	13	23	9	45	0.02	0.99
	n (%)	-28.9	-51	-20			
	Allele frequencies	0.54		0.46			
Semi	N	15	15	6	36	0.22	0.9
	n (%)	-41.7	-42	-16.7			
	Allele frequencies	0.63		0.38			

Table 2: Genotype frequencies of FADS1 rs174547 among 4 vegetarian groups. Genotypes CC, TC and TT indicate, respectively, mutant homozygote, heterozygote and wild type homozygote of rs174547 T>C of Δ5 fatty acid desaturase gene (FADS1). *P: Chi square test.

Japanese vegan and lactovegetarian C-allele carriers had lower relative concentrations of plasma DHA (TT=1.00, TC=0.80, CC=0.63), EPA (TT=1.00, TC=0.88, CC=0.48) and AA (TT=1.00, TC=0.77, CC=0.55) [4] (Figure 8). In all dietary patterns, the C-allele carriers tended to have higher precursor (ALA and LA) and lower product (DHA and AA) levels (Figure 8) [4].

In the case of Japanese omnivores, the rs174547 C-allele carriers had significantly lower AA and higher LA levels in plasma and erythrocyte membrane PL (Table 3) (15% and 6% AA reduction, respectively, per C-allele), suggesting a low LA to AA conversion rate in erythrocyte membrane and plasma PL of C-allele carriers (Table 3) [10].

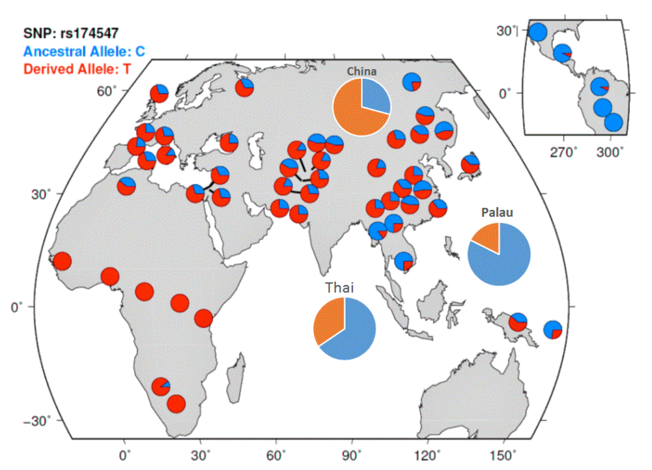

Figure 7: Geographic distribution of the allele frequency of Δ5 fatty acid desaturase polymorphism, rs174547 http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/

In omnivores, mean values of dietary lipid intakes (EPA, DHA and AA) did not significantly differ among the three genotype groups (Kruskal-Wallis test) [10]. Together with the result of low EPA/DHA intake (Figure 5) this excludes hypothesis 2, in which there is a preference for EPA and DHA by C-carriers. ALA levels were higher in the plasma PL of C-allele carriers, whereas levels of EPA or DHA were unchanged in both erythrocyte membrane and plasma PL of omnivores (Table 3) [10]. These results partially support hypothesis 3 in which both endogenous and exogenous EPA+DHA are preferentially preserved, or hypothesis 4 in which the synthesis of endogenous EPA+DHA is to some extent increased.

Fatty acid (%, total)	TT (n=47)		TC (n=58)		CC (n=19)		P
	Mean ± SD		Mean ± SD		Mean ± SD		
Plasma PL							
SFA	48	± 1.83	47.9	± 1.38	47.6	± 1.61	0.7545
MUFA	13.6	± 1.56	13.9	± 1.36	14.1	± 1.06	0.8729
PUFA	38.5	± 2.04	38.2	± 1.64	38.3	± 1.64	0.9659
LCPUFA	20	± 1.84^{a}	18.5	± 2.28^{b}	16.6	± 2.04^{c}	<0.0001
n-6 PUFA	29.4	± 2.43	29.5	± 2.55	29.7	± 3.09	0.8455
LA	18.2	± 2.11^{a}	19.5	± 2.54^{b}	21.4	± 2.54^{c}	<0.0001
GLA	0.06	± 0.04^{a}	0.05	± 0.03^{a}	0.03	± 0.02^{b}	0.0002
DGLA	1.85	± 0.49^{a}	1.96	± 0.50^{a}	1.49	± 0.29^{b}	0.0006
AA	8.9	± 1.19^{a}	7.53	± 0.98^{b}	6.33	± 1.06^{c}	<0.0001
DTA	0.15	± 0.04^{a}	0.14	± 0.04^{ab}	0.12	± 0.03^{b}	0.0126
n-3 PUFA	9.03	± 1.59	8.67	± 2.10	8.56	± 2.12	0.839
ALA	0.18	± 0.05^{a}	0.21	± 0.07^{b}	0.25	± 0.06^{b}	0.0008
EPA	2	± 0.82	1.95	± 1.02	1.89	± 1.28	0.9714

DHA	6.08	± 1.17	5.74	± 1.25	5.71	± 1.19	0.5892
AA/LA ratio	0.49	± 0.09^a	0.39	± 0.08^b	0.3	± 0.07^c	<0.0001
AA/DGLA ratio	5.12	± 1.52^a	4.04	± 0.98^b	4.37	± 0.90^{ab}	0.0006
Erythrocyte Membrane PL							
SFA	47.1	± 1.25	47.3	± 2.22	47.2	± 1.93	0.9363
MUFA	18.9	± 1.09	18.8	± 1.04	19.2	± 1.28	0.3444
PUFA	34.1	± 1.44	33.9	± 2.36	33.6	± 2.14	0.6798
LCPUFA	25.6	± 1.47^a	24.6	± 2.09^{ab}	23.7	± 1.95^b	0.0026
n-6 PUFA	23.2	± 1.89	23.4	± 2.31	23.1	± 2.36	0.8264
LA	8.38	± 1.00^a	9.13	± 1.10^b	9.73	± 1.37^b	<0.0001
DGLA	0.95	± 0.20^a	1.12	± 0.23^b	1.09	± 0.18^{ab}	0.001
AA	12.3	± 1.26^a	11.5	± 1.28^b	10.8	± 1.30^b	0.0002
DTA	1.44	± 0.35	1.38	± 0.34	1.25	± 0.30	0.2403
n-3 PUFA	10.9	± 1.49	10.5	± 1.80	10.5	± 1.78	0.8401
ALA	0.11	± 0.04	0.13	± 0.04	0.14	± 0.04	0.0506
EPA	1.38	± 0.53	1.36	± 0.58	1.4	± 0.65	0.9558
DHA	7.44	± 1.03	7.15	± 1.23	7.15	± 1.15	0.7547
AA/LA ratio	1.48	± 0.25a	1.3	± 0.17^b	1.14	± 0.16^c	<0.0001
AA/DGLA ratio	13.2	± 2.11a	10.6	± 1.92^b	9.99	± 1.28^b	<0.0001

Table 3: Fatty acid composition of phospholipids (PL) according to FADS rs174547 genotypes. TFA: Total Fatty Acid; SFA: Saturated Fatty Acid; MUFA: Monounsaturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; LCPUFA: Long Chain PUFA; LA: Linoleic Acid; GLA: Gamma-Linolenic Acid; DGLA: Dihomo-Gamma-Linolenic Acid; AA: Arachidonic Acid; DTA: Docosatetraenoic Acid; ALA: α-Linolenic Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid. ^{a,b,c}Mean values within a row with unlike superscript letters are significantly different (P<0.05; ANCOVA). Definition of genotypes, CC, CT, and TT is identical to that described in Table 2.

However, in the case of Japanese vegans, CC-carriers showed a significant decrease in both AA and DHA/EPA in plasma PL (Figure 8) [4]. The plasma fatty acid concentrations of VL in the metabolic pathway before the Δ5 desaturase step, which is the crossover point (red arrow in Figure 8), precursors ALA and ETA levels were increased, while after the crossover point, products EPA and DHA levels were decreased especially in CC-carriers compared to TT-carriers (Figure 9) [4].

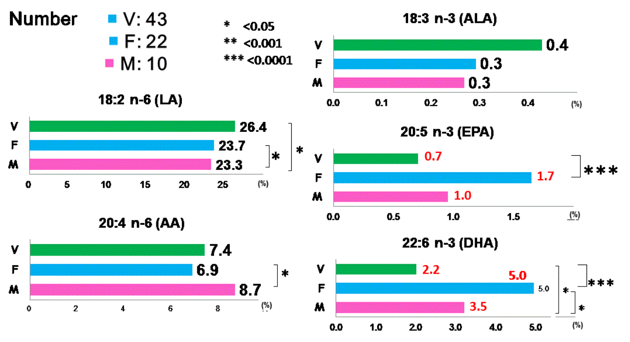

Figure 8: Fatty acid composition of plasma phospholipids of 3 vegetarian groups [4]. V, Vegans+Lacto-vegetarians; F, Pesco-vegetarian, M, Omnivores.

C allele carriers tended to have more precursors, and less fatty acid products. Especially, the CC genotype had about 60% more ALA and about 30% less DHA than the TT genotype (Figure 9). This partially discounts hypotheses 3 and 4, because both the preservation and synthesis are not deemed sufficient. On the other hand, in pesco-vegetarians, there were no significant differences in EPA and DHA composition between genotypes (Figure 10) [4]. In the case of pesco-vegetarians, the plasma concentrations of products ALA and ETA were increased, but those of products EPA and DHA were only slightly changed, in CC-carriers compared with TT carriers (Figure 10) [4]. This is because the intake of preformed EPA and DHA from fish or seafood replaces the decrease of synthesized EPA and DHA. Therefore, this indicates that dietary intake has a greater influence than the polymorphism [4].

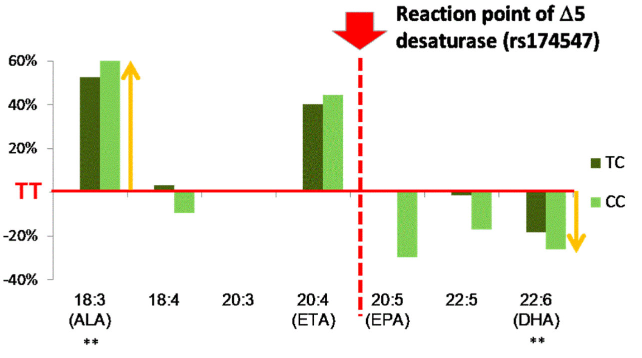

Figure 9: Decrease of EPA and DHA synthesis from ALA by C allele carriers in vegans+lacto-vegetarians (VL) group beyond Δ5 desaturase step. The synthesis of EPA and DHA proceeds from ALA via ETA (left to right direction) according to the metabolic pathway shown in Figure 4. The plasma fatty acid concentration of TT homozygotes is the base line (x-axis), and the relative concentrations of fatty acids in TC heterozygotes and CC homozygotes expressed in % are plotted on y-axis. The red arrow indicates the crossover point, where Δ5 desaturase is located on the metabolic pathway. **P<0.01.

Dietary deprivation of n-3 PUFA does not alter the half-life of AA [30], but prolongs that of DHA [31]. Positron emission tomography of ^{11}C-DHA revealed that the half-life of DHA in the human brain is approximately 2.5 years [32]. AA is used in the synthesis of prostaglandins or leukotrienes, which are involved in inflammatory responses and only very small amounts of DHA, are converted to neuroprotectins and resolvins. Moreover, the expression of DHA cascade enzymes often changed in the opposite direction to the expression of AA cascade genes [33]. This partially explains hypothesis 3, in which C-homozygote vegans preferentially preserve EPA/DHA by lowered catabolism of these fatty acids into bioactive mediators [30-32], thus ameliorating the deficiency.

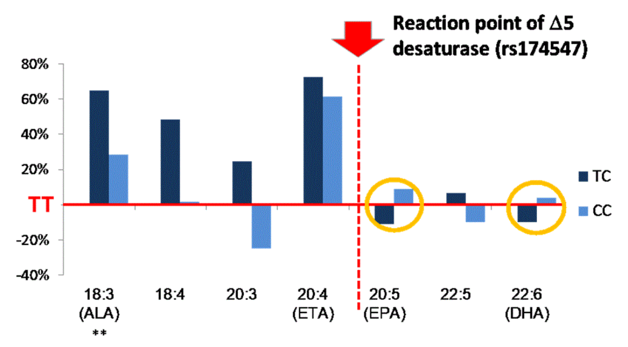

Figure 10: Decrease of EPA and DHA synthesis from ALA by C allele carriers in pesco-vegetarian group beyond Δ5 desaturase step. The synthesis of EPA and DHA proceeds from ALA via ETA (left to right direction) according to the metabolic pathway shown in Figure 4. The plasma fatty acid concentration of TT homozygotes is the base line (x-axis), and the relative concentrations of fatty acids in TC heterozygotes and CC homozygotes expressed in % are plotted on y-axis. The red arrow indicates the crossover point, where Δ5 desaturase is located on the metabolic pathway. ** P<0.01 on the other hand, in Pesco-vegetarians, there were no significant differences in EPA and DHA composition between genotypes. This is because intake of preformed EPA and DHA from fish or seafood alternates the synthesized EPA and DHA. Therefore, it could be said that dietary intake was more influential than polymorphism.

The low-fat, low-caloric diet of vegetarians increases Δ5 desaturase activities because SREBP-1 repression by AA, EPA and DHA is decreased, and SREBP-1 enhances the expression of Δ5 and Δ6 desaturase genes [26]. This partially explains hypothesis 4, in which Δ5 desaturase of C-homozygote vegans is activated by specific factors such as SREBP-1.

The most important finding was the increased EPA/AA ratio in C allele carriers of vegans and lactovegetarians (VL) compared to TT homozygotes (Figure 11). This supports hypothesis 5, in which the increase in EPA/AA ratio prevents cardiovascular diseases [28], and decreases the harmful pro-inflammatory and coagulation activity of AA-derived prostaglandins [29], and augments the beneficial anti-inflammatory activity of EPA and DHA through mediators termed resolvins and protectins [34].

There are E-series resolvins from EPA, D-series resolvins and neuroprotectins derived from DHA [34]. A significant reduction in the risk of coronary events was observed at an EPA/AA ratio of >0.75 [35]. Furthermore, the ratio of prostaglandin I_3 and prostaglandin I_2 to thromboxane A_2 has a linear relationship with the EPA/AA ratio. Prostaglandin I_3 not only inhibits platelet aggregation and vasoconstriction, but also reduces cardiac ischemic injury and arteriosclerosis and promotes angiogenesis [35].

Thus, the risk-reducing effect of EPA in cardiovascular disease is mediated by the biological action of prostaglandin I_3, in addition to the serum lipoprotein-improving action of EPA [35].

More generally, genome-wide genotyping (n=5,652 individuals) and targeted resequencing (n=960 individuals) of the FADS region in five European population cohorts, and genomic data from human populations, archaic hominins, and more distant primates, revealed that present-day humans have two common FADS haplotypes defined by 28 closely linked SNPs across 38.9 kb - that differ dramatically in their ability to generate LCPUFAs [36]. This human-specific haplotype increases the efficiency of essential long-chain fatty acids synthesis from precursors, and thereby might have provided an advantage in environments with limited access to dietary LCPUFAs [36].

Excellent health of vegetarians

The health status of vegetarians deficient in EPA and DHA was surveyed [4]. Of the total 143 vegetarians, 52 were classified as vegans +lacto-vegetarians: all animal products are excluded from the diet except dairy (VL), 39 were fish-eating vegetarians: marine products are included but not animal meat (piscivores). A cardiovascular health check revealed that more than 90% of all vegetarians including CC-carriers showed BMI <25, blood pressure <130/80 mm Hg, LDL-cholesterol <140 mg/dl and total cholesterol <220 mg/dl [4]. The majority of vegetarians had a BMI in the normal to underweight range, with only a few >25 (Figure 12). The majority of vegetarians were also characterized by low systolic blood pressure compared with the average Japanese omnivore (Figure 13) [4].

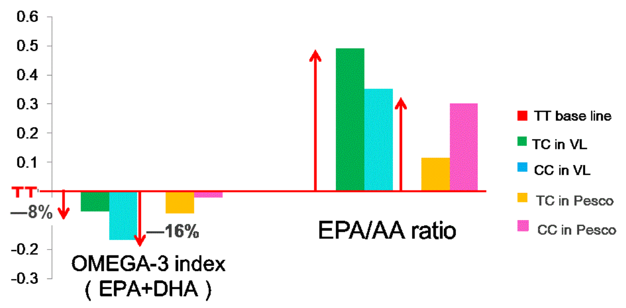

Figure 11: Effects of C-allele of Δ5 fatty acid desaturase rs174547 on omega-3 index and EPA/AA ratio [4]. Genotypes CC, TC and TT indicate, respectively, mutant homozygote, heterozygote and wild type homozygote of rs174547 T>C of Δ5 fatty acid desaturase gene (FADS1). VL, vegans+lacto-vegetarians; Pesco, pesco-vegetarians. The unit of y axis is ratio.

Plasma LDL cholesterol concentrations of vegetarians were also lower than Japanese omnivores according to Japan Atherosclerosis Society (JAS) criteria (Figure 14) [4]. Low n-3 LCPUFA intake decreases LDL-C especially in persons with CC-genotype [37]. Each C-allele of rs174547 associated with 0.05 mmol/L lower LDL

Citation: Kagawa Y, Nishijima C, Nakayama K, Iwamoto S, Tanaka A, et al. (2016) Nutrigenetics of Japanese Vegetarians with Polymorphism in the Fatty Acid Desaturase. J Nutr Food Sci 6: 498. doi:10.4172/2155-9600.1000498

concentration (P trend=0.03) [37]. Considering that a number of chronic diseases are related to obesity, high blood pressure and elevated cholesterol, this indicates that most Japanese vegetarians are characterized by good health.

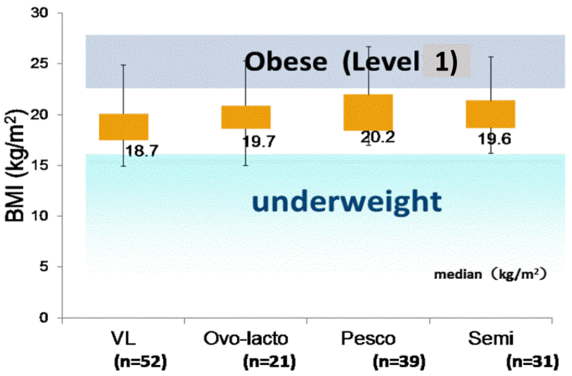

Figure 12: Body mass index (BMI) of Japanese vegetarians (kg/m^2) [4]. BMI criteria are defined by the Japan Society for the Study of Obesity. VL, Vegans+Lacto-vegetarians; Ovo-lacto, Ovo-Lacto-vegetarians; Pesco, Pesco-vegetarians, Semi, Semi-vegetarians.

Thus, the health indices of the CC-genotype support hypothesis 5, in which high EPA/AA (Figure 11) compensates for the low intake and synthesis of EPA+DHA (Figures 8 and 9).

Ethnic difference of genes

A vegan diet without cereals or a fruitarian diet is the original diet of primates, and the human lineage is thought to have diverged from the chimpanzee lineage 6 million years ago [39]. During the Paleolithic age, several types of the so-called "Paleo diet" developed depending on the environment, i.e., omnivores, carnivores and pesco-vegetarians [39]. During the Neolithic age, agriculture supplied humans with starch as a staple, and the amylase gene was duplicated in both Caucasoids and Mongoloids except for hunter-gatherers [40]. Livestock farming resulted in the persistence of lactase in Caucasoids, enabling the digestion of milk even in adults [41].

Since Mongoloids are genetically different from Caucasoids as a result of dietary evolution [6,40-42], Westernization of Mongoloids may increase the incidence of non-communicable diseases [6,7,18,42]. The thrifty allele Arg64 of Δ3-adrenergic receptors (Δ3AR), for example, is found in 100% of non-human primates in tropical regions under food shortage (Figure 2) [42]. The Arg64 allele found in 9% to 33% of Mongoloids (Figure 2, penultimate line) [18] is associated with obesity and/or non-insulin-dependent diabetes mellitus under high food availability, but was beneficial during historical periods of famine [43]. In contrast, the thermogenic allele Trp64 of Δ3AR was advantageous for increasing the body temperature of Caucasoids during the glacial period [42].

The obesity-prone Pro12 allele of peroxisome proliferator-activated receptor Δ2 (PPARγ) is found in 100% of primates [42] and most Mongoloids (Figure 2, first line) [43]. In contrast, diabetes-resistant Ala12 allele of PPARγ is found among some Caucasoids [18,43], and the Ala12 allele frequency of Japanese Americans is greater than for mainland Japanese, perhaps owing to the decreased fertility of diabetics [43].

The salt-saving allele, the Thr/Thr homozygote of the angiotensinogen gene (AGT), is found in 100% of chimpanzees living in sodium-deficient tropical rainforests [44] and in 63% of Japanese living on rainy land regions (Figure 2,5th line) [18]. In modern society with a sufficient salt supply, the Thr/Thr homozygote of AGT Met235Thr has become a risk factor for essential hypertension and visceral obesity compared to the Met/Met homozygote [45].

Impact of westernization on diseases

Westernization includes an American-type diet, less work, and an irregular activity pattern. In 1975, the major differences between average Japanese and American daily dietary intakes (kcal/day; American/Japanese) were meat (659/102.9=6.4), milk and dairy products (384/86.0=4.5), fish (27/99.3=0.27) and cereals (652/1175=0.55) [8]. In Hawaii, the overall, age-adjusted prevalence of diabetes was 18.4/1,000 about 50 years ago, and the most striking ethnic differences were that the rates of diabetes for unmixed Hawaiians (paleomongoloids) and Japanese (neomongoloids) were about six and three times that of Caucasians, respectively, owing to the westernization of Mongoloids carrying thrifty genes [6] in Hawaiian modern society [7].

In addition to diabetes, hypertension has increased in natives after Westernization. Populations of the Polynesian islands Rarotonga and Pukapuka are ethnically similar, but observe different economic and social structures and dietary habits [45]. In Westernized Rarotonga, the mean blood pressure increases with age owing to AGT polymorphism, whereas in primitive Pukapuka, age-related increases of blood pressure are slight [45]. The impact of Westernization on Japanese nutrition becomes evident after the 1970s [8].

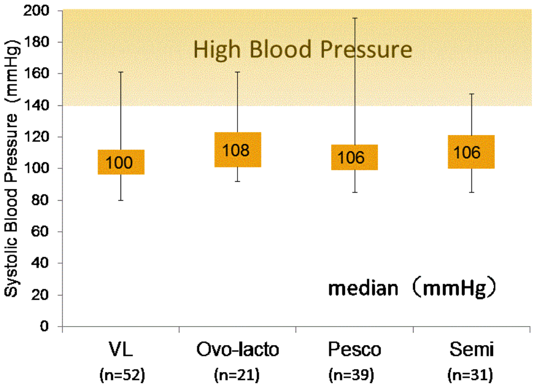

Figure 13: Systolic blood pressure of Japanese vegetarians (mmHg) [4]. Blood pressure criteria are defined by the Japan Society of Hypertension. VL, Vegans+Lacto-vegetarians; Ovo-lacto, Ovo-Lacto-vegetarians; Pesco, Pesco-vegetarians, Semi, Semi-vegetarians.

In the 1980s, the lifespan of Okinawans was the longest in Japan (1985, male 76.34 year, female 83.70 year); however, the rates of obesity, diabetes and hypertension increased more rapidly in Okinawa than in mainland Japan, and the relative lifespan ranking among Japanese males dropped to 31st.

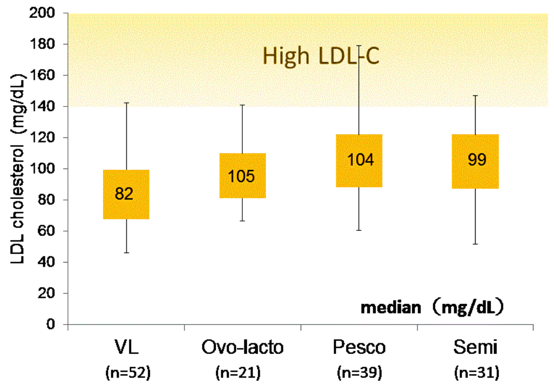

Figure 14: Serum low-density lipoprotein cholesterol (LDL-C) concentrations (mg/dl) of Japanese vegetarians. LDL-cholesterol level is defined by the Japan Atherosclerosis Society [4]. VL, Vegans +Lactovegetarians; Ovo-lacto, Ovo-Lacto-vegetarians; Pesco, Pesco-vegetarians, Semi, Semi-vegetarians.

The fat intake of Japanese increased from 18 g in 1950 [8] to 58.5 g in 2011 [2]. In 1982, the fat/energy ratio was 22.5% and 29.6% in Japan as a whole and Okinawa, respectively, and the prevalence of obesity (BMI>25) was 30.3% and 46.9%, respectively [2,46]. The prevalence of type 2 diabetes per 100,000 Japanese was only 163 (55-64 year) [8] in 1965, but increased to 26,500 (60-69 year, male) in 2011 [2], representing a 163-fold increase with Westernization (Figure 15).

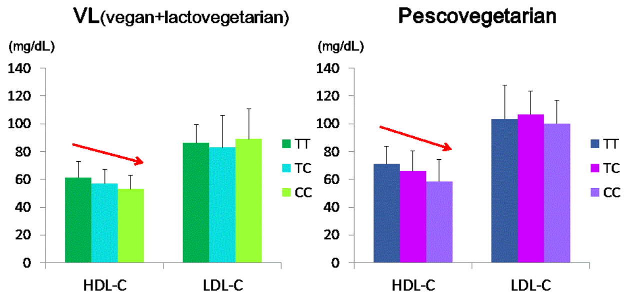

Figure 15: Effects of C-allele of Δ5 fatty acid desaturase rs174547 on HDL-C and LDL-C in vegan-lactovegetarian and pesco-vegetarian groups. Genotypes CC, TC and TT indicate, respectively, mutant homozygote, heterozygote and wild type homozygote of rs174547 T>C of Δ5 fatty acid desaturase gene (FADS1) [4].

The preference for a traditional Japanese diet (caloric density of 1.0 kcal/g) to a Western diet (caloric density of 1.7 kcal/g) is explained by the unique taste of fat, termed oleogustus [47]. Fatty acids have a taste sensation that is distinct from the other 5 basic tastes (sweet, sour, salty, bitter, and umami) [47]. Even those living in Kyoto, where a traditional Japanese pescovegetarian diet is superficially conserved, and the consumption of bread (63 kg/year, Japanese average 45 kg/year) are the highest in Japan according to Japanese government statistics [48].

Short lifespan of piscivores

The Inuit have long consumed a specialized high-fat diet rich in n-3 PUFAs. They belong to a group of carnivores specialized to eat piscivores, including aquatic mammals such as sea lions. Inuit youth have a mortality rate 3 times that of non-Aboriginal youth, and are 4 times more likely to suffer an injury [49]. Statistics Canada 2006 revealed that of 50,485 Inuit, only 1,845 (3.7%) were 65 year and over, and 535 (1.1%) were 75 year and over [50].

The most pronounced allele-frequency difference between the Inuit and other populations is found in a cluster of fatty acid desaturases-FADS1, FADS2, and FADS3A that determine PUFA levels [51]. Analyses of erythrocyte membrane lipids of the Inuit revealed modulation of fatty acid composition: EPA/AA ratio and DHA/AA were 0.91 and 1.42, respectively [52], compared to those of Japanese at 0.11 and 0.60, respectively [10] (Table 3).

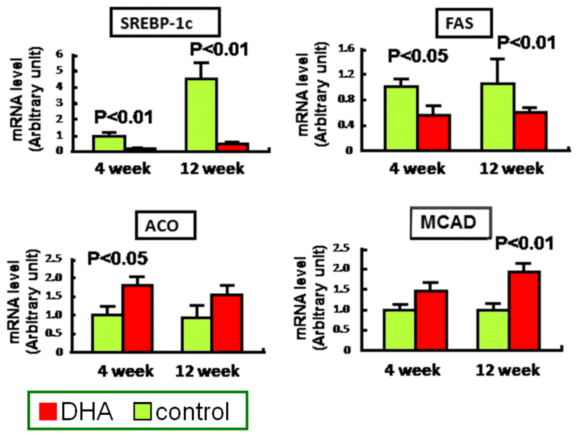

Figure 16: Down regulation of SREBP-1c and fatty acid synthase, and upregulation of acyl-CoA oxidase and medium-chain acyl-CoA dehydrogenase by feeding DHA. Male Crlj: CD-1 (ICR) mice were fed experimental diets containing 1.5%DHA+4.5% lard (red bars) or 6% lard (green bars) for 12 weeks [73]. Expressed mRNA in liver in the mice was isolated with the commercial kits, Wako ISOGEN (Wako). The mRNA level was determined by using the ABI PRISM 7700 System (PE Applied Biosystems, Foster City, CA) as reported [74]. SREBP-1c, Sterol regulatory element-binding protein 1c; FAS, fatty acid synthase; ACO, acyl-CoA oxidase; MCAD, medium-chain acyl-CoA dehydrogenase.

Unlike short- and medium-chain fatty acids, PUFAs cannot be transported across the mitochondrial membrane by simple diffusion. PUFAs are first converted to their co-A esters, and in combination with carnitines, they are converted to acylcarnitines by the CPT1A enzyme and transported across the mitochondrial membrane.

In fact, an association between liver-specific polymorphism of CPT1A and liver-specific desaturases has been found in Alaskan Inuit [53]. Since Inuit rarely consume vegetables and fruits, the incidence of congenital sucrase-isomaltase deficiency is extremely high; the allele frequency of the frame-shift mutation c.273_274delAG is 17.2% [54]. Thus, the Inuit have genetic and physiological adaptations to a diet rich in LPUFAs.

Factors supporting the longevity

Vegetarian dietary patterns are associated with lower body mass index (Figure 12 and Table 4) [3,4,16], lower prevalence and incidence of diabetes mellitus [3,4,16], metabolic syndrome and its component factors (Table 4) [16], including hypertension (Figure 13 and Table 4) [4] and dyslipidemia (Figure 14 and Table 4) [4], and lower all-cause mortality [16]. It is important to note that metabolic syndrome is accompanied by decreased PUFA n-6 family content and decreased Δ5 desaturase activity (both $p<0.001$) [55].

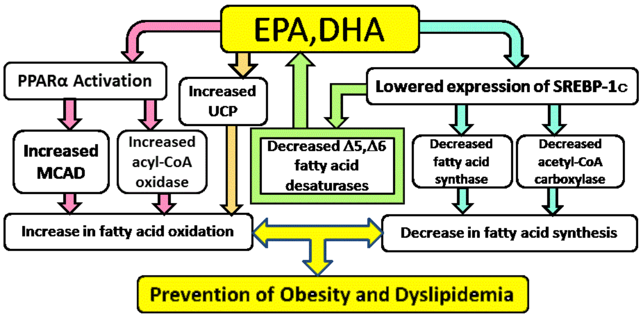

Figure 17: Upregulation of fatty acid oxidation, and downregulation of fatty acid synthesis, and Δ5- and Δ6-desaturation by both EPA and DHA. Data summarized from [26,73,74]. SREBP-1c, Sterol regulatory element-binding protein 1c; PPARγ, peroxisome proliferator-activated receptor-α.

Thus, in vegetarians, Δ5 desaturase activities may be increased to compensate for the deficiency in EPA and DHA from dietary fish. Plasma levels of all coagulation or fibrinolytic factors and natural inhibitors synthesized in the liver were lower in lactovegetarians and lacto-ovo-vegetarians than in omnivore controls [56]. Vegetarians showed significantly shortened bleeding time, and increased blood platelet count and *in vitro* platelet function (aggregation and secretion) [56]. Statistically significant decreases in certain blood coagulation factors were also observed (fibrinogen, factor VIIc, antithrombin III, protein S, plasminogen) [56].

Greater adherence to a plant-based diet is associated with lower all-cause mortality [57]. In addition to the beneficial effects of vitamins, minerals and dietary fibers from plants, the component flavonoids also have effects on human health [58]. The plant-based diet is associated with a high intake of flavonoids in vegetarians [58].

Emerging evidence highlights dietary flavonoids as candidates that may explain, at least in part, the cardio protective effect of a fruit- and vegetable-based diet [58]. Epidemiological studies indicated that the regular intake of fruits, vegetables and tea, which contain high polyphenol levels, is associated with a reduced risk of cardiovascular disease [59].

The beneficial effect of polyphenol-rich natural products is attributed, at least in part, to their direct effect on blood vessels, and in particular on endothelial cells [59]. Indeed, polyphenols from tea, grapes, berries, and plants are shown to activate endothelial cells to increase the formation of potent vasoprotective factors, including nitric oxide (NO) and endothelium-derived hyperpolarizing factor [59]. The diet of Japanese vegetarians depends largely on soy bean.

Soy protein with intact isoflavones was associated with significant decreases in serum total cholesterol (by 0.22 mmol/L, or 3.77%), LDL cholesterol (by 0.21 mmol/L, or 5.25%), and triacylglycerols (by 0.10 mmol/L, or 7.27%) and significant increases in serum HDL cholesterol (by 0.04 mmol/L, or 3.03%) [60].

The lowest mortality of pesco-vegetarians

Vegetarian diets are associated with lower all-cause mortality and some reductions in cause-specific mortality compared to omnivore diets according to a large American cohort [61]. Moreover, the varied vegetarian dietary patterns [1] are associated with different mortality rates [61]. The mortality rate of 73,308 participants during a mean 5.79 years follow-up was 6.05 (95% CI, 5.82-6.29) deaths per 1000 person-years [61] (Figure 18).

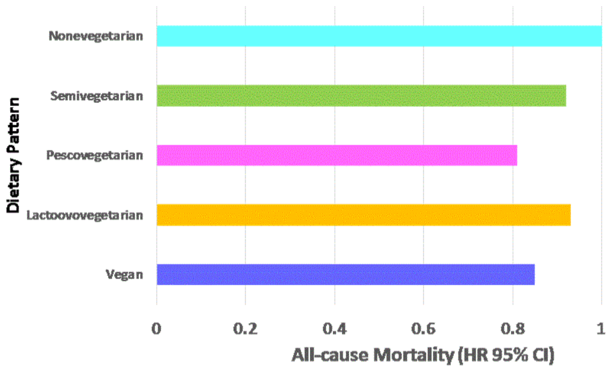

Figure 18: The association between vegetarian dietary patterns and all-cause mortality. Modified from Orlich and Fraser [61].

The adjusted hazard ratio (HR) for all-cause mortality in all vegetarians combined vs non-vegetarians was 0.88 (95% CI, 0.80-0.97) [61]. The adjusted HR for all-cause mortality in vegans was 0.85 (95% CI, 0.73-1.01); in Lacto-ovo-vegetarians, 0.91 (95% CI, 0.82-1.00); and in pesco-vegetarians, 0.81 (95% CI, 0.69-0.94) compared to non-vegetarians [61]. Significant associations with vegetarian diets are detected for cardiovascular mortality, renal mortality, and endocrine mortality [61].

A collaborative analysis of 5 prospective studies revealed that in comparison with regular meat eaters, mortality from ischemic heart disease was 34% lower in pesco-vegetarians and 26% lower in vegans [62]. In a Taiwanese population, the risk of metabolic syndrome was the lowest in pesco-vegetarians compared with other vegetarians and omnivores [63]. A total of 93209 participants were classified as vegans (n=1116), pesco-vegetarians (n=2461), lactovegetarians (n=4313) and non-vegetarians (n=85319) using the food frequency list of a self-administered questionnaire at baseline [64]. The association between metabolic syndrome (MS) and different dietary groups was evaluated using a Cox proportional-hazards regression model with adjustment for confounders [63]. During the mean 3.75 years of follow up, a total of 8006 MS incident cases occurred, and the incidence of MS was 229 (95% CI, 224, 234) per 10000 person-years.

	All subjects		VL		Ovo-lacto		Pesco		Semi		P*
	M	SD	M	SD	M	SD	M	SD	M	SD	
BMI (kg/m^2)#											
TT	20	± 2.3	19	± 1.5	22	± 2.5	20	± 2.4	21	± 2.4	0.02
TC	20	± 2.5	19	± 2.1	20	± 2.3	20	± 2.9	20	± 2.6	
CC	20	± 2.9	19	± 3.1	18		21	± 2.7	21	± 2.9	
P**	0.329		0.93		0.088		0.627		0.654		
SBP (mm Hg)											
TT	110	± 14	110	± 17	114	± 13	109	± 13	108	± 13	0.29
TC	105	± 13	102	± 12	110	± 19	106	± 11	106	± 13	
CC	113	± 32	102	± 15	111		112	± 32	129	± 48	
P**	0.101		0.924		0.591		0.108		0.142		
DBP (mm Hg)											
TT	66	± 10.7	65	± 11.9	65	± 10.9	67	± 10.9	66	± 9.7	0.47
TC	64	± 11.8	61	± 10.9	67	± 17.6	65	± 10.6	64	± 7.7	
CC	66	± 15.1	62	± 11.2	70		62	± 13.3	75	± 20.3	
P**	0.555		0.942		0.591		0.135		0.471		

Table 4: Relationship between rs174547 genotypes and BMI and blood pressure (BP). * One-way ANOVA between vegetarians (*) and genotypes (**). BMI: Body Mass Index; SBP and DBP: Systolic and Diastolic Blood Pressure; VL: Vegan+Lacto-vegetarian; Ovolacto, Ovo-Lacto-vegetarian; Pesco, Pesco-vegetarian; Semi: Semi-vegetarian. Definition of genotypes, CC, CT, and TT is identical to that described in Table 2. #For Asians obesity means BMI>25, and pre-obesity, 23<BMIH<25.

Compared with vegans, hazard ratios for MS in non-vegetarians, pesco-vegetarians, lactovegetarians were 0.75 (95% CI, 0.64, 0.88), 0.68 (95% CI, 0.55, 0.83) and 0.81 (95% CI, 0.67, 0.97) after adjusting for sex, age, education status, smoking, drinking, and physical activity [63]. Pesco-vegetarians had 0.70 (95% CI, 0.57, 0.84) times the risk of developing low HDL-C [63].

Molecular evaluation of vegetarian diets

In order to compare the effects of several kinds of dietary habits, changes in body weight [3,4], serum lipoproteins and lipids [3,4], blood pressure [3,4], blood sugar and other clinical parameters are conventionally used. However, these values can fluctuate over the short term, and can be artificially altered by drugs, etc. The overall target of this article is to determine how adopting certain dietary habits can attain the best health span. For this purpose, we needed to investigate the underlying molecular mechanisms including telomere length (TL) [11,13,64]. A vegetarian diet rich in vegetables, fruits, nuts, olive oil, and fish was employed as the working recipe for health span, as reflected by TL [64]. In women, vegetable intake was positively associated with leucocyte TL (P=0.05) [12,13,65]. Vegetables contain vitamins, minerals, fibers and flavonoids that maintain TL [64]. Recent studies have suggested that dietary folate can influence TL because it is required for DNA synthesis, repair, and one-carbon metabolism within the cell [66]. Further, folate reduces harmful serum homocysteine. The difference in multiply-adjusted leucocyte TL between the highest and lowest tertile of homocysteine levels was 111 base pairs (p=0.004), corresponding to 6.0 years of telomeric aging. This relationship was further accentuated by decreased concentrations of serum folate [67].

Vegetarian diets are rich in dietary fiber, the intake of which is positively associated with TL (Z score), specifically cereal fiber, with an increase of 0.19 units between the lowest and highest quintiles (P=0.007, P for trend=0.03) [68].

On the other hand, the vegetarian diet is low in lipids. In fact, total fat and SFA intake (P=0.04 and 0.01, respectively) are inversely associated with leucocyte TL in men after adjusting for age and energy intake [65]. PUFA intake (-0.26 units, quintile 5 compared with quintile 1: P=0.002, P for trend=0.02), specifically linoleic acid intake, was inversely associated with TL (-0.32 units; P=0.001, P for trend=0.05) [66]. Intake of short-to-medium-chain saturated fatty acids (SMSFA: aliphatic tails of ≤ 12 carbons) is inversely associated with TL [69]. Compared with participants in other quartiles of SMSFA intake, women who were in the highest quartile (median: 1.29% of energy) had shorter TL [mean: 4.00 kb (95% CI: 3.89, 4.11 kb)], whereas women in the lowest quartile of intake (median: 0.29% of energy) had longer TL [mean: 4.13 kb (95% CI: 4.03, 4.24 kb); P-trend=0.046] [69]. Except for lauric acid, all other individual SMSFAs are inversely associated with TL (P<0.05) [69]. Notably, the pesco-vegetarian diet is rich in n-3 LCPUFAs such as DHA and EPA [3,4]. The rate of telomere shortening over 5 years is inversely related to baseline blood levels of marine n-3 fatty acids [70]. Each 1-SD increase in DHA+EPA levels was associated with a 32% reduction in the odds of telomere shortening (adjusted odds ratio, 0.68; 95% CI, 0.47-0.98) [70].

A vegetarian diet reduces waist circumference [3,4], which is inversely associated with TL [0.15-unit difference in z score in a comparison of the highest (≥ 32 in, 81.28 cm) with the lowest (≤ 28 in, 71.12 cm) category (P=0.01, P for trend=0.02) in the multivariate model] [68].

Detailed metabolic analysis of DHA, EPA and ALA revealed that limited storage of the n-3 fatty acids in adipose tissue [71]. Thus, a continued dietary supply of n-3 fatty acid is needed. A large proportion of dietary ALA is oxidized as shown by experiments using uniformly labeled [^{13}C] ALA or [^{2}H] ALA as a tracer [71]. Because only less than 1% of exogenous ALA is converted to endogenous EPA in humans, ALA supplementation does not result in appreciable accumulation of n-3 LCPUFA in plasma [71]. EPA but not DHA concentrations in plasma increase in response to dietary EPA [71]. Evidence from molecular research studies indicates that the cardio protective effects of n-3 PUFA result from a synergism between multiple, intricate mechanisms that involve ant inflammation, pro resolving lipid mediators, modulation of cardiac ion channels, reduction of triglycerides, influence on membrane micro domains and downstream cell signaling pathways and antithrombotic and antiarrhythmic effects [72]. The n-3 PUFAs inhibit inflammatory signaling pathways (NF-κB) [72] and down-regulate fatty acid synthesis gene expression (SREBP-1c) [26,73] and up-regulate gene expression involved in fatty acid oxidation (PPAR-γ) [26,72]. DHA causes profound effects on glucose and lipid metabolism including acceleration of glucose uptake and glycerol synthesis in the liver via increased adiponectin [73]. The regulation of lipid metabolism by DHA and EPA are summarized in Figures 16 and 17 [26,73,74].

As shown in Figure 14, DHA strongly inhibits expression of mRNA of SREBP-1c [26,73] (Figure 16 left upper) and thus expressions of the downstream fatty acid synthase (Figure 16, FAS, right upper) and acetyl-CoA carboxylase are down regulated (Figure 17, right) [73]. On the other hand, the activation of PPARγ by DHA causes up regulation of peroxisomal acyl-CoA oxidase and middle chain fatty acid dehydrogenase (Figure 16, bottom, ACO and MCAD Figure 17, left) [26,73]. In addition, dietary DHA+EPA reduced both PL and TG in plasma of C57BL/6N Jcl mice, perhaps via activation of the PPAR system, and increased UCP2 and UCP3, which enhance fatty acid oxidation (Figure 17, UCP) [74].

The liver (n-3) PUFA levels of mice fed with DHA+EPA were higher than those with control; EPA was 29-fold greater in males ($P<0.001$) and 18-fold greater in females ($P<0.001$); DHA was 6 fold greater in males ($P<0.001$) and 5 fold greater in females ($P<0.001$), and palmitic and stearic acids were significantly increased ($P<0.001$) compared with those of the control, and MUFA and (n-6) PUFA levels in were lower than those in the control [74]. The regulatory effects of EPA+DHA on fatty acid metabolism are summarized in Figure 17 [26,72-74]. The gene expression profiles of both EPA+DHA and control groups were analyzed using cDNA expression array [74]. Of 588 genes surveyed in the array, the EPA+DHA showed 12 genes (2%) including those for glucose regulators and tumor suppressors that were expressed 100-340% more than those of control group [74]. Furthermore, 28 genes (4.8%), including growth factors and immune regulators were expressed 50-90% less in the EPA+DHA group than in the control group. These results explain in part the important pleiotropic effects of EPA+DHA [74].

Measures to supply EPA

The conversion rate of ALA to EPA and DHA is slow even in persons with normal Δ5 desaturase activity; thus, a pesco-vegetarian diet is generally the best for health span [61-63]. However, the world's fish supply is decreasing [75]. On the other hand, from the 144 million tons produced in 2006 by capture fisheries (53%) and aquaculture (47%), about 110 million tons were used for food directly and 33 million tons indirectly. This represented a record level of per capita supply of 16.7 kg (13.6 kg excluding China and 13.8 kg in low-income food-deficit countries). In the next 40 years, the marine capture of fish will dramatically decrease [75] In addition to updated policies, new science is needed to best address those challenges.

Seaweeds are a characteristic Japanese food consumed by vegans. Since fish EPA is originally synthesized by seaweed, the intake of nori (Rhodophyceae: *Pyropia tenera*), wakame (Phaeophyceae: *Undaria pinnatifida*) and kombu (Phaeophyceae: *Laminaria hyperborean*) is recommended for both omnivores and vegans. The EPA content of nori, for example, is 54.2% of total fatty acids (1.6 g/100 g food).

Another measure is the use of genetically modified foods, including stearidonic soy bean [76], n-3 pork [77] and n-3 beef [78]. Stearidonic acid (SDA: 18:4n-3) is efficiently converted into EPA and DHA [76]. Thus, we tested the effects of genetically modified stearidonic soybean oil on the lipid metabolism of rats fed the following diets: FFC diet (fish-oil-free control diet), C diet (control group, assuming a Japanese diet), SDA25 diet (25% SDA soybean oil in the C diet), SDA50 (50% SDA soybean oil in the C diet), ALA diet (34% flaxseed oil in the C diet), and EPA+DHA diet (34% fish oil in the C diet) [76]. SDA intake increased the relative efficiency of EPA accretions in serum and liver triacylglycerol and significantly decreased the serum triacylglycerol level in rats [76]. The results suggested that the consumption of SDA soybean oil might modify the lipid and fatty acid profiles, even when fish-derived EPA and DHA is absent [76].

An alternative approach to increase the levels of n-3 PUFAs in meat is to generate transgenic livestock animals. Livestock cannot convert n-6 fatty acids into n-3 fatty acids because they lack the n-3 fatty acid desaturase fat-1 gene. Transgenic pigs harboring the fat-1 gene from *Caenorhabditis briggsae* accumulated high levels of EPA and DHA [77]. Analysis of fatty acids demonstrated that cbr-fat-1 transgenic pigs produced high levels of n-3 fatty acids from n-6 analogs; consequently, a significantly reduced ratio of n-6/n-3 fatty acids was observed. Similarly, transgenic cattle expressed a *Caenorhabditis elegans* gene, mfat-1, encoding an n-3 fatty acid desaturase [78]. Fatty acid analysis of tissue and milk showed that all of the examined n-3 PUAFs were greatly increased, while the n-6 PUAFs were simultaneously decreased in the transgenic cow. Significant reductions of n-6/n-3 ratios ($P<0.05$) in both tissue and milk were observed.

The merits of washoku

Life expectancy in Japan is high (men 80.50 year, women 86.61 year, 2014), suggesting that the Japanese diet (Washoku), which is based on a pesco-vegetarian diet, has significant health benefits. In 2013, the United Nations Educational, Scientific, and Cultural Organization (UNESCO) agreed to register Washoku as an intangible cultural heritage. One of the reasons for this designation is because it is an exceptionally well-balanced and healthy pesco-vegetarian diet. However, these benefits have been called into question over the past 50 year, during which time Washoku has become increasingly Westernized and the prevalence of non-communicable disease has increased. Although the Japanese lifespan is long, the health span is estimated to be about 9 year and 13 year shorter than the lifespan of men and women, respectively [79]. In order to assess the influence of Westernization, rats were fed weekly menus from the years 1960, 1975, 1990, and 2005, which were reproduced based on the National Health and Nutrition Survey in Japan [80]. In the group fed the typical 1975 Japanese diet, lifespan was prolonged, senescence was delayed, and learning and memory capacities were maintained compared with the group fed the 2005 Japanese diet [80]. In Kohama islands of Okinawa, the islanders are characterized by high fish intake (147.7 g/day), high serum EPA (6.82 ± 2.54 mg/dl) and good health indices with very low signs of cardiovascular diseases [81].

The 20 year follow-up studies revealed significantly low standardized mortality ratio (SMR: male 84.99, female 79.20; *vs.* all Okinawa 100), ischemic heart diseases and pneumonia [82]. Similarities between Kohama pesco-vegetarian diet [81] and the Cretan Mediterranean diet (MD) [83] have been pointed out because of the high intakes of fish and vegetables, and similar blood biochemistry [81]. Cardiovascular protective effects are related to changes observed in plasma fatty acids: an increase in EPA/DHA and oleic acid and a decrease in linoleic acid, resulting from higher intakes of fish and olives, but lower meat intake [83]. Like the Japanese who follow the Washoku diet, Caucasoid individuals who adhere to the principles of the traditional MD tend to have a longer lifespan [84]. A meta-analysis showed that adherence to a MD can significantly decrease the risk of overall mortality, and mortality from cardiovascular diseases in Caucasoids [85]. Both men and women who reported consuming foods closest to the MD were about 10–20% less likely to die over the course of a study of heart disease, cancer or any other cause [84].

Conclusion

Following the work of Dyerberg et al. [86] and Kagawa et al. [81], which indicated the protective effect of EPA+DHA on risk of developing cardiovascular diseases, metabolomics elucidated the detailed lipid metabolism [87]. Molecular biology [70-74] and epidemiology [23-25,27] established the importance of EPA+DHA for healthspan [11]. Thus, the daily intake of 1 g of EPA+DHA is recommended [25] to maintain the omega-3 index [27]. But, vegans, especially those with the CC allele of rs174547, have less plasma DHA, EPA and AA than TT carriers and omnivores (Table 3) [4,10]. However, cardiovascular risk factors are greatly reduced in all vegetarian groups (Figures 10-12), including CC allele carriers (Table 4) [4], compared with the average Japanese [2]. Despite the low DHA/EPA intake, the health span of vegans, especially those with the C allele of rs174547, can be maintained by a mechanism to lower LDL-C only when exogenous EPA+DHA is low, and to increase HDL-C when ALA/LA ratio is high [37], and by the increased EPA/AA ratio described by hypothesis 5 (Figure 11, right) [4]. The higher the EPA/AA ratio, the lower the risk of cardiovascular disease [28,35]. Owing to the mutant Δ5 desaturase activity, the decrease of AA level is much faster than that of EPA level in the C allele carriers [4,10].

The health indices of vegans were found to be excellent (Figures 12-14), but in the long time scale, their health span is not as good as that of pesco-vegetarians [61-64]. The health of vegans might be improved under the following conditions: 1. Adaptation to decrease EPA catabolism and maintain DHA levels in the brain [30,32], and increase weak mutant Δ5 desaturase activity by removal of EPA/DHA +AA inhibition of SREBP-1 [26]. Notably, dietary n-3 PUFA deprivation does not alter the half-life of AA [30], but prolongs that of DHA [31]. 2. Efficient use of EPA from seaweed. 3. Increased intake of substrate α-linoleic acid from vegetables, which is ineffective for omnivores [69] 4. The use of genetically modified stearidonic soy bean [74]. In future, genotype-specific personalized dietary recommendations for optimal health span, based on telomere length, will be developed. At present, epidemiological analysis has revealed that a pesco-vegetarian diet similar to the traditional Washoku diet results in the lowest death rate among all vegetarian types [59-61], which is also supported by molecular level experiments [68-72].

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References

1. Craig WJ, Mangels AR; American Dietetic Association (2009) Position of the American Dietetic Association: vegetarian diets. J Am Diet Assoc 109: 1266-1282.
2. Ministry of Health, Labor and Welfare, Japan (2011) National Health and Nutrition Survey. National Institute of Health and Nutrition, Tokyo. (Partly English and Japanese).
3. Nakamoto K, Watanabe S, Kudo H, Tanaka A (2008) Nutritional characteristics of middle-aged Japanese vegetarians. J Atheroscler Thromb 15: 122-129.
4. Nishijima C (2012) Effects of Nutritional Intake and a Fatty Acid Desaturase Gene Polymorphism on Blood Lipid Profiles and Risk Factors for Cardiovascular Diseases in Japanese Vegetarians. Master's Thesis, Kagawa Nutrition University, Japan.
5. Tishkoff S (2015) Genetics. Strength in small numbers. Science 349: 1282-1283.
6. Kagawa Y, Yanagisawa Y, Hasegawa K, Suzuki H, Yasuda K, et al. (2002) Single nucleotide polymorphisms of thrifty genes for energy metabolism: evolutionary origins and prospects for intervention to prevent obesity-related diseases. Biochem Biophys Res Commun 295: 207-222.
7. Sloan NR (1963) Ethnic distribution of diabetes mellitus in Hawaii. JAMA 183: 419-424.
8. Kagawa Y (1978) Impact of Westernization on the nutrition of Japanese: changes in physique, cancer, longevity and centenarians. Prev Med 7: 205-217.
9. Nakayama K, Bayasgalan T, Tazoe F, Yanagisawa Y, Gotoh T, et al. (2010) A single nucleotide polymorphism in the FADS1/FADS2 gene is associated with plasma lipid profiles in two genetically similar Asian ethnic groups with distinctive differences in lifestyle. Hum Genet 127: 685-690.
10. Horiguchi S, Nakayama K, Iwamoto S, Ishijima A, Minezaki T, et al. (2016) Associations between a fatty acid desaturase gene polymorphism

and blood arachidonic acid compositions in Japanese elderly. Prostaglandins, Leukotrienes and Essential Fatty Acids 105: 9-14.

11. Kagawa Y (2012) From clock genes to telomeres in the regulation of the healthspan. Nutr Rev 70: 459-471.
12. Mukherjee M, Brouilette S, Stevens S, Shetty KR, Samani NJ (2009) Association of shorter telomeres with coronary artery disease in Indian subjects. Heart 95: 669-673.
13. Lee JY, Jun NR, Yoon D, Shin C (2015) Association between dietary patterns in the remote past and telomere length. Eur J Clin Nutr 69: 1048-1052.
14. Mori H, Kudo N (1988) Studies on the lifespan of Japanese Buddhist priests: From Nara era (AD700) to Meiji era (AD1911). J Health and Human Ecol (Minzoku Eisei) 54: 134-135.
15. Ogata M, Ikeda M, Kuratsune M (1984) Mortality among Japanese Zen priests. J Epidemiol Community Health 38: 161-166.
16. Rizzo NS, Sabaté J, Jaceldo-Siegl K, Fraser GE (2011) Vegetarian dietary patterns are associated with a lower risk of metabolic syndrome: the adventist health study 2. Diabetes Care 34: 1225-1227.
17. Fraser GE, Shavlik DJ (2001) Ten years of life: Is it a matter of choice? Arch Intern Med 161: 1645-1652.
18. Kagawa Y, Dever GJ, Otto, CTY, Charupoonphol P, Supanatas S, et al. (2003) Single nucleotide polymorphism and lifestyle-related diseases in the Asia-Pacific Region: Comparative study in Okinawa, Palau and Thailand. Asia-Pac J Public Health 15: S10-S14.
19. Komatsu F, Kagawa Y, Kawabata T, Kaneko Y, Purvee B, et al. (2008) Dietary habits of Mongolian people, and their influence on lifestyle-related diseases and early aging. Curr Aging Sci 1: 84-100.
20. Komatsu F, Kagawa Y, Sakuma M, Kawabata T, Kaneko Y, et al. (2006) Investigation of oxidative stress and dietary habits in Mongolian people, compared to Japanese people. Nutr Metab (Lond) 3: 21.
21. Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, et al. (2012) Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med 172: 555-563.
22. Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A (2009) Meat intake and mortality: a prospective study of over half a million people. Arch Intern Med 169: 562-571.
23. Mozaffarian D, Rimm EB (2006) Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 296: 1885-1899.
24. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, et al. (2006) Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) study cohort I. Circulation 113: 195-202.
25. Ministry of Health, Labour and Welfare, Japan (2010) Dietary Reference Intakes for Japanese 2010. Daiichi Shuppan Publishing Co., Ltd., Tokyo.
26. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Yoshikawa T, et al. (2002) Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPARalpha. J Lipid Res 43: 107-114.
27. Harris WS, Von Schacky C (2004) The Omega-3 Index: a new risk factor for death from coronary heart disease? Prev Med 39: 212-220.
28. Ninomiya T, Nagata M, Hata J, Hirakawa Y, Ozawa M, et al. (2013) Association between ratio of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: The Hisayama Study. Atheroscler 23: 261-267.
29. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, et al. (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 91: 3527-3561.
30. Green JT, Liu Z, Bazinet RP (2010) Brain phospholipid arachidonic acid half-lives are not altered following 15 weeks of N-3 polyunsaturated fatty acid adequate or deprived diet. J Lipid Res 51: 535-543.
31. DeMar JC Jr, Ma K, Bell JM, Rapoport SI (2004) Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. J Neurochem 91: 1125-1137.
32. Umhau JC, Zhou W, Carson RE, Rapoport SI, Polozova A, et al. (2009) Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. J Lipid Res 50: 1259-1268.
33. Ryan VH, Primiani CT, Rao JS, Ahn K, Rapoport SI, et al. (2014) Coordination of gene expression of arachidonic and docosahexaenoic acid cascade enzymes during human brain development and aging. PLoS One 9: e100858.
34. Serhan CN, Chiang N (2008) Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol 153 Suppl 1: S200-215.
35. Ohnishi H, Saito Y (2013) Eicosapentaenoic acid (EPA) reduces cardiovascular events: relationship with the EPA/arachidonic acid ratio. J Atheroscler Thromb 20: 861-877.
36. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, et al. (2012) Genetic adaptation of fatty-acid metabolism: A human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. Am J Hum Genet 90: 809-820.
37. Hellstrand S, Sonestedt E, Ericson U, Gullberg B, Wirfält E, et al. (2012) Intake levels of dietary long-chain PUFAs modify the association between genetic variation in FADS and LDL-C. J Lipid Res 53: 1183-1189.
38. Murff HJ, Edwards TL (2014) Endogenous Production of Long-Chain Polyunsaturated Fatty Acids and Metabolic Disease Risk. Curr Cardiovasc Risk Rep 8.
39. Turner BL, Thompson AL (2013) Beyond the Paleolithic prescription: incorporating diversity and flexibility in the study of human diet evolution. Nutr Rev 71: 501-510.
40. Coyne JA, Hoekstra HE (2007) Evolution of protein expression: new genes for a new diet. Curr Biol 17: R1014-1016.
41. Itan Y, Jones BL, Ingram CJ, Swallow DM, Thomas MG (2010) A worldwide correlation of lactase persistence phenotype and genotypes. BMC Evol Biol 10: 36.
42. Takenaka A, Nakamura S, Mitsunaga F, Inoue-Murayama M, Udono T, et al. (2012) Human-specific SNP in obesity genes, adrenergic receptor beta2 (ADRB2), Beta3 (ADRB3), and PPAR γ2 (PPARG), during primate evolution. PLoS One 7: e43461.
43. Yanagisawa Y, Hasegawa K, Dever GJ, Otto CTO, Sakuma M, et al. (2001) Uncoupling protein 3 and peroxisome proliferator-activated receptor γ2 contribute to obesity and diabetes in Palauans. Biochem Biophys Res Commun 281: 772-778.
44. Dufour C, Casane D, Denton D, Wickings J, Corvol P, et al. (2000) Human-chimpanzee DNA sequence variation in the four major genes of the renin angiotensin system. Genomics 69: 14-26.
45. Prior IA, Evans JG, Harvey HP, Davidson F, Lindsey M (1968) Sodium intake and blood pressure in two Polynesian populations. N Engl J Med 279: 515-520.
46. Okinawa Local Government (2011) Okinawa Health and Nutrition Survey, Okinawa.
47. Running CA, Craig BA, Mattes RD (2015) Oleogustus: The Unique Taste of Fat. Chem Senses 40: 507-516.
48. Ministry of Internal Affairs and Communications (2014) Survey on Japanese Household.
49. Wilson K, Rosenberg MW, Abonyi S (2011) Aboriginal peoples, health and healing approaches: the effects of age and place on health. Soc Sci Med 72: 355-364.
50. Statistics Canada (2006) Aboriginal peoples in Canada 2006.
51. Fumagalli M, Moltke I, Grarup N, Racimo F, Bjerregaard P, et al. (2015) Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science 349: 1343-1347.
52. Paunescu AC, Ayotte P, Dewailly E, Dodin S, Pedersen HS, et al. (2013) Polyunsaturated fatty acids and calcaneal ultrasound parameters among Inuit women from Nuuk (Greenland): a longitudinal study. Int J Circumpolar Health 72: 20988.
53. Voruganti VS, Higgins PB, Ebbesson SO, Kennish J, Göring HH, et al. (2012) Variants in CPT1A, FADS1, and FADS2 are Associated with Higher Levels of Estimated Plasma and Erythrocyte Delta-5 Desaturases in Alaskan Eskimos. Front Genet 3: 86.

54. Marcadier JL, Boland M, Scott CR, Issa K, Wu Z, et al. (2015) Congenital sucrase-isomaltase deficiency: identification of a common Inuit founder mutation. CMAJ 187: 102-107.
55. Zák A, Tvrzická E, Vecka M, Jáchymová M, Duffková L, et al. (2007) Severity of metabolic syndrome unfavorably influences oxidative stress and fatty acid metabolism in men. Tohoku J Exp Med 212: 359-371.
56. Mezzano D, Muñoz X, Martínez C, Cuevas A, Panes O, et al. (1999) Vegetarians and cardiovascular risk factors: hemostasis, inflammatory markers and plasma homocysteine. Thromb Haemost 81: 913-917.
57. Bamia C, Trichopoulos D, Ferrari P, Overvad K, Bjerregaard L, et al. (2007) Dietary patterns and survival of older Europeans: The EPIC-Elderly Study (European Prospective Investigation into Cancer and Nutrition). Public Health Nutr 10: 590-598.
58. Bondonno CP, Croft KD, Ward N, Considine MJ, Hodgson JM (2015) Dietary flavonoids and nitrate: effects on nitric oxide and vascular function. Nutr Rev 73: 216-235.
59. Schini-Kerth VB, Etienne-Selloum N, Chataigneau T, Auger C (2011) Vascular protection by natural product-derived polyphenols: in vitro and in vivo evidence. Planta Med 77: 1161-1167.
60. Zhan S, Ho SC (2005) Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr 81: 397-408.
61. Orlich MJ, Singh PN, Sabaté J, Jaceldo-Siegl K, Fan J, et al. (2013) Vegetarian dietary patterns and mortality in Adventist Health Study 2. JAMA Intern Med 173: 1230-1238.
62. Key TJ, Fraser GE, Thorogood M, Appleby PN, Beral V, et al. (1999) Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. Am J Clin Nutr 70: 516S-524S.
63. Shang P , Shu Z, Wang Y, Li N, Du S, et al. (2011) Veganism does not reduce the risk of the metabolic syndrome in a Taiwanese cohort. Asia Pac J Clin Nutr 20: 404-410.
64. Boccardi V, Paolisso G, Mecocci P (2016) Nutrition and lifestyle in healthy aging: the telomerase challenge. Aging (Albany NY) 8: 12-15.
65. Tiainen AM, Männistö S, Blomstedt PA, Moltchanova E, Perälä MM, et al. (2012) Leukocyte telomere length and its relation to food and nutrient intake in an elderly population. Eur J Clin Nutr 66: 1290-1294.
66. Moores CJ, Fenech M, O'Callaghan NJ (2011) Telomere dynamics: the influence of folate and DNA methylation. Ann N Y Acad Sci 1229: 76-88.
67. Richards JB, Valdes AM, Gardner JP, Kato BS, Siva A, et al. (2008) Homocysteine levels and leukocyte telomere length. Atherosclerosis 200: 271-277.
68. Cassidy A, De Vivo I, Liu Y, Han J, Prescott J, et al. (2010) Associations between diet, lifestyle factors, and telomere length in women. Am J Clin Nutr 91: 1273-1280.
69. Song Y, You NC, Song Y, Kang MK, Hou L, et al. (2013) Intake of small-to-medium-chain saturated fatty acids is associated with peripheral leukocyte telomere length in postmenopausal women. J Nutr 143: 907-914.
70. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, et al. (2010) Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. JAMA 303: 250-257.
71. Arterburn LM, Hall EB, Oken H (2006) Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 83: 1467S-1476S.
72. Adkins Y, Kelley DS (2010) Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J Nutr Biochem 21: 781-792.
73. Higuchi T, Shirai N, Saito M, Suzuki H, Kagawa Y (2008) Levels of plasma insulin, leptin and adiponectin, and activities of key enzymes in carbohydrate metabolism in skeletal muscle and liver in fasted ICR mice fed dietary n-3 polyunsaturated fatty acids. J Nutr Biochem 19: 577-586.
74. Cha SH, Fukushima A, Sakuma K, Kagawa Y (2001) Chronic docosahexaenoic acid intake enhances expression of the gene for uncoupling protein 3 and affects pleiotropic mRNA levels in skeletal muscle of aged C57BL/6NJcl mice. J Nutr 131: 2636-2642.
75. Garcia SM, Rosenberg AA (2010) Food security and marine capture fisheries: characteristics, trends, drivers and future perspectives. Philos Trans R Soc Lond B Biol Sci 365: 2869-2880.
76. Kawabata T, Shimoda K, Horiguchi S, Domon M, Hagiwara C, et al. (2013) Influences of stearidonic acid-enriched soybean oil on the blood and organ biochemical parameters in rats. Prostaglandins, Leukotrienes and Essential Fatty Acids 88: 179-184.
77. Zhou Y, Lin Y, Wu X, Feng C, Long C, et al. (2014) The high-level accumulation of n-3 polyunsaturated fatty acids in transgenic pigs harboring the n-3 fatty acid desaturase gene from Caenorhabditis briggsae. Transgenic Res 23: 89-97.
78. Wu X, Ouyang H, Duan B, Pang D, Zhang L, et al. (2012) Production of cloned transgenic cow expressing omega-3 fatty acids. Transgenic Res 21: 537-543.
79. Minister of Health, Labour and Welfare, Japan. (2012) Ministerial Notification No. 430 of the Ministry of Health, Labour and Welfare, Japan.
80. Yamamoto K, Hatakeyama Y, Sakamoto Y, Honma T (2016) The Japanese diet from 1975 delays senescence and prolongs life span in SAMP8 mice. Nutrition 32: 122-128.
81. Kagawa Y, Nishizawa M, Suzuki M, Miyatake T, Hamamoto T, et al. (1982) Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. J Nutr Sci Vitaminol (Tokyo) 28: 441-453.
82. Okinawa Local Government Department of Health (2005) Standardized mortality ratio (SMR) of diseases in cities, towns and villages of Okinawa Prefecture.
83. Renaud S, de Lorgeril M, Delaye J, Guidollet J, Jacquard F, et al. (1995) Cretan Mediterranean diet for prevention of coronary heart disease. Am J Clin Nutr 61: 1360S-1367S.
84. Pérez-López FR, Chedraui P, Haya J, Cuadros JL (2009) Effects of the Mediterranean diet on longevity and age-related morbid conditions. Maturitas 64: 67-79.
85. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A (2008) Adherence to Mediterranean diet and health status: meta-analysis. BMJ 337: a1344.
86. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 2: 117-119.
87. Rajalahti T, Lin C, Mjøs SA, Kvalheim OM (2016) Changes in serum fatty acid and lipoprotein subclass concentrations from prepuberty to adulthood and during aging. Metabolomics 12: 51.